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CHAIRS OF THE CONFERENCE

Prof. Françoise Barré-Sinoussi, Institut Pasteur, Paris, France
Dr Jack Whitescarver, Office of AIDS Research, NIH, Bethesda, USA

SCIENTIFIC PROGRAM COMMITTEE

Dr Carl Dieffenbach (USA), **Dr Marie-Lise Gougeon** (France)**, Prof. Olivier Lambotte** (France)**, Dr Cliff Lane** (USA)**, Dr Gary Nabel** (USA)**, Dr Julie Overbaugh** (USA),

Dr Gianfranco Pancino (France), **Prof. Giuseppe Pantaleo** (Switzerland), **Dr Felix Rey** (France), **Prof. Olivier Schwartz** (France), **Prof. Simon Wain-Hobson** (France)

THEME OF THE CONFERENCE

May 2013 will mark the 30th year since the publication in Science reporting for the first time the identification of a retrovirus associated with AIDS-related syndromes, now referred to as human immunodeficiency virus. To celebrate this anniversary, the **Institut Pasteur** in collaboration with the **U.S. National Institutes of Health, ANRS and Sidaction** is organizing an international symposium. The objective of this symposium is not to trace the history of the discovery of the virus, but to focus on the critical challenges and the future priorities that remain in HIV science as a result of 30 years of fantastic achievements. Distinguished international speakers will share their findings and their vision of the priorities of HIV research in the coming years. The symposium will gather a broad array of participants: senior scientists, young investigators, students and clinical researchers working in HIV and related areas from developed as well as resource-limited countries.

Institut Pasteur
Centre d'Information Scientifique
25-28 rue du Dr Roux - 75724 Paris cedex 15 - France
http://www.30yearsHIV.org - 30yearsHIV@pasteur.fr

WELCOME DESK • OPENING HOURS

If your registration is fully covered, you will receive your complete congress kit in a ViiV Healthcare bag, including your badge, the certificate of attendance, the conference program and if pre-booked ahead, the voucher for the conference dinner on Wednesday night.

If registration was not fully covered, please come directly to the registration desk "on site payment". We accept payment by VISA or MASTERCARD (not AMEX) or cash.

Registration desk opens at 4:30 pm on May 21st, please come early to pick-up your badge and congress kit in order to avoid delays in handing-out badges and avoid queues.

Tuesday May 21st, 2013	Wednesday May 22nd, 2013	Thursday May 23rd, 2013
Registration 4:30-6:30 pm	Registration 7:45 am-6:45 pm	Registration 8:15 am-4:15 pm
Cloakroom *4:30-09:00 pm*	*Cloakroom* *7:45 am-7:00 pm*	*Cloakroom* *8:15 am-7:15 pm*

ORAL SESSIONS

Scientific sessions are taking place in the main auditorium of *the "Centre d'Information Scientifique" (CIS)*, **building 15A (CIS)**, which can host up to 529 conference delegates.

POSTER SESSIONS

Two poster sessions will be displayed in the exhibition area (tent) located outside in front of the CIS entrance, **building 15A**, as following:

Poster session I: Wednesday May 22nd from 1:45 pm to 2:45 pm // Posters 33P to 90P

Poster session II: Thursday May 23rd from 1:45 pm to 2:45 pm // Posters 91P to 148P

Poster numbers are in the program; check the matching number on the board to display your poster in the right place. Magnets are available at the welcome desk to fix your poster.

LUNCHES AND COFFEE-BREAK

Wednesday 22nd and Thursday 23rd, buffet lunches will be served in the hall of CIS and under the tent.

During breaks, refreshments and coffee will be available at several buffet points in the hall of CIS.

WIFI

Complimentary Wifi is available in the "CIS", no password is needed, just use "CIS" connection. Computers with internet access are also at your disposal on the 2nd floor (use the lift) in the Library.

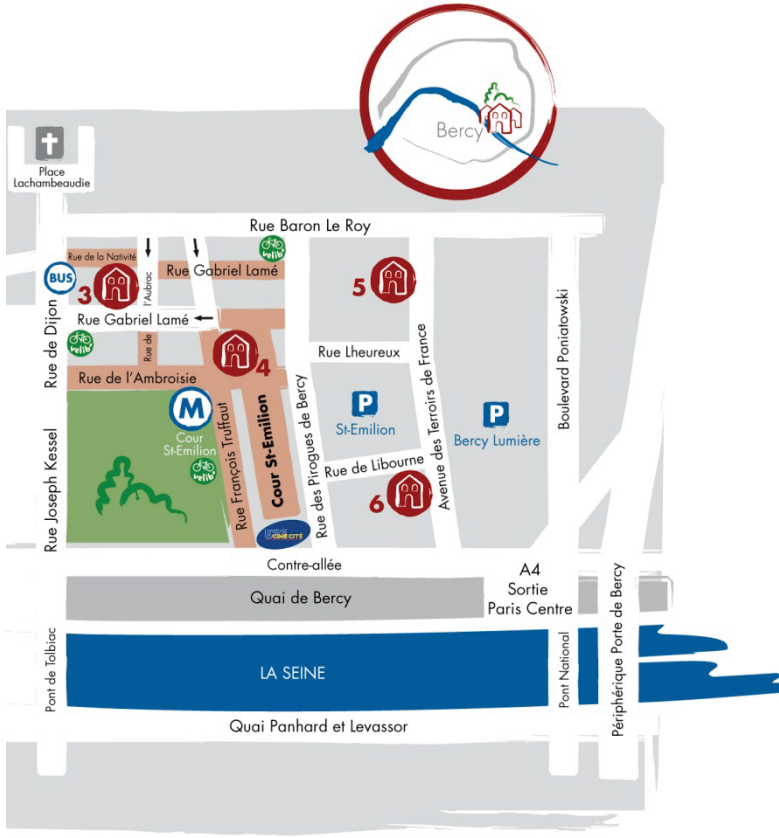
SOCIAL PROGRAMME

You are invited to join us for the Welcome reception in the hall of CIS on Tuesday 21st at 7:00 pm.

Conference dinner (submit to paying registration) is scheduled on **Wednesday 22nd** at 8.00 pm at "Les Pavillons de Bercy - Le Théâtre du Merveilleux".

Participants who have registered to this conference-gala dinner will find their voucher together with their name badge in the registration envelope.

Important: This ticket must be presented to the hostess upon arrival at "Les Pavillons de Bercy"



Les Pavillons de Bercy - Le Théâtre du Merveilleux
53 avenue des Terroirs de France, 75012 Paris
Car Park Vinci: Saint Emilion (recommanded) or Bercy Lumière
Metro station: Cours Saint-Emilion (Line 14) – 20 minutes from Institut Pasteur


Building 15 (CIS)
Welcome Desk
Cloakroom
Auditorium
Coffee break, lunches
and welcome reception
Tent
Posters exhibition
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Tuesday, May 21, 2013

4.30-6.30 pm Welcome desk opening // Registration of participants

Opening Session **1os**

06:30 **Welcome Remarks**

Alice Dautry, (General Director, Institut Pasteur, Paris, France)

Jack Whitescarver, (NIH Associate Director for AIDS Research and Director, Office of AIDS Research, Bethesda, USA)

Line Matthiessen, (European Commission, Directorate-General for Research and Innovation, Brussels, Belgium)

Jean-François Delfraissy, (General Director, French National Agency for Research on AIDS and viral hepatitis (ANRS), Paris, France)

07:00 **Special Opening Lecture:**

"The science of HIV/AIDS: much accomplished, much to do"
Anthony Fauci
NIAID/NIH, Bethesda, USA

07:30 **Social Programme**

Welcome Cocktail and light dinner buffet in the hall of the CIS

Wednesday, May 22, 2013

7.45 am Welcome desk opening

Plenary session	Interactions at molecular levels: Viral strategies of replication and host restriction mechanisms	2PL

Chairs: Simon WAIN-HOBSON
Robin WEISS

1 08:40
Innate mechanisms of HIV-1 restriction and their viral countermeasures
M. Malim
Dept of Infectious Disease, King's College London, London, United Kingdom

2 09:05
HIV assembly andmaturation: preparing the virus for infecting a new cell
H.G. Kräusslich
Universität Heidelberg, Germany

3 09:30
From HIV inhibition to HIV escape: CCR5 conformations are differentially exploited by chemokines and R5 HIV-1
P. Colin[1], Y. Bénureau[1], I. Staropoli[1], Y. Wang[1], O. Hartley[2], A. Brelot[1], F. Arenzana-Seisdedos[1], **B. Lagane[1]***
[1]Institut Pasteur, Paris, France [2]University of Geneva, Geneva, Switzerland

4 09:45
RNR2 repression by p21 restricts reverse transcription of HIV-1 and related-lentiviruses in macrophages
A. Allouch[4]*, A. David[4], S. Amie[6], H. Lahouassa[3], F. Margottin-Goguet[3-1-5], F. Barré-Sinoussi[4-2], A. Saez-Cirion[4], B. Kim[6], G. Pancino[4]
[1]CNRS, UMR8104 [2]INSERM [3]Inserm, U1016, Institut Cochin [4]Unité de régulation des infections retrovirales, Institut Pasteur de Paris [5]Univ Paris Descartes, Paris, France [6]Microbiology and Immunology, University of Rochester Medical Center, Rochester, United States

Plenary session	Interactions at molecular levels: Viral strategies of replication and host restriction mechanisms	3PL

Chairs: Eric HUNTER
Felix REY

5 10:00
Molecular mechanisms of HIV-1 post-integration latency
C. Van Lint
Université Libre de Bruxelles, Brussels, Belgium

6 10:25
Using systems approaches to study HIV biology
N.J. Krogan
University of California, San Francisco, United States

7 10:50
Cyclin T1 and CDK9 T-Loop Phosphorylation are downregulated during establishment of HIV-1 latency in Primary Resting Memory CD4$^+$ T Cells
M. Famiglietti[1]*, S. Budhiraja[2], A. Bosque[3], A. Rice[2], V. Planelles[3]
[1]San Raffaele Scientific Institute, Milano, Italy [2]Baylor College of Medicine, Houston [3]University of Utah, Salt Lake City, United States

11:05 ***Coffee break***

*** Selected speaker**

Plenary session	Interactions at tissue & systemic levels: Viral strategies of infection and host responses	4PL

Chairs: Thomas HOPE
Gianfranco PANCINO

8 11:30 **Virus-host interactions during the first phases of infection and design principles for prevention of HIV-1 transmission to women**
A. Haase
University of Minnesota, Minneapolis, United States

9 11:55 **Innate immune responses in acute HIV-1 infection: protective or pathogenic?**
P. Borrow
Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom

10 12:20 **The replicative capacity of transmitted HIV-1 contributes significantly to CD4 decline independent of VL and host contributions**
J. Prince[3], D. Claiborne[3], T. Yu[2], S. Lakhi[6], W. Kilembe[6], L. Yue[3], J. Gilmour[1], J. Tang[5], R. Kaslow[5], S. Allen[4], **E. Hunter**[3-6]*
[1]International AIDS Vaccine initiative, London, United Kingdom [2]Biostatistics [3]Emory Vaccine Center [4]Pathology and Laboratory Medicine, Emory University, Atlanta [5]Medicine and Epidemiology, University of Alabama at Birmingham, Birmingham, United States [6]Zambia Emory HIV Research Project, Lusaka, Zambia

11 12:35 **HIV exploits seminal cytokine network to promote its transmission to cervic0-vaginal tissue. Ex *vivo* study**
L. Margolis*
NICHD, Section of Intercellular Interactions, NIH, Bethesda, United States

12:50 **Lunch Buffet**

13:45 **Poster Session I**

*** Selected speaker**

Afternoon

Plenary session	Interactions at tissue & systemic levels: Viral strategies of infection and host responses	5PL
Chairs:	Rafick SÉKALY Olivier SCHWARTZ	

12 02:45 **A role for immune cell migration in the dissemination of HIV infection?**
T. Mempel
[1]Harvard Medical School [2]Massachusetts General Hospital, Boston, United States

13 03:10 **The human microbiome in health and immunodeficient states**
F.D. Bushman
The Perleman School of Medicine at the University of Pennsylvania, Philadelphia, United States

14 03:35 **Deconvoluting the molecular arm race between HIV and the CD8+ T-cell response**
M.C. Iglesias[2], M. Hashimoto[3], K. Ladell[4], P. Wilmann[1], M. Takiguchi[3], J. Rossjohn[1], D. Price[4], **V. Appay[2]***
[1]Monash University, Victoria, Australia [2]U945 Infection and Immunity, INSERM, Paris, France [3]Center for AIDS Research, Kumamoto, Japan [4]Cardiff University School of Medicine, Cardiff, United Kingdom

15 03:50 **Widespread expression of HIV-1 p24-Gag protein in tissues of patients receiving combination antiretroviral therapy**
R. Fox[2]*, B. Johnson[3], K. Wong[2], B. Larsen[2], D. Westfall[2], C. Fervet[3], J. Elliott[1], P. Anton[1], J. Mullins[2]
[1]AIDS Institute, Department of Medicine, University of California Los Angeles, Los Angeles [2]Department of Microbiology, University fo Washington [3]Department of Comparative Medicine, University of Washington, Seattle, United States

04:05 ***Coffee break***

Plenary session	Inflammation, immune activation & pathogenesis	6PL
Chairs:	Clifford LANE Marie-Lise GOUGEON	

16 04:30 **The good, the bad and the ugly of immune activation**
D. Douek
Vaccine Research Center, NIH/NIAID, Bethesda, United States

17 04:55 **The enigma of immune activation control in natural hosts**
M. Müller-Trutwin
Unité des Régulations des Infections rétrovirales, Institut Pasteur, Paris, France

18 05:20 **Linking inflammation & immune activation with non-AIDS disease: is the link causal?**
J. Lundgren
Rigshospitalet / Copenhagen Univ Hospital University of Copenhagen, Copenhagen, Denmark

*** Selected speaker**

19 05:45 **CD4+ T cells lacking SAMHD1 expression are highly proliferative in vivo and decrease during HIV-1 infection**

N. Ruffin[2-3], V. Brezar[2-3], D. Ayinde[4], O. Schwartz[4], J.D. Lelievre[1-2], Y. Levy[1-2-3], **N. Seddiki[2-3]***

[1]Clinical immunopathology, Hôpital Henri Mondor, Faculté de Médecine [2]U955 Equipe 16, INSERM [3]Vaccine Reaserch Institute, Créteil [4]Unité Virus et Immunité, Département de Virologie, Institut Pasteur, Paris, France

20 06:00 **Experimental CD4 depletion prior to SIV infection in macaques results in encephalitis, massive macrophages and microglia infection, and rapid turnover of infected cells**

L. Micci[2], R. Iriele[2], X. Alvarez[3], R. Geleziunas[4], D. Hazuda[7], A. Ortiz[2], S. Pahwa[6], M. Davenport[1], J. Estes[5], A. Lackner[3], G. Silvestri[2], **M. Paiardini[2]***

[1]The University of New South Wales, Sydney, Australia [2]Emory University, Atlanta [3]Tulane National Primate Research Center, Covington [4]Gilead Sci, Inc., Foster City [5]AIDS Cancer Virus Program, NCI-Frederick, Frederick [6]University of Miami, Miami [7]Merck Res Labs, West Point, United States

21 06:15 **Activation and gut-homing of plasmacytoid dendritic cells persists in the absence of HIV/SIV replication and contributes to residual chronic immune activation**

H. Li[2], T. Evans[2], J. Gillis[2], P. Goepfert[1], **R K. Reeves[2]***

[1]University of Alabama at Birmingham, Birmingham [2]Harvard Medical School, Southborough, United States

06:30 **Community speaker remarks**

G. Gonsalves
Yale Law School, New Haven, USA

06:45 **End of the session**

08:00 **Social programme**

Congress dinner at Théâtre du Merveilleux (Les Pavillons de Bercy)

With Guests of Honor and Special Guests

Opening address given by Dr Robert Gallo, Guest of Honor
Closing address given by Prof. Luc Montagnier, Guest of Honor

*** Selected speaker**

Thursday, May 23, 2013

Plenary session | **Clinical & translational Research** | **7PL**

Chairs: Carl DIEFFENBACH
Salim ABDOOL KARIM

22 09:00 **Gene Therapy approach for HIV-1 infection**

M. Cavazzana-Calvo[2-7-5], P. Frange[3], C. Parolin[1], G. Pancino[6], F. Spavanello[1], S. Blanche[3], E. Oksenhendler[4], for the ANRS HIV Gene Therapy Consortium

[1]Dept of Histology, Microbiology & Biotechnology, University of Padua, Padua [2]Biotherapy Dept [3]Pediatric Immuno-Haematology Unit, APHP Hôpital Necker-Enfants Malades [4]Clinical Immunology Unit, APHP, Hôpital Saint-Louis [5]GHU Ouest Biotherapy Clinical Investigation Center, INSERM, APHP [6]Regulation Unit for Retroviral Infections, Institut Pasteur [7]IMAGINE Institute, Université Paris Descartes, Paris, France

23 09:25 **HIV-1 Neutralizing Antibodies: Understanding Nature's Pathways**

J. Mascola

NIH/NIAID/VRC, Bethesda, United States

24 09:50 **Antiretroviral agents prevent HIV transmission: Where do we go from here?**

M. Cohen

University of North Carolina, Chapel Hill, United States

25 10:15 **Impact of an enhanced prenatal HIV counselling on male partner HIV testing, couple counselling and HIV-free survival of infants in Yaoundé, Cameroon. ANRS 12127-Prenahtest trial**

P. Tchendjou Tankam[1-6]*, J. Orne-Gliemann[4], P. Koki Ndombo[2], T. Mossus[1], F. Eboko[5], E. Balestre[4], A. Ngo Essounga[1], D. Amassana[1], A.C. Bissek[3], M. Tejiokem[1-6], F. Dabis[4], &. For The Prenahtest Anrs 12127 Study Group.

[1]Epidemiologie et Sante Publique, Centre Pasteur Cameroun [2]Centre Mère Enfant, Fondation Chantal Biya [3]Division de la Recherche opérationnelle en santé, Ministère de la Santé Publique, Yaoundé, Cameroon [4]INSERM U897, Institut de Santé Publique, Epidémiologie et Développement. Université Bordeaux Segalen, Bordeaux [5]UMR 912 IRD-INSERM-U2, Institut de Recherche pour le développement, Marseille [6]Réseau International des Instituts Pasteur, Institut Pasteur Paris, Paris, France

26 10:30 **Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production**

M. Perreau[1]*, A.L. Savoye[1], E. De Grignis[1], J.M. Corpataux[2], R. Cubas[5], E.K. Haddad[5], L. De Leval[3], C. Graziosi[1], G. Pantaleo[1-4]

[1]Division of Immunology and Allergy [2]Division of Thoracic Surgery [3]Institute of Pathology, Lausanne University Hospital [4]Swiss Vaccine Research Institute, Lausanne, Switzerland [5]Vaccine and Gene Therapy Institute, Port St Lucie, United States

27 10:45 **Four Days a Week and Less on Proper Antiviral Combinations Provided Long-Term Maintenance on 84 Patients' HIV / The ICCARRE PROJECT (1)**

J. Leibowitch[1]*, D. Mathez[1], P. De Truchis[1], D. Le Dû[1], J.C. Melchior[1], B. Autran[2], C. Perrronne[1], J. Izopet[3], J. David[4]

[1]Infectious Disease Dept, Raymond Poincaré Hospital, Garches [2]Immunity and Infection Laboratory, Pitié-Salpétrière Hospital, Paris [3]Microbiology, Purpan Hospital, Toulouse, France [4]Harvard School of Public Health, Boston, United States

11:00 **Community speaker remarks**

J. Gapiya Niyonzima

ANSS, Centre Turiho, Kigobe, Burundi

11:15 ***Coffee break***

*** Selected speaker**

Plenary session	Strategies for HIV control & cure	8PL
Chairs:	Christine ROUZIOUX Giuseppe PANTALEO	

28 11:40 **Why curing HIV might be easier than assumed**
S. Deeks
UCSF, San Francisco, United States

29 12:05 **Natural and treatment induced control of HIV/SIV infections**
A. Saez-Cirion
Unité des Régulations des Infections rétrovirales, Institut Pasteur, Paris, France

30 12:30 **Association between negative regulators and HIV persistence during ART: Identifying potential therapeutic targets for eradication strategy**
R. Fromentin*, S. Dafonseca, M.B. Lawani, W. Bakeman, C. Vandergeeten, R.P. Sekaly, N. Chomont
Vaccine and Gene Therapy Institute-Florida, Port St Lucie, United States

12:45 **Lunch Buffet**

13:45 **Poster Session II**

Afternoon

Debate	Which strategy to achieve a cure for HIV-1 infection?	9D
Chair:	Olivier LAMBOTTE	

02:45 **Judith Currier** (University of California at Los Angeles, USA)
Sharon Lewin (Alfred Hospital, Melbourne, Australia)
Brigitte Autran (Hôpital Pitié-Salpétrière, Paris, France)
David Margolis (University of North Carolina at Chapel Hill, USA)
Paula Cannon (University of Southern California, Los Angeles, USA)

Community speaker debater
Alain Volny-Anne (EATG - European AIDS Treatment Group)

03:45 ***Coffee break***

Plenary session	Future vaccine strategies	10PL
Chairs:	Dan BAROUCH Julie OVERBAUGH	

31 04:10 **Taming cancer and HIV with dendritic cells**
A.K. Palucka, J. Banchereau
Baylor Institute for Immunology Research, Baylor University Medical Center, Dallas, TX, United States

32 04:35 **Beyond RV144 efficacy results: update and future plans**
P. Pitisuttithum
Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

*** Selected speaker**

Debate — Which strategy to achieve a cure for HIV-1 infection? — 11D

Chair: Gary NABEL

05:00

Yves Levy (Hôpital Henri Mondor and INSERM U955, Créteil, France)
Michel Nussenzweig (The Rockefeller University, New York, USA)
Jeff Lifson (NIH/NCI, Bethesda, USA)
Louis Picker (Oregon Health & Science University, Portland, USA)

Community speaker debater
Warren Mitchell (AVAC: Global Advocacy for HIV Prevention, New York, USA)

Closing Lecture by — 12CL

06:00

Key steps to an AIDS-free generation
Quarraisha Abdool Karim (University of KwaZulu-Natal, Durban, South Africa)

Closing Remarks by — 13CR

Françoise Barré-Sinoussi (Institut Pasteur, Paris, France)

ORAL COMMUNICATIONS

Interactions at molecular levels: Viral strategies of replication and host restriction mechanisms

Oral presentations

Innate mechanisms of HIV-1 restriction and their viral countermeasures

M. Malim
Dept of Infectious Disease, King's College London, London, United Kingdom

Viruses such as HIV-1 carry relatively very few genes of their own. Viral growth and spread are therefore completely dependent on multiple factors and functions supplied by the infected host. Conversely, hosts promote their own survival by leveraging an array of innate and adaptive anti-viral immune mechanisms that aim to control viral growth and disease. The balance between pro- and anti-viral factors therefore dictates the outcome of infection. This presentation will summarise our current understanding of two classes of innate, intracellular factors that are capable of suppressing viral growth. First, the classic “restriction factors” comprising the APOBEC3 proteins, TRIM5alpha, tetherin and SAMHD1 are ubiquitously present throughout the body, can potently suppress HIV-1 replication, but are largely evaded in human cells either through the direct action of viral accessory proteins or as a result of evolutionary adaptation. Second, interferon-stimulated genes (ISGs), of which there are ~1000 in humans, collectively impose a severe impediment to HIV-1 replication, even in the presence of the full complement of virus-encoded proteins. Recent work addressing the importance and mechanisms of action of these genes will be presented. Ultimately, it is hoped that therapeutic enhancement or manipulation of innate effector proteins will offer opportunities for developing novel therapeutics.

HIV assembly andmaturation: preparing the virus for infecting a new cell

H.G. Kräusslich
Universität Heidelberg, Germany

From HIV inhibition to HIV escape: CCR5 conformations are differentially exploited by chemokines and R5 HIV-1

P. Colin[1], Y. Bénureau[1], I. Staropoli[1], Y. Wang[1], O. Hartley[2], A. Brelot[1], F. Arenzana-Seisdedos[1], B. Lagane[1]
[1]*Institut Pasteur, Paris, France* [2]*University of Geneva, Geneva, Switzerland*

Background: CCR5 is a G-protein coupled receptor for chemokines (R5-CHKs) and the coreceptor for R5 HIV-1 entry into $CD4^+$ T-cells. R5-CHKs have anti-HIV activity *in vitro*, both by competing with the viral envelope glycoprotein gp120 for binding to CCR5 and by promoting CCR5 endocytosis, suggesting that they act as a natural barrier against HIV *in vivo*. But, attempts to correlate chemokine production in infected individuals and progression of infection gave contradictory results. Moreover, R5-CHKs often showed antiviral activities *in vitro* at concentrations exceeding their affinity constant values for binding to CCR5, even though R5-CHKs have higher affinities for CCR5 than gp120. This suggests an escape of R5 HIV-1 from R5-CHKs, which we hypothesized to result from the differential use of distinct CCR5 conformations by gp120 and R5-CHKs.

Methods: Molecular pharmacological and virological approaches were used to investigate how CCR5 conformation regulates its interactions with R5-CHKs and gp120 and determines the efficiency of the anti-HIV activity of R5-CHKs.

Results: In competition binding assays, R5-CHKs used as competitors have different affinity constants for binding to CCR5 depending on whether ^{125}I-CCL3 or ^{35}S-gp120 is used as a tracer, indicating that ^{125}I-CCL3- and ^{35}S-gp120-binding receptors represent different CCR5 forms, which R5-CHKs bind to with different affinities. These distinct CCR5 forms are differentially influenced by CCR5/G-protein interactions. While high affinity binding of natural agonist R5-CHKs requires G-proteins, gp120 binds to both G-protein coupled and uncoupled CCR5, explaining why these R5-CHKs are weak inhibitors of gp120 binding. Furthermore, high affinity binding of R5-CHKs to G-protein coupled CCR5 is dispensable for R5-CHK-mediated endocytosis, on which the anti-HIV activity of R5-CHKs depends. This, and the interaction of R5 HIV-1 with G-protein uncoupled CCR5, which are of low affinity for R5-CHKs, account for the inherently weak ability of R5-CHKs to inhibit infection. Finally, we found that the increased anti-HIV activity of CCL5 derivatives is due to recognition of gp120-binding conformations of CCR5.

Conclusion: Differential interaction of R5-CHKs and HIV-1 with distinct CCR5 forms could be the cause of HIV-1 escape to R5-CHKs in anatomical sites where HIV-1 replicates and provides new clues for the development of new anti-HIV molecules.

RNR2 repression by p21 restricts reverse transcription of HIV-1 and related-lentiviruses in macrophages

A. Allouch[4], A. David[4], S. Amie[6], H. Lahouassa[3], F. Margottin-Goguet[3-1-5], F. Barré-Sinoussi[4-2], A. Saez-Cirion[4], B. Kim[6], G. Pancino[4]
[1]CNRS, UMR8104 [2]INSERM [3]Inserm, U1016, Institut Cochin [4]Unité de régulation des infections retrovirales, Institut Pasteur de Paris [5]Univ Paris Descartes, Paris, France [6]Microbiology and Immunology, University of Rochester Medical Center, Rochester, United States

Objective: Macrophages play crucial roles in HIV/AIDS pathogenesis as they are important targets for HIV-1 replication and contribute to viral spread and viral reservoir formation. We have previously reported that p21 inhibits HIV-1, HIV-2 and SIV replication in macrophages by a major block at the level of reverse transcription (Bergamaschi et al-2009-J Virol). This study aimed at understanding the molecular mechanisms involved in p21-mediated restriction.

Methods and Results: Through overexpression and knockdown experiments in primary monocyte derived macrophages, we demonstrated that p21 inhibits HIV-1 reverse transcription by down-regulating the intracellular dNTP pool available for viral cDNA synthesis. We found that p21 represses the expression of ribonucleotide reductase subunit R2 (RNR2), an enzyme indispensable for dNTP biosynthesis. Accordingly, depletion of RNR2 by p21 induction or siRNA transfection inhibits reverse transcription of HIV-1 in macrophages. SIVmac251, which express the vpx protein that causes the degradation of SAMHD1, was also restricted by p21 and by RNR2 knockdown. We further explored the mechanisms underlying the inhibition of RNR2 expression by p21 and found that p21 represses, independently from Retinoblastoma protein RB, the expression of the RNR2 transcriptional transactivator E2F1. Consistently, the depletion of E2F1 by p21 induction or siRNA transfection represses RNR2 transcription and inhibits HIV-1 reverse transcription in macrophages.

Conclusion: Here we describe a new mechanism of restriction of lentiviral replication in macrophages: p21 inhibits HIV-1 reverse transcription by affecting dNTP biosynthesis through repression of RNR2 expression. This mechanism restricts other primate lentiviruses, such as HIV-2 and SIV, since it affects dNTP anabolism, differently from SAMHD1 restriction that promotes dNTP catabolism. Our findings describe a novel metabolic pathway of HIV-1 restriction in macrophages and open on new potential cellular targets for therapy research.

Interactions at molecular levels: Viral strategies of replication and host restriction mechanisms

Oral presentations

Molecular mechanisms of HIV-1 post-integration latency

C. Van Lint
Université Libre de Bruxelles, Brussels, Belgium

The current antiretroviral therapy cART is effective and life-prolonging but does not eradicate HIV-1 from infected patients. A reduction of HIV-1 RNA levels in the plasma in cART-treated individuals to less than 50 copies/ml is frequently achieved but low-level viremia persists as detected by ultrasensitive assays. The sources of this persistent viremia are still not fully understood but could arise from ongoing cycles of residual viral replication and/or from the reactivation of viral expression from latently-infected cells. These latently-infected cells contain stably-integrated, transcriptionally-silent but replication-competent proviruses, thereby representing latent reservoirs of HIV-1. They are a permanent source for virus reactivation and could be responsible for the rebound of plasma viral load observed after cART interruption.

HIV-1 transcriptional repression is crucial to the establishment and maintenance of postintegration latency. Several elements contribute to HIV-1 transcriptional repression including: 1) the site of integration and mechanisms of transcriptional interference, 2) the absence of crucial inducible host transcription factors, 3) the presence of transcriptional repressors, 4) the nucleosomal organization of the HIV-1 promoter, 5) the epigenetic control of the HIV-1 promoter (histone posttranslational modifications, such as histone acetylation and methylation, and DNA methylation), 6) the sequestration in an inactive form of the cellular Positive Transcription Elongation Factor b (P-TEFb), composed of cyclin-dependent kinase 9 (CDK9) and human cyclin T1, 7) the absence of the viral transactivator Tat, which promotes transcription via recruitment to the HIV-1 promoter of P-TEFb, histone-modifying enzymes and ATP-dependent chromatin-remodeling complexes required for nucleosomal disruption and transcriptional processivity. In her talk, Dr Carine Van Lint will review the epigenetic and non-epigenetic mechanisms regulating HIV-1 transcriptional repression and reactivation, as well as present recent data from her laboratory. Further understanding of these mechanisms should help devise novel strategies to eliminate latent HIV-1 reservoirs or to restrict the latent pool to a size bearable by the host immune system.

Using systems approaches to study HIV biology

N.J. Krogan
University of California, San Francisco, United States

There is a wide gap between the generation of large-scale biological data sets and more-detailed, structural and mechanistic studies. However, recent work that explicitly combine data from systems and structural biological approaches is having a profound effect on our ability to predict how mutations and small molecules affect atomic-level mechanisms, disrupt systems-level networks and ultimately lead to changes in organismal fitness. Our group aims to create a stronger bridge between these areas primarily using three types of data: genetic interactions, protein-protein interactions and post-translational modifications. Protein structural information helps to prioritize and functionally understand these large-scale datasets; conversely global, unbiasedly collected datasets helps inform the more mechanistic studies. Our efforts in this respect are presently focused on model organisms, including yeast and bacteria, as well as in mammalian cells, with a particular focus on pathogenesis.

Cyclin T1 and CDK9 T-Loop Phosphorylation are downregulated during establishment of HIV-1 latency in Primary Resting Memory CD4$^+$ T Cells

M. Famiglietti[1], S. Budhiraja[2], A. Bosque[3], A. Rice[2], V. Planelles[3]
[1]San Raffaele Scientific Institute, Milano, Italy [2]Baylor College of Medicine, Houston [3]University of Utah, Salt Lake City, United States

Objective: P-TEFb, a cellular kinase composed of Cyclin T1 and CDK9, is necessary for processive HIV-1 transcription. In addition to other crucial cellular factors, P-TEFb has been proposed to play a role in the establishment of HIV-1 latency in resting CD4$^+$ T cells, where its levels would be inadequate to allow full viral expression. Here we investigate the regulation of P-TEFb in a model of HIV-1 latency based on *in vitro*cultured central memory CD4$^+$ T cells (T_{CM}).

Methods: Latently infected cultured CD4$^+$ T_{CM} cells were established from naive CD4$^+$ T cells from healthy donors, as described in Bosque and Planelles, Blood 2009. Infection levels were monitored at different time points by detection of intracellular p24Gag. Cyclin T1 mRNA and Cyclin T1 targeting miRNAs were evaluated by quantitative reverse transcription (qRT)-PCR. Cell lysates were prepared at different time points and probed via immunoblot assays for detection of CyclinT1, total CDK9, pCDK9 and HEXIM1. P-TEFb interaction with HEXIM1 was evaluated by immunoprecipitation.

Results: Cyclin T1 and pCDK9 levels were low in naive, latently infected and uninfected cultured T_{CM}, whereas cellular activation significantly increased their expression. Cyclin T1 levels were downmodulated in naive, latently infected and uninfected cultured T_{CM} by multiple mechanisms, including proteasome-dependent proteolysis and post-transcriptional regulation mediated by miR-150. We also found that HEXIM1 expression was modulated in cultured T_{CM} in a similar fashion as that of Cyclin T1 and pCDK9 and, in line with these results, little P-TEFb was associated in the 7SK snRNA-HEXIM1 inactive complex. Moreover, cell activation resulted in increased formation of such a complex.

Conclusion: Sequestration of P-TEFbinto the inactive complex with 7SK RNP does not appear to be a mechanism contributing to the establishment of HIV-1 latency in primary cultured T_{CM}. Rather, the 7SK-RNP complex acts as a reservoir of pre-activated P-TEFb, from which HIV-1 protein Tat can recruit the cellular factor in order to promote full viral transcription. Drug screenings geared toward the discovery of antilatency drugs performed in primary memory CD4$^+$ T would favor the discovery of substances that trigger increases in Cyclin T1 and/or pCDK9 levels.

Interactions at tissue & systemic levels: Viral strategies of infection and host responses

Oral presentations

Virus-host interactions during the first phases of infection and design principles for prevention of HIV-1 transmission to women

A. Haase
University of Minnesota, Minneapolis, United States

Objective: Vaccines to prevent HIV-1 transmission are particularly urgently needed to protect women in the pandemic's epicenter in Africa. Studies of the early events in the SIV-rhesus macaque model reveal favorable opportunities for a vaccine at the portal of entry to prevent transmission by blocking the establishment and expansion of initially small populations of infected cells. We sought correlates of protection that target these early stages of infection in animals vaccinated with a live attenuated SIV vaccine.

Methods: Tissues collected at time points corresponding to the maturation of vaccine protection, and following vaginal challenge, were analyzed by in situ methods to characterize the impact of vaccination on infection at the portal of entry and the immune responses at that site.

Results: IgGantibodies to trimeric gp41 produced locally in spatial proximity to neonatal Fc receptor (FcRn)-expressing mucosal epithelium correlated with prevention of establishment and local expansion of infected founder populations in cervical vaginal tissues.

Conclusions: Developingimmunogens and adjuvants that reproduce this organized system of antibody production and FcRn-mediated delivery to mucosal frontlines on the path of virus entry with could contribute to development of an effective and safe HIV-1 vaccine.

Innate immune responses in acute HIV-1 infection: protective or pathogenic?

P. Borrow[2], A. Stacey[2], A. Fenton-May[2], O. Dibben[2], E. Haygreen[2], T. Emmerich[2], N. Kim[6], E. Marshall[2], K. Lavender[2], M. Cohen[4], P. Goepfert[3], I. Williams[1], D. Wallace[6], G. Shaw[7], B. Hahn[7], C. Ochsenbauer[3], J. Kappes[3], P. Norris[8], A. Mcmichael[2], B. Haynes[5]
[1]University College London, London [2]Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom [3]University of Alabama at Birmingham, Birmingham, Al [4]University of North Carolina, Chapel Hill, Nc [5]Duke University [6]RTI International, Durham, Nc [7]University of Pennsylvania, Pittsburgh, Pa [8]Blood Systems Research Institute, San Francisco, United States

Objective: The importance of events in acute HIV-1 infection (AHI) in determining the subsequent disease course prompts a need to understand the virus-immune system interactions in this phase of infection that impact on concurrent and ensuing viral replication and pathogenesis. The speed with which innate responses can be mobilized following pathogen exposure suggests they may play critical roles in AHI. We sought to characterise the innate responses activated during AHI and identify components of the response that contribute to control of viremia or conversely promote immune activation and virus replication.

Methods: Peripheral blood samples cryopreserved at serial timepoints post-infection were used to characterise the dynamics of systemic up-regulation of cytokines/chemokines and activation of innate cell subsets in AHI and address the impact of these factors on viral control.

Results: The increase in viremia in AHI was associated with rapid activation of systemic innate responses, evidenced by a reduction in the circulating dendritic cell frequency, elevations in plasma levels of type 1 interferon (IFN) and other soluble factors and natural killer (NK) cell activation and proliferation. Some components of the innate response, e.g. strong NK cell responses, correlated with establishment of lower set-point viral loads. However the association between any one component of the innate response and set-point viremia was confounded by other interlinked innate variables.

To seek evidence that type 1 IFN exerts selective pressure on HIV replication during the establishment of infection, the IFN-resistance of HIV isolates derived from AHI subjects and founder virus infectious molecular clones (IMCs) was compared to that of virus isolates/IMCs generated from the same subjects in chronic infection. Founder viruses were found to be more IFN-resistant than matched chronic viruses, suggesting that type I IFN plays an important role in HIV control during the initial stages of infection and may drive selection for IFN-resistant founder viruses from the transmitted virus pool.

Conclusions: Identification of type 1 IFN-mediated antiviral activity and NK effector activity as mechanisms that contribute to HIV-1 control during the initial stages of infection could enable harnessing of these activities to complement the protection afforded by vaccine-elicited adaptive responses.

The replicative capacity of transmitted HIV-1 contributes significantly to CD4 decline independent of VL and host contributions

J. Prince[3], D. Claiborne[3], T. Yu[2], S. Lakhi[6], W. Kilembe[6], L. Yue[3], J. Gilmour[1], J. Tang[5], R. Kaslow[5], S. Allen[4], E. Hunter[3-6]
[1]International AIDS Vaccine initiative, London, United Kingdom [2]Biostatistics [3]Emory Vaccine Center [4]Pathology and Laboratory Medicine, Emory University, Atlanta [5]Medicine and Epidemiology, University of Alabama at Birmingham, Birmingham, United States [6]Zambia Emory HIV Research Project, Lusaka, Zambia

Objective: Selection of CTL epitope escape mutations in the *gag* gene of HIV-1 during adaptation to the host's immune system frequently results in decreased replicative fitness. We have shown previously that transmission of such mutated viruses is associated with lower early set-point (ESP) VL in the seroconvertors. The goal of this study was to determine the role of host and viral characteristics that contribute to HIV-1 pathogenesis.

Methods: The *gag* genes of HIV-1 from 149 seroconvertors were cloned into a proviral backbone and the chimeric viruses were assayed for replicative capacity (RC). Spearman correlations were performed to determine RC associations with ESP VL in the seroconvertors. Kaplan-Meier survival analyses and Cox-proportional hazard models were applied to determine the impact of RC and various host factors known to modulate set point viral load on CD4 decline over the first 3 years of infection.

Results: Viral RC, determined by the transmitted Gag sequence, correlated significantly with ESP VL ($p=0.02$). Moreover, in a Kaplan-Meier survival analysis, recipients infected with highly replicating viruses reached an endpoint of 350 CD4 T cell counts almost one year earlier than those infected with more attenuated viral strains. Using Cox proportional hazard models, we showed that this effect of RC on CD4 decline was independent of set point viral load. In addition, gender, the presence of favorable HLA alleles, and the sharing of HLA-B alleles between donor and recipient were all independent risk factors that modulated the effect of RC on CD4 decline. Kaplan-Meier analyses further showed that the combination of being female and low VRC is highly protective, while sharing HLA alleles with the transmitting partner and receiving a virus exhibiting high VRC is hazardous.

Conclusions: The RC of transmitted HIV-1 appears to define the trajectory of disease independently of its impact on ESP VL. Viruses with high RC may establish a more inflammatory environment, or may more extensively deplete memory T cell subsets prior to the onset of adaptive immunity. Ongoing studies aimed at investigating these possibilities will further our understanding of the complex interactions between HIV-1 and its host.

HIV exploits seminal cytokine network to promote its transmission to cervic0-vaginal tissue. Ex *vivo* study

L. Margolis
NICHD, Section of Intercellular Interactions, NIH, Bethesda, United States

Background: The majority of HIV transmissions occur heterosexually, in particular through vaginal intercourse, mediated by HIV-containing semen. Semen is a biologically active fluid containing soluble factors, in particular cytokines. Here, we studied changes in seminal cytokines of HIV-1 infected men and their effect on HIV-1 transmission to cervical tissue *ex vivo*.

Methods: Semen obtained from therapeutically naïve HIV-1 infected and control men from India were analyzed for the presence of 21 cytokines. HIV-1 transmission was simulated *ex vivo* by depositing virus in semen or PBS on cervico-vaginal explants.

Results: In HIV-1-infected individuals concentrations of several individual cytokines have been changed. Also, HIV-1 infection locally changed the entire seminal cytokine network, which paradoxically became more “orderly” as evidenced by the increase of inter-cytokine correlations from 21 to 72 and strengthening of pre-existing correlations One of the seminal cytokines most up-regulated in HIV-infected men was IL-7 with the concentration ~200 times higher than in blood. At this concentration IL-7 enhanced transmission of HIV-1 to cervico-vaginal tissue. Cellular mechanism of this enhanced transmission includes suppression of apoptosis as evaluated by the expression of apoptotic markers, decrease of the infected cells depletion as evaluated by flow cytometry, and increase in cell cycling as evaluated by Ki67 staining.

Conclusions: Semen and blood are two separate immunological compartments, and this compartmentalization is increased by HIV-1 infection. HIV-1 infection imposes a high rigidity on the cytokine network, which may impair the immune system capacity to respond successfully to new microbial challenges. Cytokine changes in semen may alter HIV-1 replication and evolution in the male genital tract and the probability of HIV-1 transmission in the female genital tract. In particular, upon deposition on cervico-vaginal tissue, IL-7 prolongs the life of cells that replicate virus allowing a continuous release of HIV-1 and expands the pool of HIV-1 targets thus increasing the risk of HIV-1 acquisition. IL-7 together with other seminal soluble factors may be a key determinant of the efficiency of HIV-1 transmission from infected men to his uninfected female partners through vaginal intercourse and may become a new target for transmission preventive strategies.

Interactions at tissue & systemic levels: Viral strategies of infection and host responses

Oral presentations

A role for immune cell migration in the dissemination of HIV infection?

T. Mempel[2-1], T. Murooka[2-1]
[1]Harvard Medical School [2]Massachusetts General Hospital, Boston, United States

A variety of cell contact-mediated mechanisms have been identified that greatly enhance the otherwise low efficiency of HIV spread among T cells *in vitro*. These mechanisms share the common principles that concentrating infectious virus at the molecularly structured interfaces between cells increases the viral 'payload' per target cell, and that physiological mechanisms of intercellular communication, such as adhesion, cell polarization, and secretion, can be exploited to facilitate virus transfer. Such interfaces have been described between dendritic cells and T cells, macrophages and T cells, and between T cells, and have been named virological synapses.

We have recently begun to use multiphoton intravital microscopy to study the migratory behavior of human T cells in the lymphoid tissues of BLT humanized mice, and their interactions with their environment *in vivo*. Our initial results suggest that HIV-infected T cells migrate at reduced speeds, but nevertheless continue to roam through lymphoid tissues, which supports the spread of infection. During their migratory activity, they undergo viral envelop-dependent tethering interactions with $CD4^+$ cells in their vicinity, which facilitate cell fusion and syncytia formation, and may also serve the formation of virological synapses.

We hypothesize that infection of migratory immune cells, such as T cells, may be a pathogen strategy that allows cell-associated virus to overcome anatomical barriers and to shield itself against humoral immune factors in the extracellular environment during its dissemination within and between different tissues through contact-dependent spread.

The human microbiome in health and immunodeficient states

F.D. Bushman, G. Wu, J. Lewis, H. Li, K. Bittinger, E. Charlson, C. Hoffmann, R. Collman
The Perleman School of Medicine at the University of Pennsylvania, Philadelphia, United States

Humans live in association with very large numbers of bacteria, fungi, Archaea, viruses, and other organisms. The numbers are so large that the numbers of microbial cells associated with humans exceeds the number of human cells. Microbes are familiar as pathogens, but our microbiota provides essential assistance with digestion, immune development, and drug metabolism. The lecture will explore the nature of the human microbiota, tools for its analysis, and present some generalizations on microbial populations in lentiviral infection and other immunodeficient states.

Deconvoluting the molecular arm race between HIV and the CD8$^+$ T-cell response

M.C. Iglesias[2], M. Hashimoto[3], K. Ladell[4], P. Wilmann[1], M. Takiguchi[3], J. Rossjohn[1], D. Price[4], V. Appay[2]
[1]Monash University, Victoria, Australia [2]U945 Infection and Immunity, INSERM, Paris, France [3]Center for AIDS Research, Kumamoto, Japan [4]Cardiff University School of Medicine, Cardiff, United Kingdom

Objective: HIV has developed multiple strategies to evade host immune surveillance. The demonstration of HIV adaptation to human leukocyte antigen (HLA) class I at a population level represents a singular example of the capacity of a pathogen to evolve in order to evade host immunity. The immune system has nonetheless the capacity to adjust to and control such rapidly evolving viruses, yet the molecular basis for this process is unclear. Our aim here is therefore to provide a mechanism for understanding the "molecular arms race" between the immune system and HIV.

Methods: Here, we investigated the archetypal protective CD8$^+$ T-cell response in HIV-1 infection, which is directed against the immunodominant p24 Gag-derived epitope KK10 (KRWIILGLNK$_{263\text{-}272}$) presented by human leukocyte antigen (HLA)-B*2705. We combined *ex vivo* analyses from patient samples, together with *in vitro* functional assays as well as molecular / crystal structure data to reach analytical depths not previously achieved in human studies of individual CD8$^+$ T-cell clonotypes (i.e. *TCR* gene rearrangements).

Results: We show that particular KK10-specific CD8$^+$ T-cell clonotypes are highly selected *in vivo* and shared between individuals. These "public" clonotypes exhibit high levels of TCR avidity and antigen sensitivity, conferring functional advantages that enable effective suppression of HIV replication through robust TCR-pMHC interactions. The early and enigmatic L$_{268}$M mutation at position 6 of the KK10 epitope emerges specifically to avoid recognition by these highly effective CD8$^+$ T-cell clonotypes. But the expansion of alternative clonotypes with variant reactivity enables the overall KK10-specific response to adapt to this viral transformation. These newly recruited clonotypes express TCRs that engage wildtype and mutant KK10 antigens with similar affinities and almost identical docking modes, thereby accounting for their antiviral efficacy in HLA-B*2705$^+$ individuals.

Conclusion: A protective CD8$^+$ T-cell repertoire therefore encompasses the capacity to control TCR-accessible mutations, ultimately driving more complex HIV escape mutations "underground" to impact on antigen presentation. Overall, these findings illuminate the intricacies of the host-virus equilibrium at the clonotypic level and provide refined mechanistic insights into the workings of an effective CD8$^+$ T-cell response against HIV (Iglesias et al, *Blood* 2011 and *Immunity* in press).

Widespread expression of HIV-1 p24-Gag protein in tissues of patients receiving combination antiretroviral therapy

R. Fox[2], B. Johnson[3], K. Wong[2], B. Larsen[2], D. Westfall[2], C. Fervet[3], J. Elliott[1], P. Anton[1], J. Mullins[2]
[1]AIDS Institute, Department of Medicine, University of California Los Angeles, Los Angeles [2]Department of Microbiology, University fo Washington [3]Department of Comparative Medicine, University of Washington, Seattle, United States

Objectives: A current challenge to our ability to eliminate HIV-1 from infected individuals is our lack of a cohesive understanding of anatomical/cellular sites of "latent" and/or active viral reservoirs and compartments. Some studies of therapy-suppressed patients describe a lack of phylogenetic evolution of the viral population over time. This observation has led to the proposition that persistent viral genomes are primarily latent. However, other studies demonstrate low-level viral evolution during apparently complete plasma viral suppression in patients receiving combination antiretroviral therapy (cART) suggesting ongoing viral replication, perhaps in tissue sites or cell populations with reduced drug penetrance.

Methods: Quantitative immunohistochemistry (qIHC) was used to investigate p24-Gag protein production in multiple autopsy-derived tissues (acquired 10,000c/mL). From living donors (N=9; 4 detectable pVL, 5 undetectable pVL), we investigated gut-associated lymphoid tissue (GALT) biopsies with matched blood from two time-points over 10 years. Ostensibly HIV-1 negative tissues were used for comparison.

Results: We present data demonstrating widespread expression of p24-Gag protein in tissues and the potential for infectious virus production during otherwise apparently complete viral suppression in the blood. HIV-1 p24-Gag protein was detected in all tissues examined and was highly co-localized with markers of lymphoid ($p24^+$:$CD4^+$) and myeloid ($p24^+$:$CD68^+$) cells. HIV sequences for viral genetic analysis by single genome amplification of HIV-1 RNA and DNA are currently being generated from these samples.

Conclusions: These data reinforce that plasma viral measurements may not be a sufficient measure of suppression of HIV-1 within tissues *in vivo*. Additional studies are required to optimize assessment and quantification of ongoing viral production. This tissue viral index can then be correlated with measured tissue drug concentrations to determine therapeutic tissue levels of cART drugs to aid in optimizing eradication efforts.

Inflammation, immune activation & pathogenesis

Oral presentations

The good, the bad and the ugly of immune activation

D. Douek
Vaccine Research Center, NIH/NIAID, Bethesda, United States

Systemic immune activation occurs during the acute phase of HIV infection. However, its persistence into the chronic phase is associated with the rate of disease progression and the development of non-AIDS related mortality, independently of virus load. Dr. Douek will discuss the causes and consequences of immune activation in HIV infection as well as therapeutic approaches to mitigate its effects.

The enigma of immune activation control in natural hosts

M. Müller-Trutwin
Unité des Régulations des Infections rétrovirales, Institut Pasteur, Paris, France

Current antiviral treatments of HIV-infected individuals are highly efficient, but fail to abolish residual chronic immune activation. The level of T cell activation already at the early stage of HIV-1 infection is predictive of the rate of progression towards AIDS. We have shown that high IP-10 levels in acute HIV-1 infection predict rapid disease progression. In order to better understand what regulates the early inflammatory levels in HIV infection, we study non human primate models (NHP) as they allow to analyze the early interactions between virus and host in lymphoid tissues. SIV infection in African NHP, such as African green monkeys (AGM), is usually non-pathogenic despite high plasma and gut viral load levels. We have shown that AGMs during the first weeks of SIV infection show robust innate immune responses, inflammation and T cell activation, which are however rapidly down-regulated before the end of the acute phase of infection. We tested here whether the IFN-α level in acute infection impacts the outcome of SIVagm infection. A recombinant rhesus macaque IFN-α2-Ig fusion protein (rmIFN-α) has been constructed. A daily subcutaneous injection of 5x10^5 IU of rmIFN-α, with a 10% increase every 2 days during 15 days, induced an up to 2 log decrease of viremia and a significant increase of Interferon-stimulated genes (ISG) expressions, including of *IP-10*, during chronic SIVagm infection. We then infected naive AGMs with SIVagm and treated them with rmIFN-α between days 9 and 25 post-infection to mimic the early high IFN-α quantities observed in SIVmac-infected macaques. The IFN-α treatment during acute SIVagm infection had no major impact on ISG expression, T cell activation and CD4 T cell numbers. This suggests that the high levels of ISG expression associated with disease progression in HIV-infection are not solely driven by IFN-I.

Linking inflammation & immune activation with non-AIDS disease: is the link causal?

J. Lundgren
Rigshospitalet/ Copenhagen Univ Hospital University of Copenhagen, Copenhagen, Denmark

HIV infection – like any chronic viral infection – activates T-, B- lymphocytes and monocytes / macrophages. Also, HIV infection activates the coagulation pathways in part as these systems are interlinked. Whereas fully suppressive antiretroviral therapy reduces these processes, some HIV+ persons remain in a state of continued activation. Knowledge gained in last 5 years have firmly established that biomarkers of inflammation and coagulation are associated with excess risk of subsequent organ disease, cancer and all-cause mortality. HIV+ persons are at excess of contracting these outcomes. This scenario is is not unique to HIV; similar elevated biomarker levels associated with clinical events are also seen in the general population and in particular in subgroups suffering from diseases where underlying pathology is linked with activation of inflammation (e.g. rheumatoid arthrithis, overt cardiovascular disease). However, although attractive to think, the fact that these associations exist, they do not establish that a causal link exist between inflammatory/coagulation markers and the clinical outcomes. More importantly, based on the existing literature, it can't be assumed that medications able to reduce inflammation and/or coagulation, and with no other modes of actions, reduces the risk of organ diseases, cancers and/or all-cause mortality. Therefore, a unmet clinical need in HIV, is to form novel research efforts aimed at establishing whether such a causal relationship do exist or not. Such efforts requires firstly to be able to identify the best candidate intervention that is able to reduce inflammation and coagulation. This drug should then be tested in a sufficiently powered randomised controlled trial with relevant clinical endpoints, to demonstrate whether it has a suitable benefit/risk ratio. Until a causal link has been etsbalished, medications known to have anti-inflammatory and/or anticoagulation properties should not be used outside their established clinical indication.

CD4+ T cells lacking SAMHD1 expression are highly proliferative in vivo and decrease during HIV-1 infection

N. Ruffin[2-3], V. Brezar[2-3], D. Ayinde[4], O. Schwartz[4], J.D. Lelievre[1-2], Y. Levy[1-2-3], N. Seddiki[2-3]
[1]Clinical immunopathology, Hôpital Henri Mondor, Faculté de Médecine [2]U955 Equipe 16, INSERM [3]Vaccine Reaserch Institute, Créteil [4]Unité Virus et Immunité, Département de Virologie, Institut Pasteur, Paris, France

Although it is well established that SAMHD1 is a restriction factor for HIV-1 in macrophages and resting $CD4^+$ T-cells, it needs to be determined whether SAMHD1 levels are modulated during HIV-1 infection.

Here we assessed SAMHD1 expression in $CD4^+$ T-cell subsets from antiretroviral therapy treated and non-treated HIV-1 infected individuals as well as in healthy controls, and addressed whether SAMHD1 expression is modified during T cell activation and proliferation.

Our results revealed that in healthy individuals (n=12) while the vast majority of T-cells express SAMHD1, a small subset of $CD4^+$ cells (11.025.36%) displays lower levels. Phenotypic characterization showed that memory $CD4^+$ T-cells, notably effector/memory $CD45RA^-/RO^+CCR7^-CD27^+CD28^+$ and terminally differentiated $CD45RA^+/RO^-CCR7^-CD27^-CD28^+$ subsets contain higher proportions of $SAMHD1^{low}$ cells (15.227.31% and 23.3610.37% respectively) as compared to naïve cells (7.084.27, $p<0.05$ and $p<0.001$ respectively). Importantly, both ART-treated (n=10) and non-treated (n=8) HIV-1 infected individuals displayed lower frequencies of $SAMHD1^{low}$ cells (5.423.90% and 5.473.09% respectively, $p<0.05$ as compared to controls). Moreover, $SAMHD1^{low}$ $CD4^+$ T-cells exhibit higher proportions of cycling $KI67^+$ cells as compared to $SAMHD1^+$ cells (6.155.24% vs 3.901.36% for treated and 27.7424.18% vs 5.484.46% for non-treated HIV-1 infected individuals; 2.202.24% vs 1.980.76% for controls). We confirmed these results by in vitro stimulation (anti-CD3/CD28) where dividing $CD4^+$ T-cells exhibited lower SAMHD1 expression as assessed by both RT-PCR and flow-cytometry.

Our results show that memory/cycling $CD4^+$ T-cells exhibit low levels of SAMHD1 which might explain their preferential productive infection and thus provide new insight in the mechanism of $CD4^+$ T-cell depletion, hallmark of HIV-1 infection.

Experimental CD4 depletion prior to SIV infection in macaques results in encephalitis, massive macrophages and microglia infection, and rapid turnover of infected cells

L. Micci[2], R. Iriele[2], X. Alvarez[3], R. Geleziunas[4], D. Hazuda[7], A. Ortiz[2], S. Pahwa[6], M. Davenport[1], J. Estes[5], A. Lackner[3], G. Silvestri[2], M. Paiardini[2]
[1]The University of New South Wales, Sydney, Australia [2]Emory University, Atlanta [3]Tulane National Primate Research Center, Covington [4]Gilead Sci, Inc., Foster City [5]AIDS Cancer Virus Program, NCI-Frederick, Frederick [6]University of Miami, Miami [7]Merck Res Labs, West Point, United States

Objective: The relative role of CD4 T cells as mediators of antiviral immune response and targets of viral replication in HIV/SIV infection is still poorly understood. We recently showedthat, in rhesus macaques (RM), experimental depletion of CD4 T cells prior to SIV infection results in higher viremia, rapid disease progression, and emergence of CD4-independent SIV-envelopes. In this new study of CD4 T cell depletion followed by SIV infection we investigated (i) the sources of the viral burden; (ii) the lifespan of productively infected cells; and (iii) the presence of SIV encephalitis (SIVE).

Methods: Twelve RM were included in this study. Eight were treated with a single administration of rhesus recombinant anti-CD4 depleting antibody (CD4R1) while four served as controls. All twelve RM were infected with SIV_{mac251} (i.v.) six weeks post-depletion, and treated with ART at day 52 post infection. We longitudinally collected blood, lymph node (LN) and rectal biopsies (RB), as well as additional tissues, including CSF and brain, at necropsy. Immunophenotype was assessed by flow cytometry; life span of infected cells was calculated by modeling viral decay during ART; productively infected cells were measured by ISH/IHC.

Results: Consistent with our previous study, CD4 T cell depleted RM showed lack of post-peak viral load decline and rapid disease progression. Remarkably, CD4-depleted RM exhibited (i) massive SIV infection in LN and mucosal macrophages, which constitute 80% of all SIVRNA+ cells; (ii) aberrant activation of microglia, which express high levels of HLA-DR and CD163; (iii) high frequency of microglial infection with early and severe SIVE; (iv) short life span (1.3 days) of productively infected cells, remarkably lower compared to that estimated for macrophages.

Conclusions: Faster disease progression induced in RM by depletion of CD4 T cells prior to SIV infection associates with massive infection of macrophages and microglia, favored by increased activation of these targets and the emergence of a CD4-independent virus. The net effect of CD4+ T cell depletion is inability to control SIV replication and shift of the pattern of infected cells to macrophages, microglia, and other CD4-low cells.

Activation and gut-homing of plasmacytoid dendritic cells persists in the absence of HIV/SIV replication and contributes to residual chronic immune activation

H. Li[2], T. Evans[2], J. Gillis[2], P. Goepfert[1], R K. Reeves[2]
[1]University of Alabama at Birmingham, Birmingham [2]Harvard Medical School, Southborough, United States

Objectives: Lentivirus infections are characterized by a dramatic loss of mucosal CD4+ T cells, breakdown of the gut mucosa, and subsequent chronic immune activation. Residual immune activation persists even during ART therapy and is the single greatest cause of ongoing disease morbidities, but the causes are unclear.

Methods: Plasmacytoid dendritic cells, primary producers of IFN-a, from naive patients and rhesus macaques, chronically HIV-infected persons and SIV-infected macaques, ART-treated humans and macaques, and Elite Controllers of HIV were enumerated and analyzed phenotypically in blood and tissues using polychromatic flow cytometry. Cells were evaluated functionally by intracellular cytokine staining and spatially by immunohistochemistry and confocal microscopy.

Results: During both HIV and SIV infections pDCs were depleted in peripheral blood compared to naïve subjects. However, correlating with plasma viremia, the remaining pDCs upregulated the gut-homing marker, a4b7. Even during ART and Elite Controllers, pDCs remained reduced in blood and a4b7 expression was still significantly upregulated compared to naïve subjects. Absolute numbers and frequencies of pDCs in jejunum and colon were 3-fold greater in infected compared to naïve macaques and were found almost exclusively in the lamina propria. Interestingly, pDCs accumulating in the mucosae during infection secreted high levels of IFN-a, MIP1-b, and TNF-a, resulting in net increases in cytokines in mucosal tissues. In both humans and macaques pDC trafficking to the gut mucosa was associated with an increase in PAMPS — microbial translocated LPS and CpG-DNA — in plasma, regardless of ART treatment or elite control status. Furthermore, pDC trafficking to the gut was associated with increased markers of activation (Ki67, HLA-DR) on circulating T cells.

Conclusions: Here we show a novel mechanism by which pDCs are not depleted during lentivirus infections, but rather traffic to and accumulate in the gastrointestinal mucosa. This accumulation occurred even in the absence of HIV and SIV replication and was associated with chronic immune activation. These data suggest that alternative stimuli could contribute to pDC activation and gut-trafficking as a prime source of residual chronic immune activation during ART.

Clinical & translational Research

Oral presentations

Gene Therapy approach for HIV-1 infection

M. Cavazzana-Calvo[2-7-5], P. Frange[3], C. Parolin[1], G. Pancino[6], F. Spavanello[1], S. Blanche[3], E. Oksenhendler[4], for the ANRS HIV Gene Therapy Consortium

[1]Dept of Histology, Microbiology & Biotechnology, University of Padua, Padua [2]Biotherapy Dept [3]Pediatric Immuno-Haematology Unit, APHP Hôpital Necker-Enfants Malades [4]Clinical Immunology Unit, APHP, Hôpital Saint-Louis [5]GHU Ouest Biotherapy Clinical Investigation Center, INSERM, APHP [6]Regulation Unit for Retroviral Infections, Institut Pasteur [7]IMAGINE Institute, Université Paris Descartes, Paris, France

Infection by HIV kills $CD4^+$ T-cells. After years of heightened T-cell turnover, the immune system's ability to maintain homeostasis is severely affected. As a consequence, patients progress towards profound immunodeficiency and the associated morbidity and mortality. Although antiretroviral therapy for HIV-induced disease is an undisputed success, the complex physiopathology of this infection does result in certain limitations due to the chronic inflammation, permanent immune dysfunction and cryptic virus replication.

The combination of a full myeloablative conditioning regimen and haematopoietic stem cell transplantation from an allogeneic donor with an HIV-resistant genotype has led to the apparent elimination of HIV in a leukaemia-affected recipient. This result constitutes proof of principle, whereby replacing an HIV-susceptible immune system by a genetically modified, HIV-resistant immune system may lower viral loads and (perhaps) prevent progression to AIDS in infected individuals. Indeed, haematopoietic stem cells (HSCs) constitute an attractive cell target for HIV-1 gene therapy, since they give rise to all the cells that can be infected by the virus *in vivo*. Hence, genetic modification may well protect the whole spectrum of susceptible cells.

Recently, the results of several clinical trials of gene therapy for inherited severe immunodeficiencies have proved that human hematopoietic stem cells can be stably gene-corrected and that the new immunological system can replenish central and peripheral lymphoid organs for at least 13 years. Successful translation of "genetic" inhibitors of HIV-replication into the clinic has been disappointing due to : 1) the low rate of gene transfer to human haematopoietic cells, 2) the cell toxicity of a multiple shRNA strategy, 3) the use of genetically modified T cells with a poor in vivo survival, instead of haematopoietic stem cells, and 4) the need to confer a selective advantage to the transduced cells. In order to provide the patient with an artificial selective advantage, a full conditioning regimen should be performed. HIV-infected patients with high-risk lymphoma benefit from autologous haematopoietic stem cell transplantation thanks to treatment regimens that become closer to those applied in the non-HIV patients. The use of myeloablative therapy with autologous stem cell transplantation is the basis for gene transfer into stem cells as treatment for both HIV infection and high-risk lymphoma. This forthcoming trial could provide the field with important clinical and biological information to improve the care of the HIV-1 infected patients.

HIV-1 Neutralizing Antibodies: Understanding Nature's Pathways

J. Mascola
NIH/NIAID/VRC, Bethesda, United States

Broadly neutralizing antibodies (bNAbs) to HIV-1 arise in some HIV-1 subjects, generally after two or more years of infection. Recent advances in antibody isolation and structural characterization have provided insights into the conserved neutralization epitopes on the viral spike and the mode of antibody recognition. Most of the well-characterized bNAbs have a high number of somatic mutations and some have unusually long CDRH3 loops, however relatively little is known about the immunological pathways that produce these antibodies. To better understand the key genetic characteristics and maturation pathway of bNAbs, we are using next generation sequencing to study antibody heavy and light chain gene transcripts in donors from whom neutralizing mAbs have been isolated. Bioinformatics analysis allows the identification of thousands of clonal family sequences, thus providing an expanded view of specific neutralizing antibody lineages. Among donors with for whom early time point and longitudinal samples are available, it is possible to trace the origin of a specific antibody lineage and infer the unmutated ancestor (naïve BCR) of the mature bNAbs. This analysis has been done for donors making bNAbs to the CD4 binding site and to the V1V2-glycan epitope. These analyses provide insights into the naïve BCR the produce broadly neutralizing antibodies, and the maturation pathway required for the antibody to acquire high affinity neutralization.

Antiretroviral agents prevent HIV transmission: Where do we go from here?

M. Cohen
University of North Carolina, Chapel Hill, United States

The transmission of HIV is dictated by the concentration of HIV-1 in relevant fluids (regardless of route of transmission) and the viral genotype and phenotype. People newly infected with HIV-1 (i.e. acute infection) and those with STD co-infections excrete such a large concentration of virus as to be “hyperinfectious”. The transmission of HIV likely occurs in the first few hours after exposure. The transmission of HIV after heterosexual intercourse is generally limited to one or a small number of founder variants which themselves may be “hyperinfectious”, and a slightly larger median number of variants with unprotected anal intercourse or parenteral HIV exposure. Synergistic behavioral and biologic HIV prevention strategies have been developed, and biological interventions have galvanized the prevention field: such interventions include treatment of inflammatory cofactors, voluntary male circumcision, and most recently use of antiretroviral agents for infected people (who can be rendered remarkably less contagious) or as pre- and post-exposure prophylaxis (PrEP and PEP). In a study conducted by the HIV prevention Trials Network (HPTN 052) HIV transmission was reduced by 96% above standard counseling and condoms by antiretroviral treatment demonstrated to suppress HIV replication. More recently ecologic and observational studies suggest that broader, earlier antiviral treatment of HIV may already be reducing incidence in some (but not all) populations. However, maximal benefit of HIV “treatment for prevention” will likely require a program of universal “test and treat”, where many more infected patients are identified, linked to care, and treated very early in disease and for life. Community randomized trials designed to support this approach are underway in Africa. The combination of tenofovir disoproxil fumarate and emtricitabine has been approved in the US for pre-exposure prophylaxis for high risk, HIV negative subjects, but maximal use of this strategy remains in development. At the same time, other oral and injectable agents are being developed for PrEP. In conclusion, use of antiretroviral agents for prevention of HIV infection has rapidly become a cornerstone of combination HIV prevention strategies, the costs of the agents and infrastructure for delivery notwithstanding. While many (but not all) mathematical modeling studies suggest that such use of antiretroviral agents will reduce incidence of HIV, the empirical results from prevention trials already launched are anxiously awaited.

Impact of an enhanced prenatal HIV counselling on male partner HIV testing, couple counselling and HIV-free survival of infants in Yaoundé, Cameroon. ANRS 12127-Prenahtest trial

P. Tchendjou Tankam[1-6], J. Orne-Gliemann[4], P. Koki Ndombo[2], T. Mossus[1], F. Eboko[5], E. Balestre[4], A. Ngo Essounga[1], D. Amassana[1], A.C. Bissek[3], M. Tejiokem[1-6], F. Dabis[4] &. For The Prenahtest Anrs 12127 Study Group.

[1]Epidemiologie et Sante Publique, Centre Pasteur Cameroun [2]Centre Mère Enfant, Fondation Chantal Biya [3]Division de la Recherche opérationnelle en santé, Ministère de la Santé Publique, Yaoundé, Cameroon [4]INSERM U897, Institut de Santé Publique, Epidémiologie et Développement. Université Bordeaux Segalen, Bordeaux [5]UMR 912 IRD-INSERM-U2, Institut de Recherche pour le développement, Marseille [6]Réseau International des Instituts Pasteur, Institut Pasteur Paris, Paris, France

Background: Improving HIV testing and HIV prevention within families remains a challenge. Within a multi-country randomized trial (Orne-Glemann, 2013) evaluating the impact of an enhanced prenatal HIV counselling intervention provided to pregnant women, couple-oriented post-test HIV counselling (COC), we investigated the consequences of COC on male partner HIV testing, couple HIV counselling and HIV-free survival among infants.

Methods: In Yaounde, 484 pregnant women with a stable partner were recruited on their first antenatal care visit and randomised to receive COC or SC (standard post-test HIV counselling). We collected data before prenatal HIV testing, eight weeks after HIV testing and six months post-partum using structured questionnaires. Impact of COC on male partner HIV testing, couple HIV counselling and HIV free survival rates were measured in intention-to-treat analysis. Factors associated with partner HIV testing and couple HIV counselling were assessed using multivariable logistic regression.

Results: Among pregnant women enrolled in the Cameroon site, 98% (476/484) completed the baseline questionnaire. Their median age was 27 years (IQR: 23-31 years). 28% (133/476) were married, the median duration of their couple relationship was 48 months (IQR 24-96) and 88% of them had at least secondary educational level. HIV prevalence was 12.0% [CI 95%: 9.15-15.4]. Couple HIV counselling was significantly more frequent among women from the COC group vs SC group (10.5% vs 2.1%; p=0.002) as well as male partner HIV testing (24.3% vs 14.4%; p=0.006). The proportion children free of HIV or alive born from HIV-infected mothers was significantly higher in the COC group (100% vs 82.6%; p=0.03). In multivariate analysis, factors associated to partner HIV testing were: COC group (OR: 1.82; 95% CI [1.09-3.05]), to be HIV-infected (OR: 2.21; CI95% [1.09-3.05]) and men's presence during delivery (OR: 2.13; CI 95% [1.20-1.70]. Factors associated with couple HIV counselling were: COC group (OR: 5.73; CI95% [2.07-15.8]), men's presence during delivery (OR: 3.77; CI95% [1.32-10.76]), and gestational age less than 16 weeks of amenorrhea (OR: 2.85; CI95% [1.17-6.92]).

Conclusion: Couple-oriented post-test counselling appears as an innovative and efficient strategy improving family HIV counselling and prevention, as well as men's involvement within reproductive health services.

Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production

M. Perreau[1], A.L. Savoye[1], E. De Grignis[1], J.M. Corpataux[2], R. Cubas[5], E.K. Haddad[5], L. De Leval[3], C. Graziosi[1], G. Pantaleo[1-4]

[1]Division of Immunology and Allergy [2]Division of Thoracic Surgery [3]Institute of Pathology, Lausanne University Hospital [4]Swiss Vaccine Research Institute, Lausanne, Switzerland [5]Vaccine and Gene Therapy Institute, Port St Lucie, United States

Background: Lymphoid organs are the primary anatomic compartments for both the generation of the immune response and for HIV replication and spreading. The recently identified T follicular helper cells (Tfh) reside within the germinal centers (GCs) and are specialized in providing B-cell help; however, limited data are available on the HIV infection of Tfh cells and their role as potential reservoir for HIV.

Methods: We have investigated the distribution of HIV-specific and HIV infected CD4 T-cells within different populations of memory CD4 T cells isolated from lymph nodes of 23 subjects with chronic HIV infection with CD4 T-cell count >400 per $mm^3$and plasma HIV RNA levels >5000 copies per mL, from 14 subjects with undetectable plasma viremia.

Results: Four memory CD4 T-cells populations were identified on the basis of the expression of CXCR5, PD-1 and Bcl-6: $CXCR5^-PD\text{-}1^-Bcl\text{-}6^-$, $CXCR5^+PD\text{-}1^-Bcl\text{-}6^-$, $CXCR5^-PD\text{-}1^+Bcl\text{-}6^-$ and $CXCR5^+PD\text{-}1^+Bcl\text{-}6^+$. On the basis of Bcl-6 expression and functional properties (IL-21 production and B-cell help), the $CXCR5^+PD\text{-}1^+Bcl\text{-}6^+$ cell population was considered to correspond to the Tfh cell population. We show that Tfh and $CXCR5^-PD\text{-}1^+$ cell populations are enriched in HIV-specific CD4 T-cells ($P<0.05$) and significantly increased (4 fold; $P<0.005$) in viremic HIV infected as compared to healthy subjects. However, only the percentage of Tfh cells correlated with the levels of plasma viremia ($R=0.6035$; $P=0.0023$) and the Tfh cell population contained the highest percentage (5%; $P<0.05$) of CD4 T-cells harboring HIV DNA, it was the most efficient in supporting productive infection *in vitro*and replication competent HIV was readily isolated from Tfh cells also in subjects with nonprogressive infection and low viremia (

Conclusions: These results demonstrate that the Tfh cell population contained the highest percentage of HIV infected cells and was the most efficient in supporting virus replication and production.

Four Days a Week and Less on Proper Antiviral Combinations Provided Long-Term Maintenance on 84 Patients' HIV / The ICCARRE PROJECT (1)

J. Leibowitch[1], D. Mathez[1], P. De Truchis[1], D. Le Dû[1], J.C. Melchior[1], B. Autran[2], C. Perrronne[1], J. Izopet[3], J. David[4]
[1]Infectious Disease Dept, Raymond Poincaré Hospital, Garches [2]Immunity and Infection Laboratory, Pitié-Salpétrière Hospital, Paris [3]Microbiology, Purpan Hospital, Toulouse, France [4]Harvard School of Public Health, Boston, United States

Background: Long term anti HIV combination therapies, marred by constraints, costs and toxicities, call for readjustment. Short cycles of antiretrovirals 4 days and less per week (d/wk) have provided effective therapy (Faseb J, 2010), confirmed now in 84 patients under discontinuous maintenance therapy over an average 3.8 treatment-years.

Patients and Treatments: Under the supervision of our Ethics Committee, volunteer patients - on established optimally suppressive treatment for 5 months or more- received written information explicit of the off-label nature of the proposal, to which they consented in writing. Treatment was reduced stepwise, after 2 bi-monthly plasma HIV < 50 copies/mL, from 7 to 5 (49 pts) or directly from 7 to 4 days per week (d/wk) in 39, then to 3, 2, and 1 d/wk in 72, 59, 12 pts. Antiviral compositions: integrase inhibitor-base 4+3 d/wk (+1 NNRTI + 1PI or + 2 or +3 NRTIs); standard triple combinations 4+3+2 d/wk (2 NRTIs +1 PI or +1 NNRTI); non-registered quadruple antiviral combinations 4+3+2+1 d/wk (1 NNRTI-base +3 NRTIs).

Results: Intermittent therapy controlled patients' HIV over 376 treatment-years, 61% crossing the 2.5 year, 37 % the 4 year line. Both sequential lymphocyte activation markers and cell-linked HIV DNA remained stable, while CD4/CD8 ratios rose >1, from 7 % of patients before, to 36 % on ICCARRE.

Viral failures: 14 viral escapes on 3, 2, 1 d/wk regimens (plasma HIV RNA >50 copies 4 weeks apart) were promptly countered by re-adjusted continuous treatment. Twelve failures were ascribable to prescriptor's or patients' humane blunders and errs: ½ daily recommended dosages of a base drug (7pts); overlooked resistant HIVs archived from antecedent viral failures (3 pts); acute erratic observance (2 pts); HIV inadvertently resurged at 2 d/wk and 1 d/wk on a quadruple combination, marking the lower antiviral boundary of our ultra-short treatment cycle strategy with that combination at 1.18 failures per 100 proper treatment-years.

Conclusion: ICCARRE may safely offer 40 to 85 % medicinal cuts to patients already on suppressive therapy, providing years of drug-free/ virus-free functional remission. In 5 sero-different couples, 7 babies were conceived (born HIV-free), leaving the un-protected parent uncontaminated.

(1) Intermittent, in Canny shortCycles, Anti Retrovirals may Retain Efficacy

Strategies for HIV control & cure

Oral presentations

Why curing HIV might be easier than assumed

S. Deeks
UCSF, San Francisco, United States

Given the challenge of delivering complex, expensive and potentially harmful antiretroviral therapy on a global level, there is intense interest in the development of short-term, well-tolerated regimens aimed at curing HIV infection. That a cure might be possible is supported by the well-known phenomenon of “elite” control, in which some infected individuals effectively control replication-competent virus for up to three decades. Support for a potential cure is also provided by four unique studies over the past four years. These studies reported (1) a potentially sterilizing cure in a subject who received an allogeneic stem cell transplant from a donor who lacked CCR5 (the “Berlin Patient”), (2) dramatic reductions in reservoir size in two subjects who remained on therapy, received ablative conditioning, and then received a allogeneic stem cell transplant from donors who had HIV-susceptible cells, (3) apparent durable control of HIV in a group of individuals who received very early antiretroviral therapy and remained on therapy for years (the ARNS “VISCONTI cohort”) and (4) an apparent cure of an infant who had high-level viremia at birth and received early and aggressive antiretroviral therapy. Common themes among these cases will be discussed. The implications of these reports for future cure research will also be discussed.

Natural and treatment induced control of HIV/SIV infections

A. Saez-Cirion
Unité des Régulations des Infections rétrovirales, Institut Pasteur, Paris, France

In the search for an HIV cure, achieving HIV remission or functional cure appears as a more reachable goal in the medium term that HIV eradication. The existence of a group of patients, HIV controllers (HIC), able to control infection for long periods of time without treatment holds promise that such a functional cure may be possible. Studies on different cohorts of these individuals have pinpointed host mechanisms that may contribute at different levels to control HIV infection. These mechanism include enhanced ADCC potential, reduced cell susceptibility to HIV infection and, specially, an effective T cell response against HIV. Control of infection is often associated with a favorable MHC background, which may be a confounding factor when evaluating the relative role of the adaptive T-cell response and other mechanisms of control. Different animal models of SIV control have been developed and they should help to evaluate not only the in vivo relevance of the mechanisms of control but also the initial steps leading to the establishment of such mechanisms. Finally, we have recently shown that long-term control of infection may be induced by early treatment initiation in some patients. Post-treatment controllers started antiretroviral treatment close to acute infection and they kept it for several years before discontinuation. These patients achieved durable HIV remission despite not being naturally predisposed to do so, and the viral reservoir shrunk without treatment in some of them.

Association between negative regulators and HIV persistence during ART: Identifying potential therapeutic targets for eradication strategy

R. Fromentin, S. Dafonseca, M.B. Lawani, W. Bakeman, C. Vandergeeten, R.P. Sekaly, N. Chomont
Vaccine and Gene Therapy Institute-Florida, Port St Lucie, United States

Objective: Antiretroviral therapy reduces HIV replication but does not cure HIV. The persistence of HIV in a small pool of long-lived latently infected resting CD4 T cells is a major barrier to viral eradication. Interfering with the immunological mechanisms that maintain latency in these cells may lead to novel therapeutic strategies to cure HIV infection. We hypothesized that negative regulators of T-cell activation may contribute to HIV persistence by actively promoting viral latency. We evaluated the potential role of 8 negative regulators in the establishment and maintenance of the HIV reservoir.

Methods: The levels of expression of PD-1, CTLA-4, LAG-3, TIGIT, TIM-3, BTLA, 2B4 and CD160 were measured by flow cytometry on PBMCs from 48 virally suppressed subjects. The frequency of CD4 T-cells harboring integrated and total HIV DNA as well as 2-LTR circles was determined by qPCRs. More specifically, the impact of PD-1 engagement and PD-1 blockade on HIV latency were evaluated in CD4 T-cells isolated from virally suppressed subjects.

Results: Absolute CD4 T-cell counts were negatively correlated with the expression of PD-1, LAG-3 and TIGIT on CD4 T-cells. More importantly, the frequency of CD4 T-cells harboring integrated HIV DNA was strongly correlated with the expression of PD-1, whether or not PD-1^+ cells expressed LAG-3 and TIGIT. TCM and TTM cells expressing high levels of PD-1 were enriched for HIV integrated DNA when compared to their negative counterparts, confirming the association between PD-1 expression and HIV persistence. Engagement of PD-1 with it ligand PD-L1 in CD4 T-cells isolated from virally suppressed subjects reduced viral transcription and viral production induced by TCR stimulation. Conversely, blocking PD-1 interaction with its ligands induced viral transcription and viral production in 50% of the virally suppressed subjects tested.

Conclusion: We identified PD-1 as a marker of HIV infected cells in virally suppressed subjects. Interestingly, our results suggest that other negative regulators (LAG-3 and TIGIT) may also be associated with HIV persistence. Our results demonstrate an active role for PD-1 in the establishment and maintenance of HIV latency and suggest that blocking the PD-1/PDL-1/2 pathway may be used to perturb HIV persistence.

Future vaccine strategies

Oral presentations

Taming cancer and HIV with dendritic cells

A.K. Palucka, J. Banchereau
Baylor Institute for Immunology Research, Baylor University Medical Center, Dallas, TX, United States

T cells can reject established tumors when adoptively transferred into patients, thereby demonstrating that the immune system can be harnessed for cancer therapy. Active immunotherapy with vaccines has the potential to induce tumor-specific effector and memory T cells that might control tumor outgrowth on the long term. Cancer vaccines are in a renaissance era due to recent phase III clinical trials showing some benefit to the patients. Vaccines act through dendritic cells (DCs) which induce, regulate and maintain T cell immunity. Critical to the design of improved vaccines is the concept of distinct DC subsets and distinct DC activation pathways, all contributing to the generation of unique adaptive immune responses. Rather than the quantity of IFN-g secreting $CD8^+$ T cells, we should aim at generating high quality high sensitivity poly-functional effector $CD8^+$ T cells able to reject tumors and long-lived memory $CD8^+$ T cells able to prevent relapse. Our pre-clinical studies actually demonstrate that Langerhans cells are superior, as compared to other DC subsets, in their capacity to prime high affinity melanoma-specific CD8+T cells able to kill authentic tumor targets. We also found that lung-tissue-resident DC subsets acquire influenza antigens in vivo and expand specific cytotoxic $CD8^+$ T cells in vitro. However, $CD1c^+$ DCs specialize in the regulation of mucosal $CD8^+$ T cells.

Several phase I/II clinical trials testing ex vivo generated DC vaccines charged with melanoma antigens were carried out at BIIR treating over 140 patients with stage IV melanoma. Vaccinations with DCs have resulted in immune and clinical responses in patients. Two immune parameters appear linked to clinical outcome of the patients: 1) objective clinical response is associated with induction of melanoma-specific effector cells; and 2) all patients display melanoma-specific IL-10 secreting CD4+T cells regulatory/suppressor function that may counteract effector cells. Thus, we need to identify the next generation DC vaccines able to generate large numbers of high avidity effector $CD8^+$ T cells and to overcome regulatory T cells and suppressive environment established by tumors, a major obstacle in metastatic disease.

A series of clinical trials conducted by ANRS testing lipidated HIV peptide-based vaccines showed the ability to significantly impact viral load and viral rebound upon HAART interruption. Together, these findings provided basis for the design of novel DC-based human vaccines as therapeutic agents in the treatment of HIV. To this end, we designed a clinical trial to test the safety and immune efficacy of a therapeutic HIV vaccine consisting of autologous DCs generated ex vivo from monocytes cultured with GM-CSF/IFN-alpha and loaded with five lipidated HIV antigens (LIPO5). This phase I clinical trial in 19 asymptomatic HIV-infected patients with undetectable viral load while treated with HAART showed the safety of DC vaccination and of analytical treatment interruption (ATI). Correlative studies revealed the induction of broad and strong T cell response to HIV antigens, in particular in CD4+ T cell compartment.

Thus, DC based vaccines have a central role in the modern HIV and cancer therapy.

Beyond RV144 efficacy results: update and future plans

P. Pitisuttithum
Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Thailand has been involved and fully committed to HIV vaccine R&D for more than 16 years. In 2009, it was shown that for the first time, an HIV vaccine provided a modest protection against HIV acquisition (31.2% at 42 months follow up) using ALVAC priming and AIDSVAX boosting regimen (RV144). However, the vaccine efficacy appeared to be higher up to 60% at 12 months, 6 months after the last boosting. This suggested early and nondurable immune response stimulated by this prime-boost regimen. Subsequent investigations demonstrated the potential immune correlates of risk antibodies directed against the V2 loop. The recent sieve study also uncovered genetic footprints in the V2 region associated with reduced risk of HIV. All these paved the way to new vaccine designs. Extension of the RV144 study-RV305 started in 2012 and vaccination phase is almost complete. Three more studies are underway. Future efficacy trials using a similar vaccine concept tested in high-risk groups are being planned.

POSTER COMMUNICATIONS

Session I

Stabilization of the integrase-DNA complex by Mg^{2+} ions and binding of HIV-1 integrase inhibitors

L. Miri[3-4], G. Bouvier[1], A. Kettani[3], A. Mikou[2], L. Wakrim[4], M. Nilges[1], T. Malliavin[1]

[1]Unité de Bioinformatique structurale, UMR 3528 CNRS, Institut Pasteur de Paris, Paris, France [2]Laboratoire de Catalyse et environnement, Faculté des Sciences Ain Chock [3]Unité de modélisation moléculaire et d'ingénierie des biomolécules, Laboratoire de recherche sur les lipoprotéines et l'athérosclérose -URAC 34-, Faculté des Sciences Ben M'Sik [4]Laboratoire de Virologie, Institut Pasteur du Maroc, Casablanca, Morocco

Objective: Recent X-ray crystallographic structures of the integrase from prototype foamy virus (PFV), in complex with DNA and inhibitor (raltegravir), allowed to investigate the role of the different partners (integrase, Mg^{2+}, raltegravir) in the complex stability and identify the protein residues essential for the ligand binding.

Methods: Molecular dynamics (MD) simulations were performed to study the complex stability in the presence of Mg^{2+} ions and/or raltegravir. Root mean square deviations, internal fluctuations, H-bond analysis and essential dynamics were analyzed.

To detect protein residues essential for ligand binding, a set of known HIV-1 integrase inhibitors were docked to the homology model of HIV-1 integrase, built from the X-ray PFV integrase structure.

The MD conformations and ligand binding poses were classified using a learning method based on self organizing maps.

Results: The analysis of MD simulations revealed that Mg^{2+} ions display a fundamental role in integrase/DNA complex stability. In the presence of Mg^{2+} ions, the raltegravir plays an additional role in the inhibition of the integrase catalytic activity by destabilizing the formation of intra-protein and DNA-protein hydrogen bonds.

The clusters of docking poses revealed the protein residues essential for inhibitor binding. Some of those residues had already been identified to be involved in the stabilization of the catalytic site during the MD simulations and could be used for virtual screening during the search of new inhibitors.

Mg^{2+} ions played an important role in binding ligands as they have been detected in all selected clusters. The best docked compounds provide the list of most interesting chemical functions for the integrase inhibition.

Conclusion: The role of Mg2+ ions in the stability of the Integrase/DNA complex, in catalytic activity and in effective binding of integrase inhibitors, have been elucidated.

The study puts in evidence the power of self-organizing maps for clustering ligand docking poses and for detecting free energy basins along MD trajectories.

The $tRNA_3^{Lys}$ packaging complex involves association of human mitochondrial LysRS with the polyprotein GagPol from HIV-1: a new pharmacologic target?

J. Dias, L. Kobbi, A. Nail, A. Szklarz, M. Comisso, M. Mirande

LEBS, CNRS, Gif-sur-yvette, France

Cytosolic and mitochondrial LysRS are encoded by alternative splicing of a single gene and can only be distinguished according to their very N-terminal sequences. Beyond its role in translation, mitochondrial LysRS (mLysRS) is also hijacked from the host cell following HIV-1 infection to carry the primer $tRNA_3^{Lys}$ into the virions [1]. Using monospecific antibodies, we previously showed that only mLysRS is taken up in viral particles along with $tRNA_3^{Lys}$, the primer for reverse transcription of the HIV-1 genome. We screened all the viral proteins to identify the partners of LysRS responsible for the formation of the $tRNA^{Lys}$ packaging complex. We showed that mLysRS associates with the Pol domain of GagPol. This interaction is highly specific, as assessed by the K_D value of about 5-10 nM between mLysRS and Pol. More specifically, the transframe (TF) and integrase (IN) domain proteins of Pol interact with the catalytic domain of LysRS [2]. A model of the assembly of the mLysRS:$tRNA_3^{Lys}$:GagPol packaging complex is proposed, which is also consistent with the release of its different components after maturation of GagPol into the virions. Maturation of the precursor of mLysRS upon its mitochondrial targeting is a prerequisite to form a complex with tRNA [3]. These data open new perspectives for the search of a new class of inhibitors of the HIV-1 development cycle that would block the packaging of $tRNA_3^{Lys}$ into viral particles.

References:

1. Kaminska et al. J. Virol. (2007) 81: 68-73.
2. Kobbi et al. J. Mol. Biol. (2011) 410, 875-886.
3. Dias et al. Biochemistry (2012) 51, 909-916.

DRAM triggers lysosomal membrane permeabilization and cell death in CD4$^+$ T cells infected with HIV

M. Laforge, S. Limou, F. Harper, N. Casartelli, V. Rodrigues, R. Silvestre, H. Haloui, J.F. Zagury, A. Senik, J. Estaquier

Université Paris Descartes, CNRS FRE3235, Paris, France

Productive HIV infection of CD4$^+$ T cells leads to a caspase-independent cell death pathway associated with lysosomal membrane permeabilization (LMP) and cathepsin release, resulting in mitochondrial outer membrane permeabilization (MOMP).

Herein, we demonstrate that HIV infection induces damage-regulated autophagy modulator (DRAM) expression in a p53-dependent manner. Knocking down the expression of DRAM and p53 genes with specific siRNAs inhibited autophagy and LMP. However, inhibition of Atg5 and Beclin genes that prevents autophagy had a minor effect on LMP and cell death. The knock down of DRAM gene inhibited cytochrome C release, MOMP and cell death. However, knocking down DRAM, we increased viral infection and production.

Our study shows for the first time the involvement of DRAM in host-pathogen interactions, which may represent a mechanism of defence via the eliminating infected cells.

Characterization of a novel antiviral effect of the Interferon Induced Transmembrane proteins against HIV-1

K. Tartour[1-2], L. Feneant[1-2], S. Durand [1-2], G. Berger[1-2], E. Beaumont[3], D. Brand[3], A. Cimarelli[1-2]

[1]ENS Lyon, Inserm U1111 [2]Université Lyon 1, Lyon [3]Université Francois Rablais, Inserm U966, Tours, France

The three members of the interferon-induced transmembrane protein family (IFITMs) are small proteins strongly induced upon interferon treatment that possess two transmembrane domains connected by a short intracellular loop. IFITMs have been involved in the regulation of developmental processes, in carcinogenesis and more recently in the protection of target cells from viral aggression. IFITMs appear to act on a number of diverse viruses: Influenza, West Nile virus, Dengue virus, Filoviruses, Coronaviruses and also HIV-1. In all these cases, the pool of IFITM proteins present in target cells appears to block viral entry by inhibiting the fusion between cellular and viral membranes via a mechanism that may affect the rigidity of the latters.

During our studies on the modifications that HIV-1 infection bore on primary macrophages, we have found that IFITMs are significantly upregulated during infection and that, as a result of this upregulation, they become incorporated onto virion particles. This incorporation can be recapitulated upon ectopic expression of IFITM proteins in HEK293T cells. IFITMs incorporation occurs at the exterior of the viral particle, as suggested by subtilisin digestion experiments and CD45 depletion assays and does not occur to the detriment of the incorporation of viral envelope proteins. In this setting, IFITMs decrease viral infectivity and significantly affect entry of the viral particle in target cells. While we have not observed a drastic defect in HIV-1 envelope incorporation upon IFITM expression, we believe that IFITMs may induce subtle differences in envelope incorporation that in turn may affect viral infectivity.

These and other results that will be presented indicate that the pool of IFITM proteins present in virion-producing cells can play a significant antiviral role, by directly inhibiting the infectivity of viral particles, in addition to the already described antiviral role played by the pool of IFITM proteins present in target cells.

Therefore, IFITM proteins provide a class of large spectrum antiviral factors that seemingly use their membrane association to interfere with replication in at least 2 key moments of the viral life cycle.

With the support of ANRS, Sidaction, FRM and the CNRS.

The interaction between $tRNA^{Lys}_3$ and the primer activation signal deciphered by NMR spectroscopy

D. Sleiman[1], P. Barraud[2], F. Brachet[1], C. Tisné[1]

[1]Cristallographie & RMN biologiques, CNRS/Université Paris Descartes, Paris, France [2]ETH, Institute of molecular biology&biophysics, Zurich, Switzerland

HIV-1 reverse transcriptase uses the host $tRNA^{Lys}_3$ as a primer for the synthesis of the minus DNA strand. The first event in viral replication is thus the annealing of the tRNA to the primer binding site (PBS) in the 5' UTR of the viral RNA. This event requires a major RNA rearrangement which is chaperonned by the viral nucleocapsid protein (NC). The binding of NC to nucleic acids is essentially non-specific, however, NC is known to bind selectively to hairpins located in the 5' region of the viral RNA. In a previous study, using an NMR approach in which the reaction is slowed down by controlling temperature, we were able to follow details in this RNA unfolding/refolding process and to uncover an intermediate state. We showed that the annealing can initiated both from the single-stranded CCA-3' end of the acceptor stem and from the bottom of the acceptor/TYC stem. Secondly, the complete annealing is reached only in the presence of NC, at physiological temperature, when the zinc fingers of NC contact the D loop of $tRNA^{Lys}_3$.

Recently, we investigated by NMR the formation of the PAS/antiPAS interaction upon annealing of $tRNA^{Lys}_3$ to the PBS. Indeed, the PAS ('Primer activation signal) motif is not involved in tRNA placement, but stimulates the initiation of HIV reverse transcription. Our NMR study provides molecular evidence of the existence of this interaction and highlights the role of the nucleocapsid protein in promoting this additional RNA-RNA annealing. This study presents the first direct observation at a single base-pair resolution of the PAS/anti-PAS association, which has been proposed to be involved in the chronological regulation of the reverse transcription.

Involvement of the nuclear form of uracil DNA glycosylase in active HIV-1 infection of primary macrophages

C. Hérate[1-2], S. Benichou[1-2], C. Guenzel[1-2]

[1]INSERM U1016 [2]Institute Cochin, Paris, France

Uracil DNA glycosylase (UNG2) is a nuclear protein that is highly expressed in dividing cells and participates in the cellular processes for the specific removal of misincorporated uracil residues from DNA. Previously we reported that UNG2 is incorporated into HIV-1 particles through direct interaction with the Vpr regulatory protein, and participated in the maintenance of the integrity of the viral genome by influencing the accuracy of reverse transcription. Using specific shRNA to target UNG2 expression, we have shown that viruses produced in UNG2-depleted 293T cells were less infectious that viruses produced in control cells, when analyzed in HeLa-CD4 cells or primary monocyte-derived macrophages (MDMs) used as target cells in a single-round infection assay. This decrease in infectivity of virus particles produced in UNG2-depleted cells was related to a defect in the reverse transcription process, as revealed by analysis of the efficiency of the viral DNA synthesis in target cells. In agreement, the kinetics of replication-competent viruses was also altered in UNG2-depleted HeLa-CD4. Interestingly, viruses produced in UNG2-depleted cells also displayed similar replication defect when analyzed in primary MDMs, where we found that UNG2 was expressed at low levels compared to dividing cells such as T cells or HeLa-CD4 cells. These observations suggest that the intracellular level of endogenous UNG2 expression may differentially modulate HIV-1 replication between non-dividing cells, such as terminally-differentiated macrophages, compared to dividing T lymphocytes

Structural and functional studies of the HIV-1 integrase in complex with Transportin-SR2 and VBP1

N. Levy, A. Schaetzel, K. Pradeau-Aubreton, S. Eiler, C. Crucifix, D. Moras, P. Schultz, M. Ruff

ISB, IGBMC, Illkirch, France

Integration of the human immunodeficiency virus type 1 (HIV-1) cDNA into the human genome is catalyzed by the viral integrase protein (IN) which is a key protein for HIV infection. It is the core protein of the preintegration complex (PIC), a large nucleoproteic complex responsible for all the infection steps from the reverse transcription to the integration of the viral genome in the host-cell chromatin. Integrase is thus required for reverse transcription, PIC migration along microtubules, transfer to the nucleus through the nuclear pore, chromatin targeting and integration.

PIC is a very dynamic complex. It varies in composition and size from one step of the infection cycle to another, making its study very challenging. Our main objective is to characterize the temporal and structural organization of this complex. Our strategy is to reconstitute *in vitro* sub-complexes of the PIC starting from individually purified recombinant proteins. A cryo-EM structure at 14 Å resolution of the HIV-1 IN in complex with the Lens Epithelium-Derived Growth Factor (LEDGF) has been solved in presence and absence of DNA (1). Recently we solved the cryo-EM structure of the ternary complex of HIV-1 IN, LEDGF, the integrase binding domain of the INtegrase Interactor 1 protein (INI1-IBD) and viral DNA (18 Å resolution).

We are now focusing on PIC sub-complexes involving Transportin-SR2, a crucial element for the PIC nuclear import. We characterized one stable complex formed by IN, Transportin-SR2 and the Von Hippel-Lindau binding protein 1 (VBP1). The design of the different constructs, the optimization of the production and purification of the individual proteins, in vitro complex reconstitution as well as preliminary data on the cryo-EM structure will be presented.

Reference:

1. Michel F., Crucifix C., Granger F., Eiler S., Mouscadet J.F., Korolev S., Agapkina J., Ziganshin R., Gottikh M., Nazabal A., Emiliani S., Benarous R., Moras D., Schultz P. and Ruff M. (2009). Structural basis for HIV-1 DNA integration in the human genome, role of the LEDGF/P75 cofactor. EMBO J., 28, 980-991.

Integrative modeling of HIV-1 provirus: From knowledge to structure

M. Machado, S. Pantano

Biomolecular Simulation Group, Institut Pasteur de Montevideo, Montevideo, Uruguay

30 years of investigation in HIV have provided a vast amount of information on its genome, proteome and interactome. Yet a cure to this disease has not been achieved. Novel ideas are required for targeting the provirus latency, which is responsible for the infection's persistence. In that sense, many efforts were done to characterize the protein interactions, epigenetic signals and chromatin organization events in the latent state of transcription. Consequently, many pieces of this huge puzzle have been collected and several interaction schemes were drawn regarding the many factors participating in this process. At the same time, advances in experimental and computational techniques have provided complete or partial structural data for many biomolecular complexes. Still, assembling macromolecular systems is a challenging issue in structural biology. Translating a scheme from molecular biology into a structural model is not a new idea, however so far there is no such attempt for HIV-1.

Objective: To furnish new insights on HIV-1 latency by integrating the huge collection of available data into 3D representations of the interactions.

Methods: The model building incorporated information from sequence, structure and interaction databases into a multiscale 3D scheme. Protein-DNA and protein-protein structures came primarily form X-ray or NMR data. Comparative modeling and/or *ab-inito* predictions was used when no direct experimental information existed. The information contained in the interaction schemes was translated into spatial restraints of the components to guide the assembly of the whole system. In that way atomic resolution structures were combined with low resolution data from FRET, ChIP, etc, present in the literature.

Results: We show for the first time a structural model of the latent HIV-1 provirus (Figure 1). The final molecular assembly contained more than 700 proteins and about 5 million atoms. The pseudo-atomic resolution made it feasibly to identify protein-protein surface contacts, exposed DNA sequences or methylable nucleotides and to determine volume excluded regions.

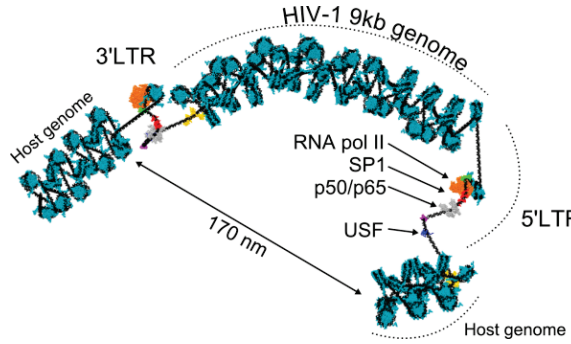

Figure 1. Structural model of latent HIV-1 provirus. The chromatin and some host proteins are highlighted.

Conclusions: We expect this approach will provide a new picture on HIV-1 latency from a perspective never seen before shedding light on its transcription and repression mechanisms.

Integrase and chromatin: Molecular bases of retroviral integration-site selectivity

M. Benleulmi, C. Calmels, M. Lavigne, M.L. Andreola, V. Parissi

UMR5234 MFP Lab, CNRS, Bordeaux, France

Objectives: DNA mobility is a central process in pathogens' replication and genome evolution which has become an essential tool for gene transfer technology. This technology, or gene therapy, aims to correct the expression of a defective gene by replacement or by insertion of an active copy of the gene *in trans*. In the latter case, the "transgene" must be properly expressed at the integration locus and must not induce any deleterious effect. Controlling the integration-site of the transgene is therefore crucial and constitutes a major issue in the field of gene therapy. In recent years, gene therapies using lentiviral vectors have shown much success but also many limitations, especially owing to the lack of data concerning the cellular behaviour of the transferred gene. During the replication of retroviruses, integration-site selectivity is mainly driven by the retroviral integrase protein and its interaction with the host chromatin (structure forming the chromosomes).

Methods: Part of our current studies focus on the understanding of this process based on data we recently obtained thanks to an *in vitro* integration system developed in-house (Lesbats *et al.*, *PLoS Pathog.* 2011), that allows analyzing the integration of model retroviral genomes into reconstituted chromatin.

Results: Based on their ability to interact with the HIV-1 intasome, we identified several cellular factors that afterwards were found to influence integration-site selectivity, presumably by increasing the local DNA accessibility at the integration locus *via* remodelling properties.

Conclusions: The impact of the chromatin structure on the activity of different retroviral integration machineries highlights the molecular bases of integration-site selectivity based on intrinsic structural constraints within the different intasomes. These retrovirus-genus specific properties could define the requirement of additional targeting cellular cofactors that may constitute good candidates for modulating the gene transfer approaches.

Cellular control of the retroviral integration: Search for specific cofactors of the HIV-1 intasome

M. Benleulmi[1], J. Matysiak[1], C. Calmels[1], S. Chaignepain[2], J.W. Dupuy[2], P. Sung[3], M.L. Andreola[1], V. Parissi[1]

[1]UMR5234 MFP Lab, CNRS [2]Plateforme Protéome - Centre Genomique Fonctionnelle, Bordeaux, France [3]Yale University School of Medicine, New Haven, United States

Objectives: The replication of retroviruses requires the stable integration of the viral retrotranscript into the infected cell genome. This integration step is catalyzed by the viral integrase protein (IN) which binds the viral DNA ends to form a nucleoprotein complex called the intasome. Within the preintegration complex, the intasome is associated with viral and cellular factors that can regulate integration at different levels: intasome stabilization, nuclear import, integration-site selectivity, catalysis and post-integration repair (PIR). To date, the known cellular IN interactors were identified by Y2H and/or co-IP independently of the presence of the viral DNA.

Methods: As an alternative to these approaches we developed a strategy to select specific partners of the HIV-1 intasome. Briefly, intasomes formed *in vitro* by incubating a recombinant HIV-1 IN with a 5' biotinylated DNA duplex mimicking the viral DNA ends were on magnetic beads carrying streptavidin, and, after washing, the proteins from 293T cells extracts interacting with the retroviral intasome were eluted and identified by mass spectrometry.

Results: Validating our strategy, virtually all the previously reported IN interactors were also selected using immobilized intasomes. In addition, several new partners were found that belong to cellular pathways expected to have a regulatory function in the retroviral integration process as DNA repair, chromatin interaction and maintenance or nuclear import. Having previously highlighted the role of the hRAD51 mediated homologous DNA repair (HR) and chromatin remodelling by the SWI/SNF on HIV-1 integration (Cosnefroy et al., *J Virol*. 2012, Lesbats et al., *PLoS Pathog*. 2011), we focused our work on these aspects of retroviral integration.

Conclusions: Biochemical, pharmacology and cellular approaches led us to depict several new HIV-1 intasomes functional cofactors that could regulate integration at chromatin interaction and post-integration repair steps. Especially, our data show that HR pathway components, including hRAD51 and the MRN complex, could constitute new HIV-1 restriction factors in addition to potentially participate to the post-integration repair process.

Evidence for a continuous drift of the HIV-1 species towards higher resistance to neutralizing antibodies over the course of the epidemic

M. Bouvin[4], M. Morgand[4], A. Moreau[4], P. Jestin[1-2], C. Simonnet[4], L. Tran[1-2], C. Goujard[1-2], L. Meyer[1-2], F. Barin[3-4], M. Braibant[4]

[1]AP-HP Hôpital de Bicêtre, Le Kremlin-Bicêtre [2]CESP Inserm U1018, Université Paris Sud, Paris [3]Laboratoire de Bactériologie-Virologie, CHU Bretonneau, Centre National de Référence VIH [4]Inserm U966, Université de Tours, Tours, France

Objective: The aim of this study was to revisit and extend the recent data obtained by *Bunnik et al.* which suggested that HIV-1, subsequently to the selective pressure exerted by the individual neutralizing antibodies (NAbs) responses, might be evolving at the population level towards an enhanced resistance to antibody neutralization, (Bunnik *et al.*, Nat. Med. 2010).

Methods: The HIV-1 population that we studied was isolated from 40 patients (all MSM) infected by subtype B viruses enrolled at time of primary infection in the French PRIMO and SEROCO ANRS cohorts at three calendar periods (1988-1991/1997-2000/2007-2010). Infectious pseudotyped viruses expressing envelope glycoproteins representative of the viral quasi-species infected each patient were generated. They were compared for their sensitivity to neutralization (TZM-bl assay) by sera from patients infected early in the epidemic (1988-1991) or more recently (2004-2008), and by a panel of 13 human monoclonal (HuMo) broadly NAbs. In addition, to check whether the evolution of the HIV-1 species towards a higher resistance to neutralization coincided with a poorer capability to induce NAbs, the neutralizing activity of sera from subtype B chronically-infected patients at the two extreme periods of the study, i.e. 1988-1991 (n=30) and 2004-2008 (n=30), was compared towards a panel of six heterologous subtype B isolates (reference “tier 2” viruses).

Results: A progressively significant enhanced resistance to neutralization was observed over time, both by human sera and by most of the HuMoNAbs tested (b12, VRC01, VRC03, NIH45-46^{G54W}, PG9, PG16, PGT121, PGT128, PGT145). However and despite this evolution, we found that one combination of two HuMoNAbs (NIH45-46^{G54W}and PGT128) still should neutralize all the most recently circulating HIV-1 variants, even at a relatively low concentration (≤1 ug/mL). In addition, we observed a significant reduction of the heterologous neutralizing activity of sera from individuals infected later in the epidemic (2004-2008) compared to patients infected earlier (1988-1991), suggesting that the increasing resistance of the HIV species to neutralization coincided with a decreased immunogenicity.

Conclusion: These data provide evidence for an ongoing adaptation of the HIV-1 species to the humoral immunity of the human population over the course of the epidemic.

Endoplasmic reticulum aminopeptidase 2 (ERAP2) haplotypes play a role in antigen presentation and resistance to HIV-1 infection

M. Biasin[5], M. Sironi[1], I. Saulle[5], M. De Luca[5], F. La Rosa[5], R. Cagliani[1], D. Forni[1], S. Lo Caputo[2], F. Mazzotta[2], D. Trabattoni[5], J. Macias[7], J. Pineda[7], A. Caruz[3], M. Clerici[6-4]

[1]Scientific Institute IRCCS E.MEDEA, Italy, Bosisio Parini [2]S. Maria Annunziata Hospital, Florence [3]Immunogenetics Unit, University of Jaen, Jean [4]Don C. Gnocchi Foundation [5]Department of Biochemical and Clinical Sciences [6]Department of Phisiopatology Medical-Surgery and Transplantation, University of Milan, Milan, Italy [7]Valme Hospital, Seville, Spain

Background: Haplotype-specific alternative splicing of the endoplasmic reticulum aminopeptidase type 2 (*ERAP2*) gene results in either full-length (FL, haplotype A) or alternatively spliced (AS, haplotype B) mRNA. As this protein trims peptides loaded on MHC class I and CD8+ T lymphocytes play an important role in protection against viral infections, we analyzed the role of this gene in resistance to HIV-infection.

Methods: *ERAP2* was genotyped in a cohort of 104 Spanish HESN individuals exposed to HIV through injection drug use (IDU-HESN). PBMC isolated from 139 healthy controls (HC) grouped according to their *ERAP2* genotype were infected with HIV-1_{Ba-L} and analyzed for: 1) mRNA specific for *ERAP2* and 96 genes involved in the antigen presentation pathway; 2) MHC class I MFI on CD45+ cells; 3) susceptibility to HIV infection; 4) ERAP2 protein expression. Finally, we investigated the co-segregation of *HLAB*57* with *ERAP2* haplotype in a cohort of Italian HESN.

Results: Genotypeanalysis indicated that *ERAP2* haplotype A is associated with protection from HIV infection (meta-analysis *p* value of 7.6 $x10^{-5}$) and that *HLAB*57* allele is significantly more common among HESN homozygous for haplotype A (homoA). *In vitro* HIV-1 infection mRNA for ERAP2-FL and a number of genes involved in antigen presentation as well as of MHC class I on CD45+ cells were significantly increased in homoA cells from HC; notably, these same cells, but not isolated CD4+ cells, were less susceptible to HIV-1 infection.

Conclusions: *ERAP2* haplotype Aconfers resistance to HIV infection possibly secondarily to its effect on antigen processing and presentation.

Tox4 and Nova-1 proteins are partners of the LEDGF/P75 PWWP domain and affect HIV-1 replication

J. Xavier[1], M. Morchikh[1-4-5], M. Naughtin[1], F. Di Nunzio[3], P. Charneau[3], Y. Jacob[2-6], M. Lavigne[1-4]

[1]Laboratoire Joliot-Curie USR 3010 CNRS, Ecole Normale Supérieure, Lyon [2]Unité de Génétique Moléculaire des Virus à ARN URA 3015 CNRS [3]Unité de Virologie Moléculaire et Vaccinologie URA 3015 CNRS [4]Unité de Virologie Structurale URA 3015 CNRS, Institut Pasteur [5]Université Pierre et Marie Curie, Paris, France [6]Dana-Farber Cancer Institute, Center for Cancer Systems Biology (CCSB) and Department of Cancer Biology, Boston, United States

The Lens Epithelium Derived Growth Factor (LEDGF/p75) contains an N-terminal PWWP domain necessary for its chromatin interaction and a C-terminal domain interacting with several proteins such as lentiviral integrases. These two domains confer a chromatin-tethering function to LEDGF/p75 and participate in the efficiency and site selectivity of lentiviral integration process. Although proteins interacting with LEDGF/p75 C-terminal domain have been extensively studied, no data exist about partners of its PWWP domain that could regulate its interaction with chromatin.

In our study, we report the identification of two new partners of LEDGF/p75 PWWP domain: the transcription activator TOX4 and the splicing cofactor Nova-1. Interactions were identified by yeast two-hybrid and confirmed in mammalian cells by protein complementation assay and co-immunoprecipitation. These interactions are specific for HDGF family PWWP domains and require PWWP amino-acids involved in its chromatin interaction. Cellular fractionation assays and immunofluorescence studies suggest a co-localization of LEDGF/p75 with the PWWP Interacting Region (PIR) of these two proteins. We also observed an inhibition of HIV-1 replication in cells overexpressing these two PIRs and, in the case of Nova-1, this inhibition can be attributed to a defect of integration. We have also identified the HMG box of TOX4 as the minimal domain interacting with LEDGF PWWP. We are now investigating if it is a general property in the HMG box family. In parallel, we are performing *in vitro* studies to confirm the interaction between the LEDGF PWWP domain and the PIRs, using purified proteins and different nucleic acid substrates (DNA, RNA, nucleosomes). Using established *in vitro* assays, we are also studying the effect of these PIRs on LEDGF/p75 interaction to chromatin and on LEDGF/p75-dependent activation of integration into DNA and chromatin templates.

Our data allow to propose a regulation of LEDGF/p75 interaction with chromatin by cellular partners of its PWWP domain that could play a role during the process of lentiviral integration.

Single Cell Imaging of HIV Provirus (SCIP)

C. Di Primio[3], V. Quercioli[3], A. Allouch[2], F. Christ[1], Z. Debyser[1], R. Gijsbers[1], D. Arosio[4], A. Cereseto[5]

[1]Laboratory of Molecular Virology and Gene Therapy, KU Leuven, Leuven, Belgium [2]Unité de Régulation des Infections Retrovirales, Institut Pasteur, Paris, France [3]Neurobiology Laboratory, Scuola Normale Superiore di Pisa, Pisa [4]Istituto di Biofisica, CNR [5]Laboratory of Molecular Virology, University of Trento, Trento, Italy

Objective: Recent advances in fluorescent microscopy provided new tools of investigation for the analysis of viral replication steps in its cellular context. HIV-1 fluorescent systems so far employed are based on labeling of viral proteins. Here we developed a method to visualize the HIV-1 proviral DNA inside a single infected cell: Single Cell Imaging of HIV Provirus (SCIP).

Methods and results: We engineered HIV DNA to contain the yeast rare cutting endonucleases I-SceI cleavage site (HIV-ISceI). In infected cells expressing ISceI enzyme, site cleavage of HIV-ISceI determines formation of double strand break and phosphorylated H2AX (γH2AX) placed on the lesion. γH2AX sub -nuclear structures are detectable by immunofluorescence, thus allowing visualization of proviruses. We showed that individual proviral DNA can be efficiently detected at level of single infected cells. Moreover, we demonstrated that SCIP is an efficient tool for the analysis of HIV replication steps by infecting cells in presence of antiretroviral compounds (AZT, Nevirapine, Raltegravir, LEDGIN), or siRNA against TRN-SR2. SCIP analysis of an infected T cell line allowed us to perform a nuclear topographic analysis of proviral DNA. From this study we obtained evidence that 48 hours post infection proviral DNA is preferentially found in the nuclear periphery while at day 13 the virus is randomly distributed inside the nucleus. Nevertheless, when SCIP was applied to cells where HIV-1 integration is retargeted toward heterochromatin, through fusion of CBX to LEDGF, topological analysis revealed that proviral DNA is randomly distributed in the nuclei. These findings strongly suggest that preferential integration sites of HIV-1, corresponding to highly transcribed genes, are placed in the nuclear periphery.

Conclusion: Here we report SCIP a new imaging technique to efficiently visualize HIV-1 DNA integrated into the host genome of individual cells. Our results shed new light on the mechanism underlying viral integration: proviral DNA localization is dynamic since integration events occur at the nuclear border but repositioning could be observed at later time points from infection. Moreover we demonstrated that SCIP is a clear-cut readout for understanding HIV-replication thus a potential tool for high throughput screening of HIV cellular co-factors and anti-retroviral molecules.

Structural and functional study of the HIV IRES located within the gag coding region

J. Deforges[2], M. Ameur, N. Chamond[1], N. Ulryck[1], B. Sargueil[1]

[1]UMR 8015, CNRS [2]UMR8015, Université Paris Descartes, Paris, France

Primate lentiviruses genomic RNA can serve both as an mRNA that encodes for Gag and Gag-Pol polyproteins and as a propagated genome. We previously reported the presence of an IRES activity embedded within Gag coding region itself that drives the production of several isoforms of the Gag polyprotein and that is conserved in HIV-1, HIV-2 and SIV_{mac}. In addition, *in vitro* reconstitution experiments revealed that the initial step of initiation complex formation is the recruitment of the 40S ribosomal subunit and eIF3. The structural and functional conservation amongst lentiviruses indicates that those properties are important for the virus cycle.

In order to define the RNA structural determinants responsible for the formation of IRES/eIF3/40S ternary complex, we have been following functional and biochemical approaches in parallel. Our results indicate that 2 distinct binding sites for the ribosome are present close to the 2 AUG codons used as initiation site for the translation. Further biochemical analyses have shown that 2 ribosomes can be recruited by the same RNA molecule.

To determine the functional role of the IRES activity on gag translation, we assayed *in vitro* the translation efficiency of mutants unable to recruit the ribosome. In parallel, we have been following a drug screening strategy to identify small molecules that would inhibit the ribosome recruitment. Such molecules would allow us to confirm the role of the IRES on gag translation, and overall to determine the functional importance of this mechanism on the HIV replicative cycle.

Polymorphisms in IL-1 and TNF-α genes: associated with the risk of Perinatal HIV transmission, in an Indian cohort

S. Ahir[2], D. Chaudhari[2], V. Chavan[2], P. Samant-Mavani[1], R. Nanavati[1], P. Mehta[1], J. Mania-Pramanik[2]

[1]Seth G S Medical College & KEM hospital, Mumbai [2]Infectious Diseases Biology, National Institute for Research in Reproductive Health, Mumbai, Maharashtra, India

Objective: Host genetic diversity plays a very important role in protecting infants exposed to HIV-1 through their mothers. Cytokines are the key regulators of immune system. IL-1 family genes are key mediators of inflammation, whereas TNF-α is a potent pro-inflammatory cytokine. Inter-individual differences in these cytokines appear to be due to the allelic polymorphism within regulatory regions of these genes. Present study aimed to evaluate if polymorphisms in IL-1 and TNF-α genes with antagonist functions are associated with perinatal HIV transmission.

Methods: Infants of HIV positive women attending the PPTCT and ICTC of K.E.M. Hospital, Mumbai, India, between January 2010 and December 2012 were enrolled with consent of their mothers for the study. Blood from the infants was collected for HIV screening and analysis of single nucleotide polymorphisms (SNPs) in IL-1 and TNF genes at 7 loci namely; IL1α (rs1800587), IL1β (rs16944), IL1β (rs1143634), IL1R (rs2234650), IL1RA (rs315952), TNF (rs1800629) and TNF (rs361525) using polymerase chain reaction with sequence specific primers (PCR-SSP) method. Haplotype block structure was determined using Haploview and statistical analysis was done using PyPop.

Results: A total of 81 children, 27 HIV positive and 54 HIV negative at the end of their 18th month follow up were considered for this study. Significant increased frequency of CT genotype at (rs2234650) in IL1R was observed in positive vs negative children (76.4% vs. 42.2%, p=0.023), while CC genotype frequency was significantly increased in exposed uninfected compared to infected children (51.1% vs.17.6%, p=0.022). The TCCCT haplotype for IL1R1 (rs2234650), IL1A (rs1800587), IL1B (rs16944), IL1B (rs1143634), and IL1RN (rs315952) was significantly associated (p=0.002) with HIV transmission. Similarly, the GG genotype, responsible for low expression of TNF-α (rs1800629) was significantly high in uninfected children as compared to infected ones (77.7% vs. 44.4%, p=0.005), while TNF-α, GA genotype frequency was high among the infected children (55.5% vs. 20.3%, p= 0.002). Also, 'G' allele frequency was high in negative children as compared to positives (p=0.016).

Conclusion: This study possibly for the first time reports association of specific SNPs of IL-1R and TNF-α genes with risk of perinatal HIV transmission. SNPs at IL1R (rs2234650) and TNF (rs1800629) can be further exploited as possible markers for prediction of perinatal HIV transmission.

Condensation of HIV-1 nucleocapsid during NCp9-p6^{Gag} separation: RNA remarkably promotes protease fast turnover in mild acidic conditions

S. Lyonnais[5], L. Dufau[3], M. Reboud-Ravaux[3], R. Marquet[4], J.C. Paillart[4], J.M. Gatell[5-1], R.J. Gorelick [6], C. Tisné[2], G. Mirambeau[5-3]

[1]Hospital Clinic, Universidad de Barcelona, Barcelona [2]Laboratoire de Cristallographie et RMN biologiques, Université Paris-Descartes, CNRS [3]Enzymologie Moléculaire et Fonctionnelle (Faculté de Biologie), UPMC Sorbonne Universités, Paris [4]Architecture et Réactivité de l'ARN (IBMC), Université de Strasbourg, CNRS, Strasbourg, France [5]AIDS Research Group, IDIBAPS, Barcelona, Spain [6]AIDS and Cancer Virus Program, SAIC-Frederick, National Cancer Institute-Frederick, Frederick, United States

The molecular mechanism of HIV-1 maturation driven by its protease (PR) remains poorly documented at the steps that control RNA condensation. The experiments shown here are mainly inspired by B. M. Alberts' concept of macromolecular machines and combine biochemical and biophysical approaches. They provide a clear *in vitro*demonstration that HIV-1 RNA behaves within the HIV-1 particle as an up-regulator of PR. The resulting fast processing of RNA-bound nucleocapsid protein (NC) from its Gag precursor clearly leads to RNA condensation after the secondary cleavage releasing the Gag C-terminal p6 domain from the NCp15 intermediate. Remarkably, such processing is optimal in more physiological conditions than classically used for *in vitro* HIV-1 PR assay, thus allowing a useful protection of the crucial NC zinc fingers. The related mechanism implies PR sequestration by clusters of NCp15 assembled along the RNA chains, highlighting a fast condensation of RNA:NC ribonucleoproteic complexes as an opportune step within the overall process of maturation, prior to the conical capsid reassembly. Full in vitro reconstitution of the maturation processes is now under development using a RNA-supporting PR assay. Finally, our data supports a new biological paradigm of a protease dramatically controlled by a RNA molecule, whereas the NCp15 processing intermediate as well as its counterpart within the Gag precursor should provide highly attractive targets for a next generation of HIV-1 maturation inhibitors.

The anterograde trafficking of CCR5 as a therapeutic target for HIV infection

A. Brelot[3], G. Boncompain[2], F. Herit[1], S. Tessier[2], A. Lescure[2], E. Del Nery[2], F. Niedergang[1], F. Perez[2]

[1]Institut Cochin [2]Institut Curie [3]virology, Institut Pasteur, Paris, France

Objective: HIV entry into target cells requires the interaction between HIV envelope glycoproteins and two cellular receptors, CD4 and a chemokine receptor (named co-receptor) CCR5 and/or CXCR4. In more than 99% of infected people, HIV with tropism for CCR5 (R5) is responsible for transmission and predominates for years during the asymptomatic phases of infection. In addition, individuals that do not express CCR5 due to a natural polymorphism (*ccr5***Δ**32/*ccr5***Δ**32) are highly resistant to R5 infection and do not suffer from the lack of CCR5.

CCR5 is thus ideally suited for therapeutic intervention but: (i) only one drug targeting CCR5 as a non-competitive antagonist (Maraviroc) has been used in clinic and escape mutants have been observed *in vitro* and *in vivo*; (ii) CCR5 agonists exist that complies with both antiviral principles of receptor occupation and receptor internalization but their structural complexity, instability and pro-inflammatory properties make them non appropriated for their therapeutic usage.

An alternative therapeutic option would be to prevent CCR5 transport to the cell surface.

Methods: We took advantage of our recently developed synchronized secretory assay, the RUSH system, to study the anterograde trafficking of CCR5. We established cellular models that allow the synchronous trafficking of CCR5 from the endoplasmic reticulum to the cell surface.

Results: It is established that a large diversity of secretory routes exists in a cell. We observed that the kinetics and the mode of transport of CCR5 are distinct from the ones of several other proteins of the secretory pathway. It seems thus possible to find specific regulators of CCR5 trafficking. In order to identify molecules that would inhibit the anterograde transport of CCR5 toward the plasma membrane, we performed a high content screening of chemical compounds libraries. Our preliminary results showed that some molecules are indeed able to block the transport of CCR5, inhibiting its presence at the plasma membrane, without affecting other secretory proteins used as reference.

Conclusion: Our study will provide clues about the trafficking mechanisms of CCR5. It could also lead to the identification of new drugs preventing the secretion of CCR5 and thus inhibiting the viral infection of target cells.

LEDGINS reveal a role for LEDGF/p75 in late stage HIV replication

Z. Debyser[2], B. Desimmie[2], J. Demeulemeester[2], R. Schrijvers[2], D. Borrenbergs[1], J. Hendrix[1], C. Weydert[2], J. De Rijck[2], R. Gijsbers[2], N. Bannert[3], F. Christ[2]

[1]Laboratory for Photochemistry and Spectroscopy [2]Molecuar Virology and Gene Therapy, KU Leuven, Leuven, Belgium [3]Robert Koch Institute, Berlin, Germany

Objective: Current clinical HIV integrase (IN) inhibitors target the active site of the enzyme. Targeted integration of lentiviruses is coordinated by the cellular co-factor lens epithelium-derived growth factor (LEDGF/p75). Recently we reported on a novel class of small molecule IN inhibitors designed to bind to the LEDGF/p75 interaction site of HIV-1 IN and referred to as LEDGINs. LEDGINs potently block HIV integration and severely impair infectivity of progeny virions produced in their presence through a multimodal mechanism of action. Here we provide evidence on the molecular basis of the late effect of LEDGINs.

Methods: We used multiple complementary techniques to investigate the infectivity of virions produced in the absence of LEDGF/p75 or presence of LEDGINs: (1) Detailed virological profiling, RT-qPCR and a nuclear import assay, (2) Electron microscopy to examine the morphology of viral particles, (3) Single molecule FRET assay to study the effect of LEDGINs on IN multimerization in mature viral particles and (4) Viral particles purified by ultracentrifugation using a step gradient fractionation were analyzed for the presence of LEDGF/p75.

Results: Viral particles produced in the presence of LEDGINs or in the absence of LEDGF/p75 display strongly impaired infectivity in various cells including primary cells without significant effect on proteolytic maturation or RNA packaging. The late effect of LEDGINs is mediated by a direct and specific interaction with IN at the LEDGF/p75 binding pocket. Only functional LEDGF/p75 but not the IN interaction-defective variant rescues infectivity of progeny virions. Using Q-PCR and fluorescently labeled PIC nuclear import assays, we pinpointed the infectivity defect in second round replication to reverse transcription and nuclear import steps. FRET analysis demonstrates that LEDGINs enhance IN multimerization in the progeny virions when added during virus production. Moreover, Western blot analysis demonstrates the specific recruitment of LEDGF/p75 into HIV particles which is prevented by LEDGINs.

Conclusion: By using complementary techniques we unambiguously demonstrate that the LEDGF/p75-IN interaction is required during both early and late stages of HIV replication. LEDGINs are a unique class of antiretrovirals that combine antiviral activities at early and late steps with a potential for further clinical development.

The RNA chaperonne activity of Vif is mainly contained in its C-terminal domain

D. Sleiman[1], S. Guerrero[2], S. Bernacchi[2], J.C. Paillart[2], C. Tisne[1]

[1]LCRB, Paris [2]CNRS, architecture et réactivité de l'ARN, Strasbourg, France

The viral infectivity factor (Vif) is an auxiliary small basic protein of 23 kDa of HIV-1. Vif has been described as an RNA binding protein. In this study, we characterized Vif and its domains for their interaction with RNA partners using several biochemical and biophysical techniques. We defined Vif domains by an In Silico approach, these domains where successfully expressed and purified. First, we showed using DLS that all domains are able to form multimers in solution. By circular dichroïsm, we then determined the percentage of secondary structure of Vif: the whole protein has around 50% of secondary structures with an N-terminal domain containing the highest percentage of secondary structure and a C-terminal domain basically in a random structures. By fluorescence spectroscopy, we investigated the ability of Vif domains to bind to RNA. Interestingly, all domains of Vif are able to bind to RNA, especially $tRNA^{Lys}_3$, the primer by HIV-1 reverse transcription. Protein footprints on $tRNA^{Lys}_3$ were determined by NMR. We showed that Vif occupied the same binding sites than the nucleocapsid protein on $tRNA^{Lys}_3$, with differential binding sites for Vif N- and C- terminal domains. Finally, we investigated the chaperon activity of the Vif domains and we showed that its C-terminal domain exhibits an RNA chaperonne activity comparable to the entire Vif protein.

Heterogeneous susceptibility of circulating SIV isolates to HIV capsid-interacting factors

J.I. Mamede[1-2-3], M. Sitbon[1-2-3], J.L. Battini[1-2-3], V. Courgnaud[1-2-3]

[1]Institut de Génétique Moléculaire de Montpellier CNRS UMR5535 [2]Université Montpellier 1 [3]Université Montpellier 2, Montpellier, France

Cross-species transmission and adaptation of SIV to humans have given rise to human immunodeficiency viruses (HIV-1 and HIV-2) in at least twelve independent occasions. So far, 45 species of African non-human primate species are known to be naturally infected with SIV and humans remain at the forefront of exposure to these viruses in Sub-Saharan Africa. Here, we evaluated the likelihood of new cross-species transmission by investigating the interactions of capsids (CA) from naturally circulating SIV isolates with cellular host factors that block (i.e. TRIM5 proteins) or enhance (i.e. cyclophilin A - CypA - and nucleopore-associated RanBP2 and Nup153 proteins) HIV replication early during the viral life-cycle.

For this purpose, we swapped the CA in a *gag-pol* SIVmac expression vector with that of circulating SIV isolates (SIVgsn lineage, SIVmnd1, SIVcol). We then evaluated by single-round infectivity assays the restriction of these CA chimeras into CHO cells stably expressing different primate TRIM5α (human, chimpanzee, African green monkey and rhesus macaque), natural TRIMCypA fusion proteins (owl monkey and cynomolgus and rhesus macaques) or a chimeric TRIM-RanBP2Cyp protein obtained by swapping the RanBP2 CypA-like motif in owlTRIMCypA. In addition, HEK-293T cells depleted for RanBP2 and Nup153 by siRNA were challenged with circulating SIV capsids. All infections were monitored by flow cytometry 48 hours post-infection.

We showed that human TRIM5α is unlikely to prevent cross-species transmission of any SIV we tested and observed that, although widespread, SIV CA-CypA interaction is not a universal phenotype. Moreover, entry in the nucleus of different SIV appeared to follow pathways that do not necessarily recruit RanBP2 or Nup153, and this regardless of their interaction with CypA. Nevertheless, we found that, alike HIV-1, human-adapted HIV-2 was dependent on the CypA, RanBP2 and Nup153 interactions for optimal infection. In contrast, we found that, unlike HIV CA, SIV CA did not require a direct interaction with the Cyp-like domain of RanBP2 to carry out successful infection. In conclusion, circulating SIV CA have distinct phenotypes with regard to CA-interacting restricting or facilitating factors, which warrants individual evaluation of circulating SIV with already identified defense mechanisms or to unveil new restricting factors and pathways.

HIV-1 induced autophagy modulation in Langerhans cells

M. Czubala, P. Mitchell, F. Blanchet, V. Piguet

Institute of Infection and Immunity, Cardiff University, School of medicine, Cardiff, United Kingdom

Aim: Our research aims to decipher the mechanism and immune consequences imposed by HIV-mediated modulation of autophagy in challenged dendritic cell (DC) subsets, in particular Langerhans cells (LC). Autophagy is an ubiquitous cellular process involved in lysosomal-mediated degradation of host components as well as invading pathogens. In previous reports, HIV-1 was shown to modulate the autophagy flux in various immune cell types, including T cells, macrophages and myeloid dendritic cells. However, very little is known about the potential autophagy-driven antiviral activity in LC.

Methods: LC used for this study were differentiated from either blood monocytes or the MUTZ-3 cell line model which phenotypically mimic LC morphology and immune features. LC were treated with wild type HIV-1, VSV-G pseudotyped HIV-1-derived lentivectors or recombinant HIV-1 envelopes. Levels of infection and cellular autophagy flux were analyzed by western blotting, FACS and confocal imaging.

Results: We observe that, as previously described for myeloid DC, autophagy flux is compromised in LC after exposure to HIV-1 envelope or infectious virus. Hence, activation of the autophagy negative regulator, mTOR, is detected, and could account for the autophagy flux inhibition. Although we could confirm the HIV-1 restriction activity of langerin and SAMHD-1 in LC, it appears that shutting down autophagy flux is an early cellular target for the virus thus strongly suggesting that it might be involved in HIV-1 clearance shortly upon viral entry.

Conclusion: In this study, we show thatLangerin and SAMHD-1 are significantly restricting HIV-1 entry and replication in LC. However, an additional barrier to infection, such as autophagolysosome-mediated degradation of virus particles, may account for the low infection rate observed in LC. Our results suggest that autophagy flux is indeed dampened upon HIV-1 challenge which might favour virus progression. We further aim to examine the effect of autophagy modulation on virus infection of LC and consequent virus transmission to $CD4^+$ T cells.

Systems Biology, HIV Disease, and RNAs big and small

M. Katze

Department of Microbiology and Washington National Primate Research Center, University of Washington, Seattle, United States

Objective: High-throughput genomic and proteomic technologies are providing us with ever deeper views into the intricacies of biological systems, and it is evident that the ability to work with vast amounts and diverse types of data is essential to deciphering biological complexity and to advancing new antiviral therapies and vaccines. My laboratory is using systems biology and computational methods to understand and model integrated views of virus-host interactions, viral evasion of host defenses, and viral pathogenesis.

Methods: We are using new experimental systems and technologies, such as mouse systems genetics, metabolomics, and next-generation sequencing (RNA-Seq), to expand our systems-level views to encompass host genetic variation, metabolic pathways, and noncoding RNAs. We are using these approaches to study early events in pathogenesis caused by a variety of viruses, including HIV and emerging pathogens such as influenza virus, SARS-coronavirus, and Ebola virus. These methods are also being applied to the early innate response to vaccination, thereby illuminating the events that lead to protective immune outcomes.

Results: Using RNA-Seq, we have observed changes in the expression of diverse classes of small and long noncoding RNAs in response to HIV and respiratory virus infection. Most recently, we have used this approach to profile microRNA expression early after HIV infection of CD4+ T cells. We defined a phased pattern of expression, which when integrated with mRNA expression data indicates a role for microRNAs (including novel unannotated microRNAs) in transcriptional regulation, T cell activation, and cell cycle control.

Conclusion: Our findings suggest that a detailed knowledge of noncoding RNA regulation and function will be necessary for a full understanding of transcriptional control and viral pathogenesis. The combination of high-throughput datasets and computational methods provides the best hope for speeding HIV vaccine and drug development and for preparedness against future viral threats.

Genetic & Phenotypic Characteristics of Subtype C Full Length Genome HIV-1 from Linked Heterosexual Transmission Pairs

M. Deymier[1], Z. Ende[1], D. Claiborne[1], W. Kilembe[2], S. Allen[1-2], E. Hunter[1-2]

[1]Emory University, Atlanta, United States [2]Zambia Emory HIV Research Project, Lusaka, Zambia

Objective: In 80-90% of heterosexual transmissions of HIV, a genetic bottleneck occurs, in which an individual, with a diverse viral quasispecies, transmits a single viral variant, the Transmitted/Founder (TF), to a naïve host. Previous genetic studies of the HIV envelope have suggested a non-stochastic process for transmission and evidence for selection of a specific TF variant. Although some genetic signatures in Env have been found, no biological correlates for TF variants have thus far been elucidated. We are analyzing infectious molecular clones (IMC) from linked transmission pairs, in order to determine whether the TF variant has distinct phenotypic properties compared to a representative sampling of the donor quasispecies near the time of transmission.

Methods: We have performed HIV-1 near full-length (NFL) single genome amplification followed by direct sequencing, from plasma of five subtype C acutely infected individuals and each of their chronically infected, virologically linked partners from the Zambia-Emory HIV Research Project. A phylogenetic analysis was performed on the 125 NFL genomes (mean 25/transmission pair). Replication of full-length IMCs derived from these amplicons was compared in PBMCs.

Results: Despite the diverse population in the donors, the transmission of a single viral variant was observed in all five transmission events. The TF variant more closely resembles the most recent common ancestor from the donor quasispecies than does the average of the donor viral variants ($p=0.031$). Preliminary experimental studies with fully infectious molecular clones of TF variants in direct comparison with variants from the linked donors show that the TF viruses can have lower replicative capacities in PBMCs than their non-transmitted counterparts. Additional analyses are needed to probe the role of replication capacity and other properties of the TF virus in the transmission process.

Conclusion: Studies of IMCs from virologically linked transmission pairs have the potential to inform on key properties linked to transmission. Phylogenetic analyses suggest that viral evolution in a chronically infected host decreases the ability to establish new infections, with transmission of an earlier evolutionary variant. The genetic bottleneck thus provides an opportunity for understanding the unique properties of TF viruses and may provide potential targets for intervention.

Duffy antigen receptor for chemokines and HIV

R. Weiss

University College London

Objective: To test Duffy Antigen Receptor for Chemokines (DARC) as (a) a co-receptor and (b) a risk factor for HIV infection because HIV and CCL5 have previously been reported to bind to DARC.

Methods:

(a) Human DARC alleles (Fy-a, Fy-b and Fy-null) and tyrosine sulphate mutants were transfected into CD4+ cells to test their ability to support HIV entry by exposure to R5 and X4 strains or pseudoviruses of HIV.

(b) We also tested whether DARC+ and DARC- healthy human volunteers had different plasma levels of CCL5 (RANTES) chemokine.

Results:

(a) 0/20 diverse strains of HIV-1 were able to utilise DARC as a functional co-receptor for vial entry. However, 8/15 HIV-2 strains could utilise DARC.

(b) DARC-null individuals had a significantly reduced mean level of CCL5 in plasma. Preliminary analysis indicates that the individuals with low CCL5 levels correlate with those exhibiting DARC-associated benign African leukopenia, which has been linked with a 2-fold increased risk of HIV-1 infection.

Conclusion: The DARC-null phenotype is determined by an African-specific allele which occurs in 85% of sub-Saharan Africans. While DARC is not an important co-receptor for HIV-1 entry, absence of DARC appears to increase risk of HIV infection by reducing protective levels of CCL5 in the plasma and at mucosal surfaces. One implication is that DARC-null status may account in part for the relatively high frequency of HIV infection in sub-Saharan Africa.

Distinct mechanisms of HIV transfer from macrophages or Langerhans dendritic cells to T lymphocytes

M. Peressin, A. Proust, S. Schmidt, M. Lambotin, B. Su, M. Biedma, G. Laumond, T. Decoville, C. Moog

Institut de Virologie, Université de Strasbourg, INSERM U1110, Strasbourg, France

Objective: In addition to T cells, dendritic cells (DC) and macrophages residing at mucosal sites are considered as first HIV target cells following sexual infection. Due to their antigen presenting cell (APCs) functions, DCs and macrophages are in close contact with T cells, which favors efficient transfer of viral particles to lymphocytes, leading to rapid HIV-1 dissemination. In this study, we aim to understand the mechanisms and the respective contribution of DCs and macrophages in HIV transfer to T cells.

Methods: Langerhans DCs and interstitial DCs (LCs/IDCs) were obtained by differentiation of $CD34^+$ cord blood cells and MDMs (monocytes-derived macrophages) were differentiated from blood monocytes. LCs/IDCs or MDMs were incubated with HIV-1_{BaL} during 2 hours before extensive wash. These HIV-loaded APCs were then cocultivated with autologous stimulated CD4 T cells in presence or absence of inhibitors. HIV infection was recorded 2 and 3 days post-infection by measurement of intracellular p24 in each cell population and analyzed by flow cytometry.

Results: We demonstrated that the mechanism of transfer from LCs/IDCs and MDMs to T cells differed in several points. First, the kinetic of HIV transfer to T cells was more rapid in MDMs, recorded within 48 hours versus 72 hours for LCs/IDCs. MDMs, that were more susceptible to infection, transfer also HIV 4 fold more efficiently compared to DCs subsets. Moreover, trans-transfer (without de novo production of virus particles) was major in MDMs cocultures, compared to LCs/IDCs that mainly transfer HIV in cis to T cells. Finally, we demonstrated that the presence of T cells in the different APCs cocultures induced modifications of the fusion kinetic and influenced HIV infection cycle in APCs: increased replication in DCs subsets, and reduced in MDMs cocultures.

Conclusion: Altogether, our results highlight the important contribution of each target cell population in HIV infection and transfer to T cells. Moreover, they emphasize the potential role of macrophages in HIV transmission to T lymphocytes at mucosal site.

Productive HIV-1 Infection of Cervical Tissue *Ex Vivo* is Associated with the Secretory Phase of the Menstrual Cycle

E. Saba[2], M. Origoni[3], C. Doglioni[3], J.C. Grivel[4], L. Margolis[4], G. Poli[2]

[1]Department of Obstetrics and Gynecology, Italy [2]AIDS Immunopathogenesis Unit [3]Department of Pathology, San Raffaele Scientific Institute, Milano, Italy, Milan, Italy [4]Program in Physical Biology, Eunice Kennedy-Shriver National Institute of Child Health and Human Development, NIH, Bethesda, Md, United States

Objective: The mucosal immune system of the lower female genital tract plays a key role in HIV-1 transmission. *Ex vivo* cervical tissue explants (CTE) have proven to efficiently support productive R5 HIV-1 infection thus allowing the investigation of the role of immune cells and factors influencing sexual HIV-1 transmission, replication and pathogenesis.

Methods: CTE were established from HIV-1^{neg} women undergoing hysterectomy for benign tumors. CTE were infected with R5 HIV-1_{BaL} virus. Productive infection of HIV-1 was documented by $p24_{Gag}$ ELISA, intracellular p24 Gag staining and detection of total viral DNA by RT-PCR. Control CTE incubated with the anti-HIV agent 3TC were included to subtract residual HIV input signals. Multiparametric FACS analysis of differentiation/activation markers of T lymphocytes and macrophages associated was performed on cells isolated from CTE.

Results: CTE from 22 seronegative women were exposed to R5 HIV-1_{BaL} virus. However, only 8 CTE (36%) were productively infected as demonstrated by HIV-1 $p24_{gag}$ release in culture supernatants whereas 14 (64%) were nonproductive. In partial contrast, accumulation of HIV_{gag} DNA and of $p24_{Gag}^{+}$ $CD4^{+}$ T cells and macrophages occurred in both productive and, although at lower levels, in nonproductive CTE. Furthermore, nonproductive CTE secreted more CCL3 and CCL5 than the productive ones. A *post-hoc* analysis revealed that all productive CTE were established from women in their secretory phase of the menstrual cycle, whereas nonproductive CTE derived from women in either their secretory (28%) or proliferative menstrual cycle phase (36%) or with an atrophic endometrium (36%).

Conclusion: Our results support the epidemiological observation that sexual transmission of HIV-1 leads to a more efficient spreading infection in women in their secretory phase of the menstrual cycle.

Molecular characterization of high avidity CD4+ T cells in HIV Controllers

D. Benati[4], M. Galperin[4], O. Lambotte[2], A. Lim[5], B. Lemercier[5], S. Hendou[3], F. Boufassa[3], D. Zucman[6], P. De Truchis[1], J. Delfraissy[2], F. Arenzana-Seisdedos[4], L. Chakrabarti[4]

[1]Département des Maladies Infectieuses et Tropicales, AP-HP, Hôpital Raymond Poincaré, Garches [2]Service de Médecine Interne et Maladies Infectieuses, AP-HP, Hôpital de Bicêtre [3]INSERM U822, Le Kremlin-Bicêtre [4]Unité de Pathogénie Virale, Institut Pasteur [5]Institut Pasteur, Département d'Immunologie, Paris [6]Service de Médecine Interne, Hôpital Foch, Suresnes, France

Background: HIV Controllers spontaneously control HIV replication to levels undetectable by standard assays in the absence of antiretroviral treatment. We previously reported that HIV Controllers harbour a pool of memory CD4+ T cells able to respond to the immunodominant Gag293 peptide with particularly high TCR avidity. We set to functionally analyze high avidity CD4+ T cells and to characterize their TCRs at the molecular level.

Methods: HIV Controllers from the ANRS CODEX CO21 cohort (n=8) were compared to efficiently treated patients (HAART group, n=8). Patients from both groups were characterized by

Results: Stimulation with low Gag293 peptide doses generated IFNg-positive CD4+ T cell lines in HIV Controllers (response rate: 6/8 at 10^{-9}M, 2/8 at 10^{-11}M), but not in HAART patients, confirming the presence of high-avidity Gag-293-specific cells in the HIC group. The immunoscope analysis revealed major amplifications of certain TCR Va and Vb chains in the sorted tetramer+ Gag293-specific population, indicating the presence of dominant clonotypes.

Conclusion: We identified a number of TCR Va and Vb chains preferentially expressed by the high-avidity population of Gag293-specific CD4+ T cells. Transfer of these TCRs to heterologous cells will help determine whether they are sufficient to confer the efficient Gag-specific responses characteristic of HIV Controllers.

HIV-1 Nef Interferes With T Lymphocyte Circulation Through Confined Environments *in vivo*

B. Stolp[2-1], A. Imle[1], F. Matos Coelho[2], M. Hons[2], R. Gorina[2], R. Lyck[2], J.V. Stein[2], O.T. Fackler[1]

[1]Dept of Infectious Diseases, Virology, Fackler lab, University Hospital Heidelberg, Heidelberg, Germany [2]University of Bern, Theodor Kocher Institute, Bern, Switzerland

Objective: HIV-1 Nef elevates virus replication and contributes to AIDS progression *in vivo*, but the understanding of the underlying molecular function remains incomplete. As one of its established *in vitro* activities, Nef interferes with T lymphocyte chemotaxis by reducing host cell actin dynamics. The goal of this study was to provide first insight into the *in vivo* impact of this Nef-mediated deficiency in host cell motility on T lymphocyte circulation.

Methods: We established a retroviral transduction/isolation strategy to achieve efficient and homogenous expression of biologically active Nef in primary murine T lymphocytes. Cells were introduced into recipient mice by adoptive transfer and homing behavior was analyzed using FACS-based cellular quantification, intravital imaging, and 3D quantitative immunofluorescence (3DQIF) reconstruction of lymph nodes. These *in vivo* analyses were complemented by *ex vivo* analyses of transendothelial migration under flow conditions and motility in complex 3D collagen matrices of different densities.

Results: We found that Nef drastically impaired *in vivo* homing of T lymphocytes to peripheral lymph nodes. This block was primarily due to a strong inhibition of extravasation through high endothelial venules, while subsequent parenchymal motility was only slightly reduced. *Ex vivo* analyses of transendothelial migration revealed that Nef disrupted T lymphocyte polarization and interfered with diapedesis and migration in the narrow subendothelial space. Using 3D collagen matrices we could show that Nef specifically affected those T cell motility modes employed in dense environments imposing high physical barriers upon migration, while leaving migration in low constrictive environments intact. Mechanistically, inhibition of lymph node homing, subendothelial migration and cell polarization, but not diapedesis, depended on Nef's ability to inhibit host cell actin remodeling.

Conclusion: Nef interferes with T lymphocyte circulation *in vivo* and predominantely acts on motility modes employed in confined environments. The use of physiological assays revealed diapedesis as a migratory activity which is inhibited by Nef independently of its ability to deregulate actin dynamics. The activity of Nef to interfere with T lymphocytes' *in vivo* recirculation to lymph nodes may compromise T cell help and thus represents an important mechanism for its function as a HIV pathogenicity factor.

Human NK cells control HIV-1 infection at a mucosal level

H. Quillay[5-3], M. Duriez[3], H. El Costa[3], C. Cannou[3], R. Marlin[1], C. De Truchis[2], A. Le Breton[2], M. Rahmati[4], F. Barré-Sinoussi[3], MT. Nugeyre[3], E. Menu[3]

[1]UMR-CNRS-5164-CIRID, Université Bordeaux 2, Bordeaux [2]Gynecology-Obstetrics Service, A. Béclère Hospital, AP-HP, Clamart [3]Regulation of Retroviral Infection Unit, Institut Pasteur [4]Gynecology-Obstetrics Service, Pitié Salpétrière Hospital AP-HP [5]Université Paris Diderot, Sorbonne Paris Cité, Cellule Pasteur, Paris, France

Objectives: Mucosa are the preferential portal for HIV-1 entry in the body. It is thus crucial to identify the immune responses necessary for an efficient control in the mucosa. The control of HIV-1 infection at the materno-fetal interface during the first trimester of pregnancy is a model to study natural protection against transmission at a mucosal level. The decidua (uterine mucosa during pregnancy) is one of the major materno-fetal interfaces. In this tissue, $CD14^+$ antigen-presenting cells (dAPC) are the main target cells of R5 tropic HIV-1. Decidual natural killer (dNK) cells account for 70% of decidual leukocytes during the first trimester of pregnancy. They display distinct phenotype and functions compared to peripheral NK cells. At the periphery, NK cells are involved in the control of HIV-1 infection. The aim of this study was to determine the role of dNK cells in the control of HIV-1 infection of dAPC.

Methods: Deciduas were obtained from HIV-1 negative women undergoing elective abortions (8-12 weeks of amenorrhea), with their written informed consent. dAPC and dNK cells were purified by magnetic beads selection. dAPC were infected with R5 HIV-1 strain and dNK cells were added (ratio 1:5) at different times. Cocultures were performed in the same well or separately in a double chamber system.

Results: When dNK cells were added before dAPC infection, a significant inhibition of dAPC HIV-1 infection was observed. This inhibition was less important when dNK cells were added one or three hours after infection. A moderate or no inhibition was observed when dNK cells were added 16 hours after infection. Maximum inhibition occurred when dAPC and dNK cells were cocultured in the same well. In contrast, no or limited inhibition was observed when dAPC and dNK cells were cocultured separately.

Conclusion: These data demonstrate that dNK cells control dAPC HIV-1 infection in the early steps of infection through cell-to-cell contacts and soluble factors. The exact mechanisms involved in this control are currently under investigation. This study will help to identify correlates of protection, which are important for the development of future preventive vaccines against HIV-1 mucosal transmission.

Whole-transcriptome profiling of women at risk for HIV acquisition defines shared risk signatures with implications for prevention interventions

V. Naranbhai[4-1-2], R. Singh[2], Q. Abdool Karim[1], S.S. Abdool Karim[1], T. Ndung'u[2-3], A.V.S. Hill[4]

[1]Centre for the AIDS Programme of Research in South Africa (CAPRISA), Nelson R Mandela School of Medicine, University of KwaZulu Natal [2]HIV Pathogenesis Programme, Nelson R Mandela School of Medicine, University of KwaZulu Natal [3]KwaZulu-Natal Research Institute for Tuberculosis and HIV (K-RITH), Nelson R Mandela School of Medicine, University of KwaZulu Natal, Durban, South Africa [4]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom

Background: Identification of correlates of HIV acquisition is required for risk stratification and to guide development of novel prevention interventions. Many cellular or genetic factors have been implicated in HIV acquisition, yet these are often derived from *in vitro*, candidate gene and/or cross sectional studies. We applied a systems biology approach to identify transcriptomic factors associated with HIV acquisition in the CAPRISA004 microbicide gel trial conducted in KwaZulu-Natal, South Africa.

Methods: RNA was extracted from unstimulated PBMC from 44 women sampled prior to acquisition (15-404 days pre-acquisition) and 38 women who remained HIV negative despite the highest behavioral risk in the trial. RNA was labeled and hybridized to Illumina HT12v4 microarrrays containing 47,231 probes covering 20,827 genes across the transcriptome. Variance-stabilising transformation and robust spline normalization was implemented in the *lumi* package(Bioconductor). Chip effects were excluded as technical confounders before unblinding acquisition outcome. After stringent quality control 13,834 probe sets from each of 39 HIV-acquirers and 34 non-acquirers were filtered for analysis in *limma*, IPA and PAMR. The Benjamini-Hochberg false discovery rate(FDR) was used to adjust for multiple comparisons.

Results: Overall, 523 gene transcripts (624 probes) were more abundant in HIV acquirers and 154(278 probes) less abundant vs. non-acquirers(log_2FC>0.37;FDR<0.05). HIV-acquirers clustered distinctly from non-acquirers in both unsupervised hierarchical clustering analysis of differentially expressed probes, or principal components analyses using all 13834 probes. Amongst the most highly differentially expressed genes(log_2FC>1;FDR<0.0001), 6/28 have previously been implicated in HIV control in GWAS, siRNA or HIV-host protein interaction screens (moesin, POLR2a, ITGB2, THBS1, ACTB,CD14). In addition, several of these genes have plausible roles in HIV immunity e.g.: *Tapasin* (log_2FC=0.80; FDR=$3x10^{-22}$), *IFN-γ*(log_2FC=-1.05; FDR=$3x10^{-7}$). Inflammatory pathways downstream of TNF (p=$1.5x10^{-15}$), PDGF (p=$1.15x10^{-14}$), TCR signaling (p=$3.2x10^{-14}$), and STAT3 (p=$4.34x10^{-12}$) were significantly over-represented in HIV acquirers. Increased expression levels of five genes (MYH9, CMIP, POL2RA, moesin, CCNK) together correctly predicts acquisition outcome with >90% precision (>100 cross validation runs). Deconvolution to define cell-type specific signatures, functional validation of novel factors and independent validation of the prediction algorithm is underway.

Conclusions: These data suggest that women who acquire HIV share a transcriptomic signature of inflammation and reduced IFN-*γ* that precedes acquisition. Therefore attempts at modifying acquisition may require targeting of multiple upstream drivers.

Effect of seminal fluid from infected and uninfected men on HIV infectivity in vitro

C. Camus[2], O. Bourry[2], D. Mahé[2], L. Bujan[4], C. Pasquier[3], D. Le Lannou[1], O. Zirafi[5], J. Munch[5], N. Roan[7], W. Greene[6], C. Pineau[2], N. Dejucq-Rainsford[2]

[1]CECOS Ouest Centre Hospitalier Universitaire [2]INSERM - U1085, INSERM, Rennes [3]Virology laboratory, Universitary Hospital of Toulouse [4]CECOS Midi-Pyrénées, EA 3694, Human Fertility Research Group, Universitary Hospital of Toulouse and University de Toulouse, Toulouse, France [5]Institute of Molecular Virology, Ulm University, Ulm, Germany [6]Gladstone Institute of Virology and Immunology, San Francisco [7]Department of Urology, University of California at San Francisco, San Franscico, United States

Objective: Semen is the main vector for HIV dissemination. Recent evidence indicates the presence of factors in seminal fluid (SF) of uninfected men modulating HIV infectivity. We previously showed that semen-producing organs are infected by HIV, which most likely modifies the composition of SF of HIV+ men, and cytokine levels in semen have been shown to differ between uninfected and infected individuals. Here we compare, for the first time to our knowledge, the effect of SF derived from infected *versus* uninfected men on HIV infectivity in cellular models.

Methods: SF from 16 uninfected and 20 therapy naïve HIV+ men in the chronic stage were tested for their effects on HIV-1 R5 SF162 infectivity on cultured PBMCs and TZM-bl (3h exposure, final SF concentration on target cells: 1, 0.2 and 0.04%), as well as for the levels of 46 cytokines, chemokines and growth factorsand PGE2, and HIV enhancing factors (SEVI, and fragments from semenogelins (SEM) 1 and 2) by Luminex and ELISA. Cytotoxicity was assessed by quantitating ATP levels.

Results: SF from both uninfected and infected men had a similar dose-dependent enhancing effect on HIV infection of TZM-bl. This effect was positively correlated with the level of SEVI but not of SEM1 or SEM2. In contrast, semen from HIV infected men had a significantly lower enhancing effect on PBMC infectivity when compared with SF from uninfected men and this effect did not correlate with the levels of either SEVI, SEM1 or SEM2. PBMC metabolic activity was not affected following 3h exposure to semen at maximum concentrations of 1% from either HIV+ or HIV- men. Several cytokines were significantly elevated in the semen of HIV+ *versus* uninfected men.

Conclusions: These results indicate a differential effect of SF from HIV infected *versus* uninfected men on PBMC infectivity. The impact of SF from infected men on PBMC HIV receptor expression, proliferation, apoptosis and immune activation is currently under study. The proteomic profile of semen from infected men will also be evaluated.

Keywords: HIV transmission, semen, cytokines, HIV infectivity modulating factors

Sources of funding: Inserm, ANRS, Sidaction, Ministère de la recherche.

Biological samples were obtained from the biological resource centre GERMETHEQUE

HBV serologic status in a cohort of HIV-positive patients from Romania

O. Streinu-Cercel[1-2], A.M. Tudor[1-2], A. Streinu-Cercel[1-2], M. Paraschiv[2], G. Ceapraga[2], D. Vlad[2], A. Streinu-Cercel[1-2]

[1]Carol Davila University of Medicine and Pharmacy [2]National Institute for Infectious Diseases "Prof.Dr. Matei Bals", Bucharest, Romania

Objective: We describe the serologic HBV status of HIV-positive patients in a particular cohort of Romanian patients, infected in their early years of life with both HIV and HBV.

Methods: We assessed HBsAg, anti-HBs, total anti-HBc, HBeAg and anti-HBe in a cohort of HIV-positive patients coinfected with HBV, monitored at the National Institute for Infectious Diseases “Prof.Dr. Matei Balş” from Bucharest, Romania, at a prespecified time point and compared this data to the available medical history for each patient.

Results: We evaluated 95 patients coinfected with HIV+HBV, with a median age (±SD) of 21±1.6 years, 51 (53.7%) of which were female. The medical records showed that 26 (27.4%) of them had been previously diagnosed with hepatitis B in our clinic, with a median disease duration of 10±3.1 years.

The median time from their last hospital visit for hepatitis B evaluation was 15 months. At this last hospital visit, 57/95 (60%) had positive HBsAg and 31/95 (32.6%), negative HBsAg; data was missing for 7 patients. Out of those positive for HBsAg, 12 were also positive for HBeAg (21.1%), 27 (47.4%) were positive for anti-HBe, and no data on HBe markers was available in 18 cases. Of the total number of patients, 15/95 (15.8%) had a serology profile suggestive for history of HBV infection (negative HBsAg but positive anti-HBc), of which 5 (33%) also associated positive anti-HBs. An extra 3 patients had negative markers and positive anti-HBs, suggestive of previous immunization.

When evaluated after a median of 15 months, 59/95 (62.1%) had positive HBsAg and 36/95 (37.9%), negative HBsAg. Of the patients with positive HBsAg, 10 (16.9%) were also positive for HBeAg, 22 (37.3%) for anti-HBe, and data on HBe markers was missing for 27 patients. We identified positive anti-HBs in 19/95 patients (20%), compared to only 8 patients at their last hospital visit.

Conclusion: This cohort of patients infected early with HIV+HBV displayed a long evolution of liver disease, but viral clearance was recorded in 20% of them, even after years of chronic infection. More data is necessary to determine which were the factors that led to clearance of HBV.

Urethral Macrophages are key players in HIV-1 infection in the human male genital tract

Y. Ganor[1-3-4], R.A. Zenak[1-4-3], Z. Zhou[1-3-4], S. Marion[1-3-4], M. Revol[2], M. Bomsel[1-3-4]

[1]Mucosal entry of HIV and mucosal immunity, CNRS UMR 8104 [2]Plastic surgery, Hopital St Louis [3]Institut Cochin Mucosal HIV and Mucosal immunity, INSERM U1016 [4]Institut Cochin, Paris Descartes University, Paris, France

Background: The male foreskin is an efficient site of human immunodeficiency virus type-1 (HIV-1) entry in men, with circumcision protecting men from heterosexual acquisition of HIV-1 by 60%, leaving the 40% unprotected. Still, the mechanisms of HIV-1 transmission across the different penile epithelia and the occurrence of penile cellular reservoirs of the virus are completely unknown.

Aims and Methods: To describe the initial steps of HIV-1 entry into the different penile epithelia, we developed *ex-vivo* polarized explants using whole normal adult penile tissues removed during elective gender reassignment. In parallel, we evaluated HIV distribution and dynamic in similar tissues removed to HIV+ individuals.

Results: In penile explants, exposure to HIV-1 infected cells for 1hr results in efficient HIV-1 entry into the urethra, while the fossa navicularis and glans are resistant to the virus transmission. CCR5+/CD4+ macrophages present in the urethra, but not T-cells, are the initial cells infected by HIV-1. Urethral macrophage infection by HIV-1 correlates with the presence of CD68+/CD163+ macrophages in the urethral epithelium that are absent from glans and fossa epithelium. HIV-1+ infected cell inoculation induces exit of urethral macrophages from the epithelial compartment accompanied with decreased production of CCL2/MCP-1. Cell-free HIV-1 is inefficient at urethral penetration.

We next evaluated whether the urethra serves as a yet unrecognized anatomical reservoir for HIV-1 in direct link with the initial HIV-1 infection of urethral macrophages. Accordingly, HIV-1 could be detected by ISH in penile urethral tissues from HIV-1 patients under effective anti-viral therapy. These HIV-1+ urethral tissues contained mucosal macrophages and T-cells with an increase in density and a change in spatial distribution as compared to normal tissue. More, urethral macrophages purified from these HIV+ individuals produced virus upon contact with activated PBMCs. Altogether, these results suggest an active role for resident macrophages in HIV transmission and/or for infection/latency/ reservoir formation.

Conclusion: Our results identify the male urethra, and in particular resident urethral macrophages, as a novel and highly efficient entry site and potential cell reservoir for HIV-1 which plays a major role in AIDS resistance to anti-viral therapy.

Lack of correlation between measurements of bacterial translocation and immune activation in virologically suppressed HIV-1-infected patients

M. Abad-Fernandez, A. Vallejo, L. Diaz, A.M. Moreno, F. Dronda, J.L. Casado, E. Navas, M.J. Perez-Elias, C. Gutierrez, B. Hernandez-Novoa, N. Madrid, S. Moreno

Department of Infectious Diseases, Hospital Ramon y Cajal, Madrid, Spain

Background: Although previous reports have shown a correlation between bacterial translocation, as measured by plasma LPS or sCD14 levels, and activated $CD4^+$ T and $CD8^+$ T cells, other authors have failed to show this association. Our objective is to analyze the correlation between different measurements of microbial translocation with CD4+ and CD8+ T cell immune activation in the context of two clinical trials.

Methods: Bacterial translocation and immune activation were measured in parallel in 126 plasma samples from 18 HIV-1 infected adults during ART intensification trials with maraviroc and raltegravir. Measurements were done at baseline, weeks 12, 24, 36 and 48 of intensification, and weeks 12 and 24 after discontinuation of the intensifying drug. Bacterial translocation was measured by LPS, sCD14, LBP and 16S rDNA levels. Activated T cells were defined by the expression of $CD38^+$ and $HLA\text{-}DR^+$. Spearman´s rank test was used to determine correlations between immune activation and bacterial translocation.

Results: No association was found between measurements of $CD4^+$ T cell activation and any measurements of bacterial translocation, including LPS (p=0.068), LBP (p=0.728), sCD14 (p=0.619) or r16S DNA levels (p=0.877). No correlation was found neither between $CD8^+$ T cell activation and measurements determining bacterial translocation [LPS (p=0.295), LBP (p=0.081), sCD14 (p=0.328) or 16S rDNA levels (p=0.152)] in this group of patients.

Conclusions: In patients with suppressed viremia, different measurements of bacterial translocation (LPS, LBP, sCD14 or 16S rDNA levels) do not correlate with the frequency of activated $CD4^+$ or $CD8^+$ T cells in peripheral blood. In this population, the persistence of immune activation may be driven by causes different to bacterial translocation.

Impact of the discontinuation of maraviroc and raltegravir intensification in suppressed HIV-1-infected patients on microbial translocation, immune activation, and dynamics of T cell subpopulations.

M. Abad-Fernandez, A. Vallejo, L. Diaz, C. Gutierrez, B. Hernandez-Novoa, N. Madrid, A. Moreno, J.L. Casado, F. Dronda, S. Moreno

Department of Infectious Diseases, Hospital Ramon y Cajal, Madrid, Spain

Background: Unlike raltegravir, maraviroc intensification showed alterations in microbial translocation and expression of gut-homing β7 receptor on T-cell subsets. The effect of discontinuation of intensification treatment on dynamics of microbial translocation, immune activation, expression of gut-homing β7 receptor and T-cell subsets in patients with high $CD4^+$ T-cell counts and undetectable plasma viral load are still poorly defined.

Materials and Methods: Eighteen long-term suppressed HIV-1-infected patients with $CD4^+$ T-cell count above 350 cells/mm^3 and undetectable plasma viral load, were analyzed. Nine patients intensified their antiretroviral treatment with maraviroc and the other nine patients with raltegravir. Samples were analyzed for both groups at baseline, at week 48 of intensification treatment and at weeks 12 and 24 after discontinuation of the intensification treatment.

Results: Plasma levels of LPS correlated significantly with sCD14 and LBP levels. Decreasing immune activation during treatment intensification continued after intensification discontinuation with levels significantly lower than baseline. Among maraviroc group, the significant increase of microbial translocation in parallel with the expression of gut-homing β7 receptor on activated $CD8^+$ T-cells, persisted after maraviroc removal with sCD14 levels and activated $\beta7^+$ T-cells significantly higher compared to baseline. Besides, levels of LPS and sCD14 correlated with CD8-TEM at week d24 during maraviroc removal. By contrast, only LPS levels increased significantly after raltegravir removal in parallel with the expression of gut-homing β7 receptor on activated $CD4^+$ T-cells. Among raltegravir group, significant correlations were also found between activated $CD4^+$ T-cells and activated $CD4^+\beta7^+$ T-cells during follow-up. But among maraviroc group, this correlation was only found at baseline and 24 weeks after maraviroc removal.

Conclusions: The reduced levels of immune activation achieved during the treatment intensification were maintained even after intensification discontinuation,regardless of the intensification drug. We are unable to ensure that levels of microbial translocation could trigger immune activation as no correlation was observed in our immunocompetent patients. However, we considered microbial translocation as an important factor of gut inflammation and immune activation as evidenced by the correlation between $CD4^+$ T-cell activation and activated $CD4^+\beta7^+$ T-cells, and the association between the dynamics of microbial translocation and activated T cells expressing the gut-homing receptor.

HIV disease progression despite viral suppression is a consequence of the exhaustion of lymphopoiesis driven by immune activation

D. Sauce[2], M. Larsen[2], S. Fastenackels[2], P. Hunt[3], C. Katlama[1], V. Appay[2]

[1]Hôpital Pitié-Salpêtrière [2]U945 Infection and Immunity, INSERM, Paris, France [3]University of California San Francisco, San Francisco, United States

Objective: Progressive decline in CD4+ T cell counts is the hallmark of HIV disease progression, however its potential link to elevated systemic immune activation is not fully understood. In particular, low CD4+ T cells despite successful viral suppression (i.e. in patients who fail to reconstitute their CD4+ T cell pool despite effective antiretroviral therapy, or in elite controllers who eventually progress towards disease despite natural control of HIV replication below detectable thresholds) remain unexplained. Here, we aim at providing new insights into the reasons for the CD4+ T cell decline and investigating the consequences of immune activation in HIV infection.

Methods: We have performed a comprehensive study of attributes related to immune activation, homeostasis and lymphopoiesis in more than 200 HIV-1 infected individuals, including patients presenting immunological failure (i.e. no CD4+ T cell recovery despite potent antiretroviral therapy) and rare elite HIV controllers with evidence of CD4+ T cell decline.

Results: Our analyses underline that progressive failure to maintain adequate CD4+ T cell count during HIV infection is associated with a general exhaustion of lymphopoiesis (i.e. observed down to the level of hematopoietic CD34+ progenitors). This alteration of lymphopoiesis is directly correlated with systemic immune activation rather than direct HIV infection or replication in untreated HIV-1 infected donors, and it can be partially reversed by antiretroviral therapy. However, successful antiretroviral therapy is ineffective at reconstituting the CD4+ T cell pool in patients with profound and persistent damage to the lymphopoietic system. Moreover, exhausted lymphopoiesis emerges as an explanation for disease progression in Elite Controllers.

Conclusion: These findings provide new insights into the consequences of persistent immune activation in HIV-1 infection, and demonstrate the importance of primary hematopoietic resources in HIV pathogenesis and the response to antiretroviral treatments. Importantly, it shows that HIV disease progression despite elite control of HIV replication or virological success on antiretroviral treatment is associated with persistent damage to the lymphopoietic system.

HIV induce a pro-inflammatory/neurotoxic response in primary cultures of nervous cells, even without infection

E. Escobar-Guevara[1-2], M. Alfonzo-Díaz[2]

[1]Centro de Medicina Experimental, Instituto Venezolano de Investigaciones Científicas-IVIC [2]Laboratorio de Inmunofisiología Celular, Escuela de Medicina "JM Vargas", Universidad Central de Venezuela, Caracas, Venezuela

Objective: HIV-1 can invade central nervous system (*CNS*) and affect its function. To study CNS response and how it could mediate pro-inflammatory/neurotoxic effects, we evaluate production of cytokines and nitric oxide (*NO*) and determined nervous cell counts/viability in primary cultures of nervous cells in the presence of HIV or HIV-envelope glycoprotein-gp41.

Methods: We used primary cultures of *Sprague-Dawley* rat-fetal brain cells, cultured in 24 wells plaques ($2.5x10^5$ cells/well), in RPMI-10%FCS (**Group A-*Control***), or in the presence of recombinant gp41, 10nM (**Group B-*gp41***), or $1.9x10^9$ copies/ml of HIV-1_{IIIB} (**Group C-*HIV***), for 3, 5 and 7 days (37°C, 5%CO_2). Culture supernatants were collected to determine IL-1α, IL-6, IL-10, TNFα (flow cytometry, *CBA, BD*) and *NO* levels (*Griess*); nervous cells were counted after trypsin treatment (*Trypan Blue/Neubauer*). Groups were compared using ANOVA or Kruskal-Wallis tests, as appropriate, with Bonferroni's or Dunn's multiple comparison tests, respectively, considering as significantly $p<0.05$.

Results: When nervous cells were cultured in the presence of HIV-1 or gp41, significantly higher levels of IL-1α, IL-6, IL-10, TNFα and *NO* were observed (**Groups B** vs. **A; C** vs. **A**); behaving gp41 as a more potent inducer of IL-6- and TNFα-production, whereas HIV of IL-1α- and IL-10-production (**Group B** vs.**C**). Moreover, the presences of HIV-1 or gp41 in cultures were associated with significantly lower nervous cell counts (**Groups B** vs. **A; C** vs. **A**).

Conclusion: In our experiments, the presence of HIV or gp41 was associated with a more pro-inflammatory environment (high *NO*, IL-1α, IL-6 and TNFα production), with a relative lower immunomodulatory power for gp41-stimulation (lower IL-10 production); and more nervous cell destruction, even with no productively infection of such rat nervous cells, suggesting that virus/viral glicoprotein-nervous cell interactions could induce responses capable of affect nervous cell survival/function, as could be in infected patients, and showing how bystander/non-infected cells could respond and be affected in HIV-infection.

High detected occult HCV infection in HIV-Positive patients with HCV Seronegative in Indonesia

S. Juniastuti[3], T. Utsumi[3-5], N. Nasronudin[3-4], L. Alimsardjono[2], M. Amin[3], M. Adianti[3], Y. Yano[5], S. Soetjipto[3-1], Y. Hayashi[5], H. Hotta[5], M. Lusida[3-2]

[1]Department of Biochemistry, School of Medicine [2]Department of Microbiology, School of Medicine [3]Institute of Tropical Disease, Airlangga University [4]Department of Internal Medicine, Dr. Soetomo General Hospital, Surabaya, Indonesia [5]Center for Infectious Diseases, Kobe University, Kobe, Japan

Objective: This study aimed to identify and describe cases of newly acquired Hepatitis C Virus (HCV) infection in HIV-positive patients.

Methods: The repositoried plasma specimens were obtained from HIV-positive patients who visited Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia for HIV viral load examination. Those patients mostly did not have any symptoms of hepatitis. The plasma were examined for : anti-HCV (by HCV EIA 3.0), HCV RNA (by RT-PCR using primers targeting a part of NS5B region/5'UTR), and HIV viral load (by rt-PCR). HCV genotype/subtype was determined by analysis of sequence of the PCR product. All data was analyzed descriptively.

Results: A total of 187 plasma samples from 153 males and 34 females were examined. One hundred and nineteen (63.6%) patients were anti-HCV positive, and of them, HCV RNA was detected in 73 (61.3%) with HCV-1a as the predominant subtype (31.5%). Of the 68 samples with anti-HCV negative, HCV RNA was detected in 26 (38.2%) and interestingly, HCV-3a was most prevalent (50%). High HCV viral load was more common in group of HCV-seropositive patients (45.2%) than group of HCV-seronegative patients (3.9%). Group of HCV-seropositive patients was more likely (73.1%) to have parenteral HCV transmission (IVDUs) than group of HCV-seronegative subjects, which was mostly with history of heterosexual transmission (54.4%). Although sexual HCV transmission is relatively inefficient, the presence of HIV could facilitate it much easier.

Conclusion: HCV infection appears to occur more frequently among HIV-positive patients with HCV seroppositive and also those with HCV seronegative (occult HCV infection) more than appreciated. The delay formation of anti-HCV antibodies in immunodeficiency in HIV/HCV coinfected patients may result in delay in diagnosis and treatment of HCV. It might also consider that the differences in HCV seropositivity and subtypes might exist between HIV-positive patients acquired HCV sexually and those acquired HCV parenterally.

HIV-1 Nef alters podosomes and promotes the mesenchymal migration in human macrophages

C. Vérollet

Maridonneau-Parini Team, IPBS-CNRS, Toulouse Cedex 4, France

Macrophages are a cell target of Human Immunodeficiency Virus-1 (HIV-1). They play a key role in AIDS pathogenesis as long-term viral reservoirs. As macrophages are able to migrate in all body tissues, they are also susceptible to participate in systemic dissemination of viruses. Here, we examined the migration ability of HIV-1-infected primary human macrophages. We show that HIV-1 infection modifies dramatically the migration of macrophages in 3-dimentionnal (3D) environements. While the amoeboid migration mode is inhibited upon infection, another migration mode specifically used by macrophages in dense 3D environments, the mesenchymal migration mode, is largely enhanced. HIV-1 negative factor (Nef) is responsible for both effects on macrophage migration modes. Consistently with an increase in mesenchymal migration capacities, Nef accumulates around F-actin structures necessary for proteolysis of the extracellular matrix, e.g. podosomes and alters their structure, function and dynamics.

Mechanistically, HIV-1-induced podosome modifications and mesenchymal macrophage migration depend on Nef's ability to activate the macrophage-specific Src tyrosine kinase, Hck.

We conclude that HIV-1, by the action of Nef, is able to force macrophages to infiltrate preferentially some tissus. Thus, interfering with Nef/Hck interaction emerge as an unexpected strategy to reduce the spread of the virus by macrophages, for example in the brain of patients where the presence of infected macrophages is associated with neurotoxicity and AIDS-associated dementia.

CCL2 blocking strategies as a novel approach to control HIV-1 replication in macrophages

M. Sabbatucci[1], C. Purificato[1], S. Gessani[1], A. Guglielmotti[2], L. Fantuzzi[1]

[1]Istituto Superiore di Sanità, Rome [2]Angelini Research Center, S. Palomba-Pomezia, Rome, Italy

Objective: Bindarit is an indazolic derivative acting through an innovative mechanism involving CCL2 synthesis inhibition. Growing evidence suggests that this chemokine plays important roles in AIDS pathogenesis. We found that CCL2 levels increase in monocyte-derived macrophages (MDM) exposed to HIV-1 gp120 and during the course of HIV-1 infection, and that this chemokine promotes viral replication in these cells. We thus foresee to assess the effect of bindarit on HIV-1 replication in macrophages.

Methods: MDM were obtained from peripheral blood CD14+ monocytes, isolated from healthy donors, upon 7 days of in vitro culture. The effect of bindarit on CCL2 secretion was tested in MDM exposed to CCL2-inducing stimuli or infected with HIV-1BaL. The effect of bindarit on HIV-1 replication was evaluated by measuring cell-associated and released p24 Gag levels by FACS analysis and enzyme-linked immunosorbent assay (ELISA), respectively, and HIV-1 protein expression by western blot analysis, and was compared to the effect of CCL2 blocking by neutralizing antibodies (Abs).

Results: Our results show that bindarit inhibits, in a dose-dependent manner, LPS-induced CCL2 production in MDM, without affecting cell viability. Furthermore, we found that bindarit does not modulate gp120-triggered CCL2 release, whereas it reduces virus-induced CCL2 production. The inhibitory effect on CCL2 expression is specific for this chemokine, since bindarit does not modify IL-8 and IL-6 production. We thus compared the effect of bindarit versus that of CCL2 neutralizing Abs on HIV-1 replication, showing that both treatment modalities result in inhibition of p24 Gag release. However, whereas CCL2 neutralizing Abs strongly reduces the percentage of p24 Gag positive cells, bindarit does not. Furthermore, bindarit does not affect HIV-1 protein expression. Statistical analysis was performed using the paired Student's t test. Values of $p < 0.05$ were considered significant.

Conclusions: Our results demonstrate that CCL2 blocking strategies represent a reliable approach to control HIV-1 replication in primary macrophages. Dissecting the cellular and molecular mechanisms underlying HIV-1 replication modulation by different modalities of CCL2 blocking could potentially contribute to the identification of new targets for therapeutic interventions.

HIV-1 Elite Controllers display a low CCR5 surface expression on CD4 T-cells

L. Brandt[2], T. Benfield[3], D. Yu[3], H. Mens[1], A. Fomsgaard[2], I. Karlsson[2]

[1]Copenhagen University Hospital [2]Statens Serum Institut, Copenhagen [3]Copenhagen University Hospital, Hvidovre, Denmark

Objective: A subset of HIV-1 infected individuals suppresses and controls HIV-1 without therapy, so called elite controllers. No single mechanism behind elite control is known. Here we tested if the expression of the HIV-1 co-receptor, C-C chemokine receptor type 5 (CCR5), or it's ligands, MIP-1α, MIP-1β and RANTES, is different among elite controllers compared with other HIV-1 infected individuals.

Methods: The CCR5 expression on CD4 T-cells was quantified using flow cytometry and the level of MIP-1α, MIP-1β and RANTES using ELISA. Three groups of in HIV-1 infected individuals: viremic individuals, individuals on antiretroviral therapy and elite controllers, as well as uninfected individuals, were compared. For accurate analysis *ex vivo* whole blood was used.

Results: We found that elite controllers display a significantly lower number of CCR5 molecules on the surface of CD4 T-cells compared with HIV-1 infected progressors and uninfected. In addition, the expression of CCR5 per cell was significantly lower in elite controllers compared with HIV-1 infected progressors and uninfected in both the CD4 T_{CM} and CD4 T_{EM} compartment. We found no differences in the concentration of the CCR5 ligands MIP-1α, MIP-1β and RANTES in unstimulated whole blood cell cultures between elite controllers and HIV-1 infected progressors.

Conclusion: Comparable levels of the CCR5 ligands in elite controllers and HIV-1 progressors and a low number of CCR5 surface molecules on CD4 T-cells and low per cell CCR5 expression in T_{CM} and T_{EM} may contribute to natural control of HIV-1 replication in elite controllers.

HIV-1 and its RNA modulate TLR expression and function in human neutrophils

D. Giraldo, J.C. Hernández, S. Urcuqui Inchima

Immunovirology, Universidad de Antioquia, Medellín, Colombia

The polymorphonuclear neutrophils are a key cellular population in the innate immune response: they are the first to arrive on the infection site, are involved in the early response to infections and amplify the proinflammatory response. Yet, little is known about the response of neutrophils to viral infection, and even less to HIV-1 infection.

Our **objective** was to evaluate the effect of HIV-1 particles and RNA on stimulation of polymorphonuclear neutrophils, on the expression of innate immune receptors such as Toll-like receptors (TLRs)2/4/7/8, and their function, measured by CD62L/CD11b expression, proinflammatory cytokine secretion and production of reactive oxygen species (ROS).

Methods: neutrophils were purified from peripheral blood of 5 healthy donors and challenged with HIV-1 or viral RNA with or without TLR2/4/7/8 agonists. After 3 hours, mRNA expression of these TLRs was quantified by real-time PCR, and after 8 hours, the protein levels of TLRs, CD62L/CD11b and ROS production, were evaluated by flow cytometry; proinflammatory cytokine secretion, such as IL-6 and TNF-α was evaluated by ELISA.

Results: An up- or down-regulation of TLRs was observed in neutrophils challenged with HIV-1 or viral RNA. HIV-1 and its viral RNA increase the production of IL-6 and TNF-α. HIV-1 decreases the CD62L production and ROS secretion. When neutrophils where stimulated with HIV-1 or its viral RNA in the presence of Pam_2csk4, LPS and R-848, agonists of TLR2/2, TLR4 and TLR7/8, respectively, we observed a change in the pattern of TLR expression compared with the effect of HIV-1 or viral RNA without TLR agonists. HIV-1 in the presence of LPS or R-848, decrease the production of IL-6 and TNF-α. Finally, HIV-1 or the viral RNA in the presence of Pam_2csk4, or HIV-1 in the presence of LPS, increases the production of ROS. Interestingly, a cross-talk was detected between TLRs that was also altered by HIV-1 particle or viral RNA stimulation.

Conclusion: HIV particle or its viral RNA can functionally activate neutrophils, altering TLR2/4/7/8 expression and function. We suggest that polymorphonuclear neutrophils may play a prominent role in HIV infection/pathogenesis.

Mucosal inflammation in African women; implications for HIV microbicide development

J. Kyongo[1], T. Crucitti[3], J. Menten[2], L. Hardy[4], S. Delany-Moretlwe[9], M. Mwaura[7], G. Ndayisaba[8], J. Van De Wijgert[10], G. Vanham[1-5-6], A. Buve[4], K. Arien[1], V. Jespers[4]

[1]Department of Biomedical Sciences, Microbiology Group, Virology Unit [2]Department of Clinical Sciences, Clinical Trials Unit [3]Department of Clinical Sciences, HIV/STI Reference Laboratory [4]Department of Public Health, ITM HIV/AIDS Centre, Institute of Tropical Medicine [5]Faculty of Pharmaceutical, Veterinary and Biomedical Sciences, University of Antwerp, Antwerp [6]Faculty of Medicine and Pharmacology, University of Brussels, Brussels, Belgium [7]International Center for Reproductive Health, Mombasa, Kenya [8]Project Ubuzima, Kigali, Rwanda [9]WITS Reproductive Health and HIV Institute, Johannesburg, South Africa [10]Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom

Background: Recent data suggests that Sub-Saharan Africa continues to bear a disproportionate burden of both incidence (69%) and prevalence (68%) of HIV infection worldwide. An effective microbicide would be a desirable female-initiated HIV prevention tool but its development and safety requires a better understanding of the female genital tract (FGT) and the factors that influence mucosal immunity.

Methods: Cervicovaginal lavage (CVL) samples were collected on six time points over a period of 8 months from 430 African women with different epidemiological profiles in Kigali, Rwanda; Mombasa, Kenya and Johannesburg, South Africa. These samples were then assayed for the presence of ten cytokines, chemokines and growth factors using a multiplex immunoassay. Clinical data was also collected and quantification of bacterial species found in the vaginal vault of these women was done using qPCR. Statistical analyses were done using STATA software.

Results: Differential expression of the soluble pro-inflammatory markers IL-1β, IL-1α, IL-6, IL-8, IL-12, MIP1β, GM-CSF and G-CSF was observed between the different groups defined in the study protocol; healthy adults, adolescents, women engaging in traditional vaginal practices, HIV-positive adults, HIV-negative female sex workers and HIV-negative pregnant women. Women with bacterial vaginosis (BV) had elevated IL-1β, IL-1α, IL-6, IL-8 and IL-12 but decreased IP-10 concentrations compared to women with normal flora. The presence of cervical ectopy was associated with increased IL-1β, IL-6, IL-8, G-CSF and MIP-1β. Clinical observations of infection in the female genital tract i.e. abnormal vaginal discharge and increased mucous thickness were also associated with increased pro-inflammatory cytokine concentrations. Unsurprisingly, commensal lactobacilli species *L.crispatus* and *L. vaginalis* were associated with lower levels of pro-inflammatory cytokines while BV-associated *A. vaginae*, *G. vaginalis* and *P. Bivia* but also *E. coli* presence clearly skewed the pro-inflammatory cytokine balance upwards.

Conclusions: We characterized the expression of soluble markers of inflammation in the FGT of this diverse African population and identified women with raised levels of immune activation as defined by their cervicovaginal concentrations of pro-inflammatory soluble markers. Potential risk factors of inflammation and consequently increased HIV infection susceptibility were defined and should be considered when assessing microbicide safety during clinical trials.

TRIM22 and HDAC4. Novel controllers of HIV-1 latency and replication

E. Vicenzi[3], M.V. Schiaffino[2], I. Palmisano[2], C. Van Lint[1], G. Poli[3]

[1]Laboratoire de Virologie Moleculaire, Universite Libre de Bruxelles (ULB) Institut de Biologie et de Medecine Moleculaires (IBMM), Bruxelles, Belgium [2]AIDS Immunopathogenesis Unit, San Raffaele Scientific Institute, Milan [3]Immunology, AIDS Immunopathogenesis Unit, San Raffaele University, Milano, Italy

Objective: To identify novel pathways and determinants controlling HIV-1 latency and replication in CD4+ T cells and/or monocyte-macrophages.

Methods: Uninfected U937 cell clones permissive (Plus) or relatively non-permissive (Minus) to HIV-1 replication, chronically infected promonocytic U1 and T lymphocytic ACH-2 cell lines were exploited to search novel factors regulating proviral latency or virus replication. Current methodologies were used to characterize the state of virus expression in these cells.

Results: TRIM22 was selectively expressed by Minus, but not by Plus U937 cell clones and its shRNA-mediated knocked-down (KD) resulted in increased levels of virus replication. TRIM22 inhibited basal and PMA+ionomycin induced proviral transcription, but was ineffective on Tat and TNF-alpha dependent stimulation of RNA synthesis. From the observation that essential aminoacid starvation resulted in increased expression of integrated transgenes, we identified HDAC4 as responsible epigenetic factor of the observed effect. HDAC inhibition by either pharmacological agents or shRNA resulted in the reactivation of proviral transcription and virus production in HDAC4+ ACH-2, but not in HDAC4-negative U1 cell lines.

Conclusion: Cell lines either acutely or chronically infected with HIV-1 are useful tools to identify novel restriction factors or modulators of proviral latency acting at both genetic and epigenetic levels.

HIV-1 Nef recruits TRAF2, TRAF5 and TRAF6, and stimulates HIV-1 replication in macrophages

W. Abbas[2], K.A. Khan[2], A. Varin[2], A. Kumar[2], V. Di Martino[1], I. Dichamps[2], G. Herbein[2]

[1]Department of Hepatology [2]Department of Virology, University of Franche-Comte, CHU, Besançon, France

Objective: TNF receptor associated factor (TRAF) signaling plays a central role in many biological activities, such as regulation of immune and inflammatory responses and control of apoptosis that are key events in the pathogenesis of human immunodeficiency virus 1 (HIV-1). The Nef protein of HIV-1 is an important factor in AIDS pathogenesis. Nef triggers activation of the T-cell receptor (TCR) cascade to facilitate virus spread and modulates cellular signal transduction pathways. Since HIV Nef and several of the TRAF proteins activate NF-kB, we investigated their respective role in HIV-1 replication in the macrophage, a cell type infected by HIV-1.

Methods: The studies were performed with the promonocytic cells U937, chronically infected U1 cells and primary monocyte-derived macrophages (MDMs). For protein interactions, we performed GST-pull down assay and immunoprecipitation. To measure NF-κB activation, *electrophoretic mobility shift assay* (EMSA) was carried out. Reporter gene expression was performed to examine NF -kB LTR-driven gene expression. MDM cultures were transfected with duplexes siRNA (Dharmacon RNAi technologies) using lipofectamine RNAiMAX (Invitrogen). P24 assay was done to measure HIV-1 replication. SPSS was used for statistical analysis.

Results: We found that the C-terminal region (55-206aa) of HIV-1 Nef binds to TRAF-2, TRAF-5 and TRAF-6. In addition to the acidic cluster domain of HIV-1 Nef involved in binding to TRAF2 (AxEE), a minor TRAF-binding consensus sequence (PxxQxxT) which could bind TRAF5, and a TRAF-6-binding consensus sequence (PxExxD/E/F/W/Y) are present in the C-terminal region of HIV-1 Nef. We observed the upregulation of TRAF-2 and TRAF-6 in MDM treated with rNef ($p < 0.001$). Further, by using siRNA, we determined that the enhanced NF-kB activation and HIV-1 replication in MDMs treated with rNef is mediated through TRAF2, TRAF5 and TRAF6 ($p < 0.05$).

Conclusion: We observed that HIV-1 Nef binds and recruits TRAF2, TRAF5, and TRAF6, activates NF-kB and enhances HIV-1 replication in monocyte-derived macrophages (MDMs). Our results reveal a mechanism by which activation of the TRAF pathway by HIV-1 Nef favors the replication of HIV-1 in MDMs.

Defect in B cell maturation and BAFFR expression in HIV-infected patients with isolated anti-hepatitis B core (a-HBc) antibodies

D. Nazzal[5], A. Felin[2], O. Godon[5], V. Mellon[6], M.N. Ungeheuer[6], C. Binquet[1-3], L. Piroth[2-4], M.L. Michel[5]

[1]CIC Epidemiologie clinique [2]Département d'infectiologie, CHU de Dijon [3]CIE1, INSERM [4]Université de Bourgogne, Dijon [5]INSERM U845, Laboratory PVHB [6]Plate-forme ICAReB, Institut Pasteur, Paris, France

Objective: Isolated antibody to hepatitis B core antigen profile occurs in about 30% of HIV-infected patients. Our aim is to examine the immune characteristics associated with this profile in HIV-infected patients.

Methods: Blood samples from 42 HAART-treated HIV-infected patients with isolated anti-HBc antibody pattern (no HBsAg, no anti-HBs) and with CD4 number>200/mm^3 were collected from 7 medical centers in France. Specific T-cell immune responses were studied in PBMC by Elispot and proliferation assays. Flow cytometry was used to assess B cell phenotype and maturation before and after in vitro non-specific stimulation. B cell Elispot was used to evaluate total or HBV-specific immunoglobulin (Ig) production. Blood samples from hepatitis B-vaccinated or unvaccinated healthy donors (HD) were used as controls.

Results: We showed that only 7.1% and 14.2% of patients had proliferative responses to HBs and HBc antigens, respectively. In *ex-vivo* IFN-g-Elispot assay, 30.2% and 16.2% had positive responses to HBV envelope and capsid peptides respectively, whereas 55.8% had T cell response to HIV Gag. Despite a similar number of total Ig-producing cells, only B cells from vaccinated-HD produced anti-HBs Ig. B cells producing anti-HBc Ig were detected in HIV-patients. By flow cytometry analysis, we showed that:

- After stimulation, the % of CD19+ B cells did not increase in PBMC from HIV patients compared to HD.
- The percentage of CD38+C27+CD20+ memory B cells before and after stimulation was significantly lower in HIV patients compared to HD.
- The percentage of CD38+CD27+CD20- Plasma cells /Plasmablasts was significantly lower upon stimulation in HIV patients in comparison with HD.
- The percentage and expression of B cell-activating factor receptor (BAFFR) decreased after stimulation in memory B cells from HD only. In contrast to HD, expression of BAFFR in HIV patient's plasma cells (CD138+) was not down regulated.

Conclusions: Our results suggested that HIV patients with isolated anti-HBc had modest HBV-specific T cells and a strongly altered B cell maturation affecting memory B and plasmablasts/plasma cells. These effects seemed related to sustained BAFFR expression. This could be implicated in the poor response to hepatitis B vaccine observed in HIV patients.

HIV-1 infection impairs phagosome maturation and macrophage activation

A. Dumas[1], J. Mazzolini[2], F. Herit[1], D. Russell[3], S. Benichou[1], F. Niedergang[1]

[1]INSERM (U1016), CNRS (UMR 8104) et Université Paris Descartes (UMR-S1016), Cochin Institut, Paris, France [2]University of Birmingham, Birmingham, United Kingdom [3]Cornell University, Ithaca, United States

Background: The Human Immunodeficiency Virus (HIV)-1 infects macrophages, but the impact of the viral infection in these cells is still poorly characterized compared to T cells. Because of their susceptibility to infection leading to a productive viral replication and because of their resistance to cytopathic effects, macrophages can be involved in the establishment of persistant viral reservoirs. In addition, HIV-infected macrophages exhibit defective functions of activation and cytokine production that contribute to the development of bacterial and fungal opportunistic diseases. We have recently shown that phagocytosis, the internalization of large particles, bacteria and fungi, is impaired in HIV-infected primary human macrophages. This inhibition was due to a Nef-dependant perturbation of the membrane remodeling that is required for a successful phagocytosis. We aim now at analysing whether and how the later steps of phagocytosis, ie phagosome maturation and macrophage activation, are impaired during HIV infection.

Materials and Methods: We use primary human macrophages infected or not with R5-tropic HIV-1 strains.

Results: We set out to study late stages of phagosome maturation by analysing the recruitment of endocytic markers and the intracellular migration of phagosomes as a reflect of their maturation as phagolysosomes. We have measured the intracellular survival of non-invasive bacteria (*E.coli*) as well as invasive intracellular bacteria (*Salmonella typhimurium*). Using fluorescent probes, we detected the production of reactive oxygen species in HIV-infected macrophages. Finally, we have analyzed the signaling pathways leading to macrophages effector functions and the secretion of cytokines.

Conclusion: Our data reveal how macrophages intracellular trafficking and functions are impaired upon HIV-1 infection and should help us to better understand the physiopathology of the infection by HIV-1.

HIV-Specific ADCC decreases NK cell NKp46 surface expression: A role for ADCC in inducing chronic NK cell dysfunction

M. Parsons, C. Tang, S. Jegaskanda, R. Center, A. Brooks, I. Stratov, S. Kent

Microbiology and Immunology, University of Melbourne, Parkville, Australia

Objective: There is interest in the role of NK cell-mediated ADCC in preventing/controlling HIV infection. However, HIV infection induces dysfunction in NK cells. Infected individuals have decreased expression of activating receptors, such as CD16 and natural cytotoxicity receptors (e.g. NKp46). Decreased expression of CD16 on NK cells presumably follows activation through that receptor. Decreased expression of NKp46 in HIV infection is paradoxical, as there is no increase in NKp46 ligands in HIV-infected cells, and thus no triggering of NKp46. As NKp46 shares signaling mechanisms with CD16, we hypothesized that activation of NK cells through CD16 could simultaneously decrease NKp46 expression.

Methods: Whole bloods from healthy controls (n=7) and treatment-naïve HIV-infected individuals (n=6) were stained for *ex vivo* NKp46 expression and were incubated with gp140 and anti-HIV antibodies. NK cell activation was assessed with fluorochrome-conjugated antibodies against CD3, CD56, NKp46, and CD107a. To assure observed alterations in NKp46 expression were due to CD16 triggering, NK cells in whole blood were activated in separate assays with anti-CD16 antibody.

Results: The expression of NKp46 was decreased on NK cells from HIV-infected individuals compared to uninfected controls (mean 62%+/-8.2 Vs. 92%+/-2.5, $p<0.01$). The activation of NK cells, from uninfected individuals, for anti-HIV ADCC was independent of NKp46 ligation, as NKp46 blocking did not alter degranulation ($p=1.00$). Anti-HIV ADCC activation of healthy donor NK cells recapitulated the decreased expression of NKp46 observed in HIV+ donors (92%+/-2.5 Vs. 83.9%+/-4.3, $p<0.05$), suggesting activation through CD16 decreases NKp46 expression. Downregulation of NKp46 occurred preferentially in activated cells, with ~48% of the $CD107a^+$ cells exhibiting NKp46 null phenotypes, compared to ~12% of the CD107a- cells ($p<0.01$). Decreased expression of NKp46 upon activation of NK cells with anti-CD16 antibody confirmed this observation.

Conclusion: These observations suggest that chronic stimulation of NK cells in HIV infected individuals through CD16 may induce an exhausted/dysfunctional phenotype characterized by NKp46 downregulation. While ADCC antibodies may assist in vaccine-induced protection from infection, this study suggests the potential of *in vivo* ADCC responses to provide protection against HIV disease progression may be restricted by HIV-mediated NK cell dysfunction.

Intestinal Parasitic Infections Among HIV Positive Person with Antiretroviral Therapy in Klinik Teratai, Bandung, Indonesia

A. Wardani[4], I.F.D. Arya[2], N. Haeruni[1], R. Wisaksana[3]

[1]Clinical Pathology [2]Health Community and Family Medicine [3]Internal Medicine [4]Parasitology, Padjadjaran University, Bandung, Indonesia

Background: The prevalence of intestinal parasitic diseases is still high in tropical developing countries. Among the HIV positive patients, gastroenteritis caused by intestinal parasites may be complicated and still become major health problem. Use of antiretroviral therapy (ART) is expected to reduce prevalence of intestinal parasitic infection through improvement of the immune status of the patients.

Methods: 27 HIV positive patients on ART aged from 26 to 44 years of both sexed participated in the study along November 2012. Each study participant was provided with a fecal container and 10% formalin was used for preservation. Microscopic examination was performed to detect ova, larvae, and cysts. The modified Ziehl-Neelsen staining method was used to detect *Cryptosporidium* spp and *Cyclospora* spp.

Results: All of study participants were on antiretroviral therapy (ART) with different period of treatment. Only two (7.40%) participants came with diarrhea at the time of study. *Blastocystyc hominis* is the only parasite that found from 15 (55.56%) fecal samples. Significant association was observed between $CD4^+$ T-cell count and the infection. *Blastocystyc hominis* found more frequent at the higher CD4 (> 200 cells/µL) than in the lower CD4 (≤ 200 cells/µL), 66.67% (10/15) and 33.33% (5/15) respectively, with the lowest CD4 was 33 cells/µL and the highest was 877 cells/µL.

Conclusions: The finding showed that the prevalence of intestinal parasitic asymptomatic infection still high in patients under ART with high $CD4^+$ T-cells count. Comprehensive study must be proceed to determine factors that contribute to this condition.

Impact of HIV-1 infection on Salmonella typhimurium survival in primary human macrophages

C. Deschamps[1], A. Dumas[1], C. Ferhi[1], S. Benichou[1], M. Gordon[2], F. Niedergang[1]

[1]Phagocytosis and Bacterial Invasion Group, Institut Cochin, INSERM, Paris, France [2]Department of Gastroenterology, Henry Wellcome Laboratories, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom

Objective: Sever pathologies caused by nontyphoidal Salmonellae, hyper-invasive and resistant to antibiotics, have developed recently in immunocompromised patients, especially in sub-Saharan Africa. While Salmonella typhimurium is responsible for classic gastroenteritis, invasive nontyphoidal *Salmonella* (iNTS) infections are characterized by bacteremia and systemic diseases and are almost always associated with HIV-1 positive individuals.

The aim of this study is to understand how some nontyphoidal Salmonella strains can be more invasive and initiate downstream signaling pathways to manipulate immune responses and disseminate.

Method: We compare the fate of iNTS D23580 strain to S. typhimurium 4/74 strain associated with classic gastroenteritis, in non-infected or HIV-1 infected human primary macrophages.

Results: We showed that clearance of bacteria is impaired in HIV-1 infected primary human macrophages and that intracellular survival of S. typhimurium is enhanced. Moreover, the iNTS D23580 strain presents a more invasive phenotype than the S. typhimurium 4/74 strain. We are currently studying the variations of cytokine profiles and activation of signaling pathways such as MAPkinases in response to the different Salmonella strains in non-infected or HIV-1 infected macrophages.

Conclusion: Our data reveal how macrophage functions are impaired upon HIV-1 infection and should help us to understand how this is hijacked by opportunistic pathogens.

Early initiation of combined antiretroviral therapy (c-ART) protects HIV-1 infected individuals from the alteration of the Treg/Th17 profile in the gut

A. Kök[1], L. Hocqueloux[5], M. Carriere[1], A. Guguin[2], P. Tisserand[1], H. Hocini[1], Y. Lévy[1-4], S. Hüe[1-3]

[1]Hopital Henri Mondor, INSERM U955, Equipe 16 [2]Plateforme de Cytométrie en flux, IMRB, UFR de Médecine [3]Hôpital Henri Mondor, Service d'Immunologie Biologique [4]Hôpital Henri Mondor, Service d'immunologie clinique, Creteil [5]Service des Maladies Infectieuses et Tropicales CHR d'Orléans, Orléans, France

Gastrointestinal mucosa is an early target for HIV replication and $CD4^+$T-cell depletion. Gut depletion of specific T cell populations, such as $IL\text{-}17^+CD4^+$T-cells (Th17) and regulatory T-cells (Tregs), may significantly impact microbial translocation which is claims to be associated with systemic inflammation and a higher risk of mortality.

Here, we show a decrease of rectal Th17 cells in the gut of individuals naïve of c-ART (NT, n=6; 1.3±0.5%) or who have initiated c-ART during chronic phase of infection (CHI, n=14; 2.8±0.2%) as compared to HIV-negative individuals (HIV-, n=7; 4.0±1.4%) (P=0.01 and P=0.005 for both comparisons, respectively). IL-22 secreting T cells were significantly decreased in NT (5.0±1.8%) but were restored in CHI patients (9.5±0.9%) as compared to HIV- (10±1.8%). In contrast, patients who initiated ARV at early phase of HIV-1 infection (PHI, n=11) maintained a normal range of IL-17 and IL-22 secreting T cells (3.7±0.4% and 7.6±1.3% respectively; P=NS for comparison with HIV- controls). The frequency of Treg was the same in all groups of HIV+ patients and HIV-. Importantly, as compared to the Treg/Th17 balance in HIV- control (0.2±0.02), the Treg/Th17 ratio remains increased in CHI despite several years of control of HIV replication and in NT (0.4±0.05 and 0.9±0.3, P=0.01 for both comparisons with HIV-) while patients treated at the PHI maintained a normal ratio (0.3±0.02).

ARV initiation at PHI leads to a better Treg/Th17 restoration as compared to CHI despite a control of HIV replication. These results provide a strong rationale for ARV initiation at the PHI.

NK immunity in HIV patients with tuberculosis: implications for immune reconstitution syndrome during TB-treatment and HAART

C.B. Giacoia-Gripp[1], J.H. Pilotto[1], T.P. Silva[1], V.C. Rolla[2], D. Scott-Algara[3], M.G. Morgado[1]

[1]Laboratory of AIDS and Molecular Immunology, Oswaldo Cruz Foundation - IOC, Niterói [2]Clinical Research Laboratory on Mycobacteriosis, Oswaldo Cruz Foundation - IPEC, Rio De Janeiro, Brazil [3]Unité de Régulation des Infections Rétrovirales, Institut Pasteur, Paris, France

Objective: To determine the implication of NK cells in the onset of inflammation linked to immune reconstitution syndrome (IRIS) in tuberculosis (TB) HIV infected patients on antiretroviral therapy (HAART) and the evolution of both infections in the context of the co-infection treatment.

Methods: Ten HIV/TB patients (3 IRIS cases), from a Brazilian Clinical Trial who started HAART four weeks after the beginning of TB treatment, were preliminary selected according to their cryopreserved cells availability and evaluated at HAART-starting time (D0), IRIS development (for 3 patients) and one time point following HAART (D30-90). We tested CD107a expression, INF-gamma/TNF-alpha production and repertoire of NK cells by flow cytometry. Although our data is still preliminary, statistical analyses were conduct among studied patients.

Results: No differences were observed for CD107a expression or cytokines production, among the D0, D30-90 and IRIS time. Of interest, a basal CD107a expression was detected for HIV/TB patients (previous and under HAART), without stimulation (culture controls), which increased against K562. At IRIS-moment, this difference in percentage of expression was not observed. Moreover, despite during HAART therapy, an expansion of CD158b+ expressing NK cells was detected among IRIS-patients, after the development of IRIS (Median: 21,8%; CI: 11,8-30,9), versus the non-IRIS group (Median: 4,6%; CI: 2,0-16,5).

Conclusions: These preliminary analyses gave us an initial scenario about the NK immunity among TB patients submitted to HAART after four weeks of TB treatment. The spontaneous NK cell degranulation observed in all patients at the inclusion, together with the expansion CD158b+ NK subset among IRIS-patients, might explain the low frequency of IRIS-cases observed in this study. However, more analyses will be done to confirm these observations.

Immunohistochemisty of granulocytic sarcoma in a patient with HIV

V. Jurisic[2], T. Terzic, N. Colovic, M. Colovic

[1]University of Belgrade, Belgrade [2]University of Kragujevac, Kragujevac, Serbia-Montenegro

Introduction: Human immunodeficiency virus (HIV)-infected patients have a greatly increased risk of developing certain malignant tumors such as Kaposi's sarcoma and non-Hodgkin's lymphoma, and a slightly increased risk of developing others such as acute myeloid leukemia (AML). Granulocytic sarcomas (chloromas) in HIV patients are rare extramedullary tumors consisting of immature myeloid cells. We presented immunohistochemistry of granulocytic sarcoma in the case of an HIV patient together with changes in lymphocyte characteristics, in peripheral blood flowing disease progression.

Material and methods: Biopsy specimen of the gingival myeloid sarcoma was analyzed by classical immunohistochemical staining using a panel of monoclonal antibodies: LCA, cytokeratin, vimentin, S-100, Melan A, HMB45, TdT, CD34, CD117, CD13, HLA-DR, CD68, myeloperoxidase, lysozyme, CD79a, CD20, CD3, CD4, CD5, CD7, CD8, CD15, CD23, CD43, CD45RO, CD56, CD57, granzyme B, CD138, ALK- 1, CD30, bcl-2, bcl-6, and Ki-67 (Dako, Carpinteria, CA, USA). The peripheral blood cell subsets were analyzed by direct coupled techniques (BD, Pharmingen, San Diego, CA, USA) on flow cytometry (Beckton Dickinson, USA), and the measured absolute CD4+ cell count. Results: The neoplastic cells showed reactivity to LCA, CD117 (fig. 2 c), CD13 (weaklypositive), HLA-DR, CD68 and lysozyme. The proliferative index determined with Ki-67+ was over 90%.Tumor cells were negative for TdT, CD34, MPO, epithelial markers, and all T, NK, and B cell markers. A diagnosis was made of a granulocytic sarcoma with an extramedullary leukemic infiltrate corresponding to AML-M5 or M4. In beginning of diagnosis CD4 count was 321 cells/ml without inversion of the Th/Ts index. Flow cytometry studies of the bone marrow aspirate did not detect any population of abnormal blast cells. Laboratory data at disease progreesion was: Hb 78 g/l, plt 48 × 109/l, WBC 6.1 × 109/l (differential formula: bands 4%, segmented neutrophils 80%, eosinophils 2%, lymphocytes 12%, monocytes 2%), and the WBC very soon dropped below 2.1 × 109/l. The number of CD19+/CD45+ was 42% (absolute lymphocyte number 290 cells/µl), CD3+ /CD45+ was 38% (262 cells/µl) and CD3++CD4+/CD45+ was 0.4% (3 cells/µl). Other peripheral blood cell subsets, commonly used for estimation of immune system function examined by Flow cytometry for CD3+/CD4+ were 0.4 % and for CD3+/CD8+ were 37%, showing evident inversion of Th/Ts index (0.01). The patient started with highly active antiretroviral therapy HAART (abacavir, lamivudin, lopinavir) but died from sepsis caused by Enterococcus cloache and disseminated herpes zoster infection.

Persistent T-cell activation can accelerate immunosenescence and contribute to disturbances of TCRVβ repertoire in visceral leishmaniasis-HIV-1 co-infected patients

J. Santos-Oliveira[2], C. Giacoia-Gripp[2], G. Cota[1], E. Marques-Paulo[2], L. Couto[2], A. Bertho[2], M. Morgado[2], A. Rabello[1], A. Da-Cruz[2]

[1]Fiocruz, CpRR, Belo Horizonte [2]Fiocruz, IOC, Rio De Janeiro, Brazil

Visceral leishmaniasis-HIV-1/AIDS patients (VL/HIV-1) have enhanced cellular activation levels, independently of clinical phase of VL, antiretroviral (ART) and anti-*leishmanial* (ALT) therapies. Indeed, we have shown that *Leishmania* infection and the plasmatic lipopolysaccharide (LPS), probably of luminal origin, were associated with the heightened cellular activation in VL/HIV-1.

Objective: Considering this, we investigated whether persistent cell activation observed in co-infected patients can accelerate the immunosenescence and disturb the T-cell repertoire.

Methods: Ten VL/HIV-1 were followed-up during active disease, at the end and at sixth month after ALT. Sixteen HIV-1/AIDS patients, 6 VL monoinfected cases and 10 healthy subjects (HS) were included. All HIV-1 cases were under ART therapy and those Leishmania-co-infected patients were submitted to secondary chemoprophylaxis to VL with amphothericin B. The immune status was assessed by $CD4^+$T-cell counts, cellular activation degree ($CD38^+HLA\text{-}DR^+$) and senescence ($CD57^+CD27^-$), as well as by the characterization of the TCRVβ repertoire.

Results: VL/HIV-1 presented low levels of $CD4^+$T-cells (<200cells/mm^3) throughout the study. Higher levels of $CD38^+/HLA\text{-}DR^+$ on $CD8^+$T cells were seen independently of VL clinical phase and even long-term after treatment when compared to VL cases alone (p<0.05). Low or undetectable RNA copies/mL were observed and had no influence on $CD38^+/HLA\text{-}DR^+$ or $CD4^+$T counts. Considering all the time points evaluated, VL/HIV-1 presented similar levels of senescent cells on $CD8^+$T (33%) compared to HIV-1/AIDS cases (38%), but higher than HS (12.6%,p<0.05). The degree of immunosenescence was higher on $CD8^+$T cells compared to $CD4^+$T lymphocytes. Consistent with the cellular activation state, a positive correlation between $CD38^+/HLA\text{-}DR^+$ and $CD57^+/CD27^+$ on $CD8^+$T cells was verified. The Vβ families more expressed by $CD4^+$T cells on VL/HIV-1 were the same used in VL and HIV-1 cases alone. However, all Vβ chains were used for less than 5% of $CD8^+$T cells from VL/HIV-1, suggesting a different and altered repertoire.

Conclusion: Our results suggest that the T-cell activation persistence, despite the control of viral load, ALT and even of the chemoprophylaxis, can contribute to the immunosenescence phenomenon and account for the TCRVβ repertoire disturbances. As consequence, $CD4^+$T depletion and probably $CD8^+$T function are worsened, impairing the immune status of co-infected patients. Support:MS/PNDST-Aids/CNPq/FAPERJ/IOC/FIOCRUZ.

Therapeutic Interventions to Modulate Microbial Translocation, Immune Activation and Cardiovascular Comorbidities. *Lessons from Non-Human Primate Models*

I. Pandrea[4], A. Landay[1], C. Wilson[3], R. Tracy[2], C. Apetrei[4]

[1]Rush University, Chicago, Il [2]University of Vermont, Colchester, Ve [3]University of Colorado, Denver, Co [4]Center for Vaccine Research, University of Pittsburgh, Pittsburgh, United States

Objective: Immune activation/inflammation (IA/INFL), rather than virus replication are the key determinants of HIV/SIV disease progression. Control of IA/INFL results in partial recovery of $CD4^+$ T cells in HIV-infected patients and SIV-infected nonhuman primates (NHPs), while control of viral load (VL) alone is not associated with mucosal immune restoration. Increased IA/INFL during chronic HIV/SIV infection is associated with microbial translocation (MT). The natural hosts maintain normal gut permeability throughout SIV infection and do not progress to AIDS when infected with SIV. Our goal was to use interventional strategies to (1) induce immune activation in natural hosts of SIV and (2) decreasing MT in progressive SIV-infection with the aim of identifying new therapeutic strategies for HIV-1 infected patients.

Methods: African green monkeys (infected and uninfected), which are a natural host of SIV were used for approaches aimed at inducing immune activation (through administration of alcohol, fat diet or LPS); SIV-infected pigtailed macaques (PTMs) were used in studies aimed at decreasing the levels of MT (administration of rifaximine/sulfasalazine and sevelamer). The levels of viral replication, T cell changes, immune activation (HLA-DR CD38 and Ki-67), MT (sCD14), coagulation (D-Dimer) and plasma cytokines iwere compared between treated and control, untreated nonhuman primates (NHPs).

Results: Our interventions successfully modulated MT and monocyte activation. Reduction of MT in SIV-infected PTMs resulted in decreased IA/INFL, lesser $CD4^+$ T cell loss and lower VLs; an opposite effect was observed after increasing MT in AGMs. These studies directly proved that MT is a critical factor in triggering IA/INFL. Modulation of the MT levels impacted coagulability in both progressive and nonprogressive NHP SIV models, confirming the relationship between MT, IA/INFL and CV comorbidities.

Conclusions: NHP models are instrumental for understanding the mechanism(s) of IA/INFL and are invaluable for testing new therapies targeting the different pathways of IA/INFL. Treatments targeting MT during acute SIV infection reduces both systemic T cell immune activation and the activation of coagulation. As prevention of HIV-related comorbidities such as cardiovascular disease requires control of immune activation during the chronic infection, additional assessment of the efficacy of MT targeting approaches is needed.

Different NK Cell Receptor-Ligand Expression in successfully treated HIV patients is associated with the occurrence of AIDS-defining Opportunistic Infections at presentation

F. Bisio[2], F. Bozzano[1], F. Marras[2], C. Viscoli[4-3], A. Di Biagio[3], L. Moretta[2], A. De Maria[4-1-3]

[1]Centre of Excellence for Biomedical Research [2]Giannina Gaslini Institute [3]IRCCS A.O. U. San Martino-IST, Genoa [4]University of Genoa, Genova, Italy

Objective: To compare innate immune response in patients with different capabilities of controlling HIV infection, not necessarily reflected by CD4+ T-cell counts alone.

Methods: We investigated by cytofluorometry after immune recovery (CD4+T-cells) the expression of NK cell receptors and ligands in 19 aviremic cART-treated HIV-infected patients who originally presented with CD4+<220/ml (11 AIDS, 8 non-AIDS). Opportunistic Infections (OI) were either Pneumocystosis (9) or Neurotoxoplasmosis (2). Uninfected healthy donors (n°10, HD) served as controls.

Results: Expression of NKp46 and NKp30 was significantly higher in non-AIDS vs. AIDS patients. Overall, the level of NKp46 expression directly correlated with the degree of NK cell cytotoxicity. No correlation was found between ultrasensitive plasma viral load and NK receptor expression. As compared to HD, in both groups, there was a similar increase of CD69 and HLA-DR expression in NK cells that directly correlated with the presence of activation markers (HLA-DR) on CD4+ and CD8+ T cells. As compared to AIDS, in non-AIDS patients in vitro activated CD4+ showed higher expression of MIC-A (NKG2D ligand), with significantly higher Nectin-2/DNAM-1 and MIC-A/NKG2D ratios.

Conclusions: NK cell responses in AIDS and non-AIDS patients with similar CD4+ counts and CD4+ recovery significantly differ after similar treatment. This suggests an involvement of innate mechanisms, in preventing AIDS-defining OI. At similar CD4+ counts, innate immune parameters may be useful to explain differences in the clinical course of HIV infection.

Keywords: HIV; NKp46; NKp30; Opportunistic infection; AIDS.

Evidence of less pathogenic HIV infection associated with higher adaptation to HLA-I mediated immune response: Insights from the study of the HIV-positive native community from Oran

D. Dilernia[3], D. Monaco[3], M. Quipildor[2], A. Di Paolo[2], L. Yue[3], H. Salomon[1], E. Hunter[3]

[1]UBA-CONICET, Instituto de Investigaciones Biomedicas en Retrovirus y SIDA, Ciudad Autonoma De Buenos Aires [2]Hospital San Vicente de Paul de la Nueva Oran, San Ramon De La Nueva Oran, Salta, Argentina [3]Emory Vaccine Center, Emory University, Atlanta, United States

Objective: Our objective was to study a recently initiated HIV epidemic in a native community from South America that exhibits a restricted HLA diversity, with the initial hypothesis that the HIV would rapidly select escape mutations to the limited number of HLA alleles and this increased adaptation would impair immune response against the infection.

Methods: We performed high-resolution HLA class I typing and near-full length genome sequencing on HIV-1 isolated from 65 chronically infected HIV-positive patients from the region of Oran in Argentina. Implementing statistical and phylogenetic based methods we identified a number of HLA-linked viral polymorphisms associated with escape to the most frequent HLA alleles. Considering them as signatures of viral adaptation, we studied their prevalence and correlated their presence with viral load and CD4 count.

Results: HLA typing confirmed the restricted diversity of HLA-A, -B and -C. We identified 24 HLA-linked viral escape mutations (p <0.05; q <0.1) distributed across the entire HIV proteome. From the phylogenetic analysis, we observed 12 monophyletic clades (3-9 isolates/clade; bootstrap support=100) that suggest independent introductions of HIV into the community with subsequent transmission clusters. Classifying the viral strains based on their prevalence at the population-level, we found that viruses present in these clades exhibit a higher number of HLA-linked mutations compared to those found in isolated introductions (p=0.0114). In addition, in a subset of 41 antiretroviral-naïve patients, we found that the number of escape mutations was positively correlated with the CD4 count (p=0.044) and negatively correlated with the viral load (p=0.023) of the patients.

Conclusion: Our results suggest a rapid adaptation of HIV to HLA-I mediated immune responses in the native community of Oran and unexpectedly also suggest that this adaptation is leading to less pathogenic HIV infections. Our current hypotheses are focused on a potential negative impact of escape mutations on viral fitness and/or redirection of immune response to novel/subdominant epitopes where escape is more difficult, our results show that after 30 years of the epidemic, the study of discrete populations like the native community of Oran, can provide new insights that inform our understanding of HIV pathogenesis.

POSTER COMMUNICATIONS

Session II

Effects of HAART on the plasma levels of regulatory T cells and their role on TCD4+ cells function in HIV+ children

M. Alfonzo[2], W. Vivas[2], E. Navarro[2], Y. Fuentes[2], M.G. Lopez[1], L. Siciliano[1], J. Garcia[1]

[1]Servicio de Infectologia, Hospital de niños JM de los Rios [2]Depto de Ciencias Fisiologicas, lab de Inmunofisiologia Celular, Universidad Central de Venezuela, Caracas, Venezuela

Background: Little is known about the role of regulatory T cells (Treg+) in the immunopathology of HIV, especially in children. Vaccination with Bacillus Calmette-Guerin (BCG) induces an immune response in healthy children against the disease (TB) but such response in HIV-infected children has not been fully characterized. The administration of highly active antiretroviral therapy (HAART) has managed to recover the immune system in most patients, but is still unknown effects on the response to BCG and role Treg+ cells in children.

Objectives: To assess the effects of HAART on plasma levels of Treg+ cells and their role on TCD4+ lymphocyte function in HIV-infected children under HAART management by 14 months.

Methodology: Peripheral blood from HIV-infected children (n=5) before and during 14 months of HAART, were used to determine T cells phenotying and Treg+ was performed by flow cytometry. In addition, peripheral blood mononuclear cells (PBMC) were cultured under the following conditions (no stimulus, PHA, BCG and Gag) to determine proliferation TCD4+ lymphocytes. Moreover, the production of IFN-g was evaluated in absence or presence of purified Treg+ cells using anti-CD4, anti-CD25 and anti-CD127 monoclonal antibodies-conjugated to magnetic beads.

Results: There was a relative reduction (31%) of plasma Treg+ cells from 7.10% at day 0 to 4.88% at month 14. Besides, a relative increase (117%) in total TCD4+ lymphocytes population was observed from 14.3% at day 0 to 31% at month 14.Furthermore, cellular proliferation in presence of BCG and Gag increased after 14 months of treatment (169% and 111%, respectively), but is not inhibited in the presence of Treg+. While IFN-g production in TCD4+ cells increased in the presence of PHA (160 %), but decreased in the presence of BCG (36%) and Gag (46%). The Treg+ cells did not affect these last parameters.

Conclusions: These results indicate that a short-term administration of HAART could exert a decrease in blood population Treg+, causing a partial improvement in the functionality TCD4+cell measured as BCG and Gag induced proliferation and increase production of IFN-g by PHA, independently of the presence or absence of Treg+ cells.

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Switching tenofovir/emtricitabine plus lopinavir/r to raltegravir plus darunavir/r in patients with suppressed viral load did not result in recovery of renal function but could sustain viral suppression: randomized multicenter trial

T. Nishijima[4-1], H. Gatanaga[4-1], T. Shimbo[5], M. Ishisaka[4], K. Tsukada[4], T. Naito[6], I. Itoda[7], M. Tei[3], H. Mitsuya[2], S. Oka[4-1]

[1]Center for AIDS Research, Kumamoto University [2]Department of Infectious Diseases and Hematology, Kumamoto University Graduate School of Medical Sciences, Kumamoto [3]Department of Integrated Medicine, Saku Central Hospital, Nagano [4]AIDS Clinical Center, National Center for Global Health and Medicine [5]Department of Clinical Research and Informatics, International Clinical Research Center, National Center for Global Health and Medicine [6]Department of General Medicine, Juntendo University School of Medicine [7]Shirakaba Clinic, Tokyo, Japan

Background: Whether tenofovir nephrotoxicity is reversible after discontinuation of tenofovir is unknown. Furthermore, no data on the viral efficacy of raltegravir (RAL) plus ritonavir-boosted darunavir (DRV/r) for patients with suppressed viral load is available.

Methods: This multicenter, randomized, open-label trial compared renal function and viral efficacy of RAL+DRV/r and ritonavir-boosted lopinavir (LPV/r) plus tenofovir/emtricitabine (TVD) in randomly allocated patients who were on LPV/r+TVD with suppressed viral load. The primary endpoint was the proportion of patients with >10% improvement in estimated glomerular filtration rate (GFR) at 48 weeks calculated with Cockcroft-Gault equation.

Results: 58 randomized and treatment-exposed patients were analyzed (28 on RAL+DRV/r and 30 on LPV/r+TVD). 6 (25%) out of 24 with RAL+DRV/r and 3 (11%) of 28 with LPV/r+TVD, respectively, experienced >10% improvement in eGFR, and the difference was not statistically significant (p=0.272). Sensitivity analyses with three other equations for eGFR showed the same results. Urinary albumin and β2 microglobulin significantly improved with RAL+DRV/r than with LPV/r+TVD (albumin: -21 versus +1.4 mg/g Cr, p=0.033; β2 microglobulin: -0.6 versus -0.1 $\log_{10}$μg/g Cr, p=0.003). Per protocol analyses showed that all patients in both arms (24 in RAL+DRV and 29 in LPV/r+TVD) were of HIV RNA.

Conclusions: This trial showed that, against expectation, switching LPV/r+TVD to RAL+DRV/r did not result in improvement of renal function among patients with relatively preserved eGFR. However, improvement in urinary albumin was observed, suggesting discontinuation of tenofovir might be beneficial in a long-term.RAL+DRV/r showed favorable viral efficacy and safety for patients with suppressed viral load, although larger studies are warranted to confirm the viral efficacy of RAL+DRV/r.

Table. The proportion of patients with >10% and any improvement in eGFR at 48 weeks from the baseline calculated with four equations.

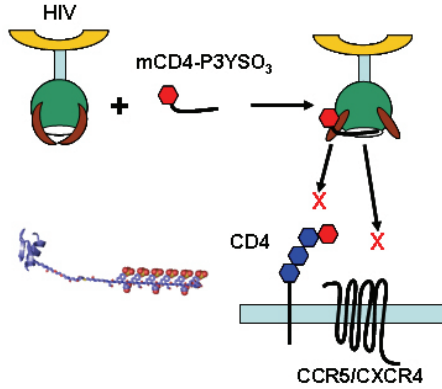
	>10% increase from the baseline	P value	Any increase from the baseline	P value
Cockroft-Gault equation				
RAL+DRV/r	6/24	0.272	14/24	0.093
LPV/rTVD	3/28		9/28	
Japanese Society of Nephrology equation				
RAL+DRV/r	4/24	0.688	12/24	1.000
LPV/r+TVD	3/29		15/29	
CKD-EPI equation				
RAL+DRV/r	2/24	1.000	12/24	0.786
LPV/r+TVD	2/29		16/29	
MDRD equation				
RAL+DRV/r	5/24	0.444	12/24	0.786
LPV/r+TVD	3/29		16/29	

Synthetic Heparan Sulfate-Mimetic Peptides Conjugated to Mini CD4 Display sub nanomolar Anti-HIV-1 Activity Independently of Coreceptor Usage

H. Lortat-Jacob[2], B. Connell[2], Y.M. Coic[3], P. Clayette[1], F. Baleux[3]

[1]Laboratoire de Neurovirologie, CEA - Bertin Pharma, Fontenay Aux Roses [2]Institut de Biologie Structurale, UMR 5075 CNRS-CEA-Université de Grenoble, Grenoble [3]Unité de Chimie des Biomolécules, Institut Pasteur, Paris, France

Objective: The HIV-1 gp120, which features the binding determinants for both CD4 and coreceptor recognition, offers multiple sites for therapeutic intervention. However, the cryptic nature of the coreceptor binding site, which becomes exposed only when the virus has bound cellular CD4, limits its vulnerability to inhibition both temporally and spatially. To pierce this defense mechanism we hypothesized that molecules composed of CD4 linked to HS-like compounds would bind to gp120 through its CD4 moiety and expose the coreceptor binding domain, which would then become available to be targeted by HS. Such molecules should simultaneously block the binding of gp120 to CD4 and coreceptors, and thus inhibit viral entry.



Methods: We used a chemical approach to synthesize a CD4-mimetic peptide (mCD4) that was covalently linked to either a HS dodecasaccharide or to a library of HS mimicking tridecapeptides. To investigate whether this new class of compounds inhibit gp120 binding to HIV coreceptors we also set up an assay in which detergent solubilized CCR5 and CXCR4 were both functionally captured on top of sensorchips. This assay enables us to follow in real time the binding of YU2 or MN gp120 to CCR5 or CXCR4 respectively, using Surface Plasmon Resonance techniques.

Results: Amongst a collection of tridecapeptides, we found that those containing sulfotyrosines target the gp120 coreceptor binding site. Covalently linked to a CD4-mimetic that binds to gp120 and renders the coreceptor binding domain available to be targeted, the conjugated tridecapeptides display low-nanomolar affinity for its target. These bivalent compounds inhibit gp120 binding to both CCR5 and CXCR4, and in peripheral blood mononuclear cells broadly inhibits the entry and replication of several clinical HIV-1 isolates (R5 and X4, clade A, B and C) replication with IC_{50} of 0.2 to 30 nM in the absence of cytotoxicity.

Conclusion: These compounds, which have the unique ability to simultaneously target two critical and highly conserved regions of gp120, establish a new type of inhibitor that neutralize both CCR5- and CXCR4-tropic HIV-1. Chemically defined, these molecules are amenable to large scale production and are currently being investigated as a microbicide in a NHP model of infection.

Fifteen years of HIV positivity and trends of associated risk factors documented at the Institut Pasteur du Cambodge

V. Phuong[1], Z. Tun[5], L. Borand[1], D. Douk[1], E. Soeur[1], K. Nong[1], E. Webb[5], I. Fournier[3], C. Mean[2], J.F. Delfraissy[3], F. Barré-Sinoussi[4], A. Tarantola[1]

[1]Institut Pasteur du Cambodge [2]National Center for HIV/AIDS, Dermatology and STD Control (NCHADS), Phnom Penh, Cambodia [3]Agence Nationale de Recherches sur le Sida et les hépatites virales (ANRS) [4]Institut Pasteur, Paris, France [5]London School of Hygiene & Tropical Medicine (LSHTM), London, United Kingdom

Background: The Institut Pasteur du Cambodge implemented the first HIV counseling and testing center (VCCT / CDAG) in Cambodia in 1995; there are now 250+ thanks to the national AIDS program.

Methods: Over the years, the same questionnaire was administered to people before testing. In 2012 we performed a cross-sectional analysis of the anonymized VCCT database, assessing risk factors' progression as the epidemic evolved over 15 years (April 1996 - December 2011, inclusive). The progression of risk ratios for sociodemographic and self-declared risk behavior associated with a positive result were analyzed. Logistic regression models were used for uni- / multivariate analyses to assess overall risk factors of HIV positivity using hierarchical modeling.

Results: The database contained 83810 HIV-tested individuals; 13295 (15.9%) tested positive. HIV positivity among VCCT users decreased from 14.2% in 1996 to 6.5% in 2011. Factors associated with HIV positivity were age 35-44, being female, being tested early in the study period, being illiterate, residing outside the capital city, occupations (police & military, basic workers, sex industry), being "Vietnamese" (likely proxy), not living with partner, age Conclusion: The analysis of the 15-year VCCT database from a single and vantage point provides a unique viewpoint. Documented seroprevalence is high compared to the general population but trends in factors' risk ratios were documented as the epidemic unfolded and changed over a 15-year period. IDUs, blood transfusion recipients and children of positive parents remain at increased risk and could benefit from further interventions. The database is being shared with the national partners and will be used to document changes associated with prevention strategies in population subgroups.

Differential regulatory T cell activity in HIV-1-exposed seronegative individuals

L. Pattacini, J. Lund

VIDD, Lund Lab, Fred Hutchnson Cancer Research Center, Seattle, United States

Objective: To address the role of regulatory T cells (Tregs) in protection from HIV infection. It has been hypothesized that by dampening general T cell activation, Tregs could limit the pool of HIV-susceptible cells but they could also limit the HIV-specific response.

Methods: To address this question, we analyzed samples from 129 HIV-exposed seronegative individuals (HESN) part of a HIV-serodiscordant couples cohort. To assess T cell and Treg function, we measured the proliferation of T cells in response to HIV peptide pools by a CFSE-based proliferation assay.

Results: The HIV-specific proliferative response rates were low for both CD4 and CD8 T cells, and surprisingly, the overall CD4 T cell proliferation response rate was not increased when Tregs were removed from cell preparations prior to culture. Of the 20 individuals that had HIV-specific CD4 T cell proliferative responses, 8 of them had Tregs that could functionally suppress this proliferation, whereas 12 of them had Tregs unable to suppress. When subjects were thus identified based on Tregs able to suppress or not suppress CD4 T cell proliferation, we found a correlation between CD4+ T cell activation and Tregs frequencies (p=0.0001). Additionally, subjects with Tregs unable to suppress proliferation had a statistically significant increase in HIV-specific production of MIP1 b by CD4+ T cells (p=0. 02).

Conclusions: Our study shows a possible mechanism of HIV resistance at least partly due to a lack of suppression of HIV specific responses and to an increased CD4+ T cell autocrine production of MIP-1b that has previously been shown to be protective from HIV infection. The novel finding could be helpful in the sesign of future HIV vaccines.

Efavirenz pharmacogenetics: plasma exposure and clinical outcomes among Ugandans

J. Mukonzo

International HIV Fellowship, The CTN, CIHR Canadian HIV Trials Network, Vancouver, Canada Pharmacology & Therapeutics, Makerere University, College of Health Sciences, Kampala, Uganda

Introduction: Efavirenz, one of the commonly used NNRTIs for HIV treatment exhibits a narrow therapeutic window. Plasma concentrations > 4 µg/ml and < 1 µg/ml are associated with CNS and virologic failure respectively. The metabolizing enzymes; *CYP2B6* and *CYP3A4/5* and *ABCB1,* a gene coding for P-glycoprotein may influence efavirenz pharmacokinetics and pharmacodynamics. All genetic factors above exhibit differences in population allelic frequencies with possible corresponding differences in both efavirenz in-vivo exposure and clinical outcomes. Population specific efavirenz pharmacogenetic considerations might inform optimization of efavirenz dosing. We determined population frequencies of relevant *CYP2B6, CYP3A*5 and *ABCB1* genotypes and their effect on efavirenz pharmacokinetic and pharmacodynamics Ugandans.

Methods: The study was conducted among healthy volunteers (HV) (n = 121) and HIV/AIDS patients (n= 263) in Uganda. The HV sub-study was cross-sectional while the patient study was a prospective cohort study with a total follow-up period of 32 weeks. HVs were genotyped for 32 SNP in *CYP2B6*, *CYP3A5* and *ABCB1* and individual SNP effect on efavirenz pharmacokinetics determined. Patients were genotyped for SNPs that influenced efavirenz pharmacokinetics according to the HV study. They included CYP2B6 (*6 and *11), CYP3A5 (*3,*6 and *7) and ABCB1 (rs 3842 and 3435C>T). SNP effect on enzyme induction and efavirenz-related CNS toxicity was determined.

Results: SNP frequencies among Ugandans were different from other populations but comparable to other African populations. CYP2B6*6 and * 11 influenced efavirenz pharmacokinetics with mutant individuals exhibiting 26% and 21% lower clearance. Bioavailability was 26% higher in ABCB1 (c4036A>G) homozygotes. CYP2B6 *6 and *11 and ABCB1 c.4036A>G SNPs frequencies were 55%, 13.6% and 16.8% respectively. Enzyme induction was generally predicted by CYP2B6*6 ($p < 0.01$) while CYP2B6*11 predicted efavirenz clearance on days 14; $p = 0.002$ and 56; $p=0.019$. 7 in 10 individuals experienced efavirenz related symptoms. Genotypes influenced efavirenz concentrations that in-turn predicted efavirenz related CNS symptoms but no direct association with efavirenz CNS toxicity was observed.

Conclusion: As a result of differences in *CYP2B6* and *ABCB1* population genotype frequency differences, efavirenz might uniquely affect the Ugandans and other African populations in terms of both pharmacokinetics and pharmacodynamics indicating probable need for population based dosing.

Spastic diplegia due to HIV encephalopathy – Is this the same as in Cerebral Palsy?

N. Langerak[1-5], J. Du Toit[3], M. Burger[5], M. Cotton[2], P. Springer[4], B. Laughton[2]

[1]Neurosurgery, University of Cape Town, Cape Town [2]Children's Infectious Diseases Clinical Research Unit [3]Orthopaedic Surgery [4]Paediatrics and Child Health [5]Physiotherapy, Stellenbosch University, Tygerberg (cape Town), South Africa

Objectives: Human Immunodeficiency Virus Encephalopathy (HIVE) is the most common clinical presentation of central nervous system disorder in children born with HIV. HIVE can present as spastic diplegia [1], which has been well described within Cerebral Palsy (CP) [2,3]. It is unclear if the physical status in HIVE is similar to, and can be managed the same as CP. The aim of this study is to investigate the gait patterns and physical impairments of children with spastic diplegia secondary to HIVE.

Methods: Socio-demographic and clinical background information was obtained, followed by 3-dimensional gait analysis (3DGA) and a physical examination including assessments of muscle tone, strength, motor control, contractures and bony deformities of the lower extremities.

Results: The study cohort consisted of 8 boys and 6 girls, with a mean age of 5.7 (range: 4.3-6.8 years). Based on distinctive, different gait patterns the cohort was divided into two groups: Group I (n=9) showed only limited abnormalities; and Group II (n=5) displayed a more pathological gait pattern including Stiff Knee and Equinus ankle abnormalities (Figure 1) [2]. 3DGA data and physical examination outcomes showed increased impairments from proximal to distal (except for hip extension).

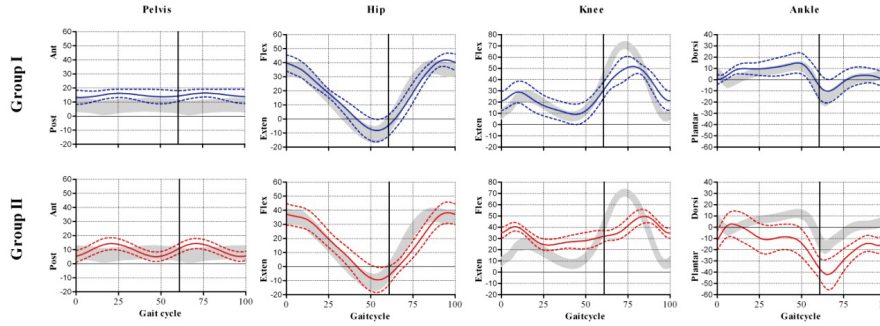


Figure 1. Sagittal gait patterns. Grey band represents mean ± 1 standard deviation (SD) for typical developing persons, while the black lines shows the mean ± 1SD for Group I and II. Abreviations: Ant: anterior tilt; Post: posterior tilt; Flex: flexion; Exten: extension; Dorsi: dorsiflexion; and Plantar: plantarflexion

Conclusion: The gait and physical manifestations of children with HIVE and spastic diplegia are unique, and different to CP. There is may be more than one neuropathogenic pathway in HIVE presenting clinically as spastic diplegia. Further investigation of natural history of HIVE is needed to design treatment protocols.

References:

1. Chiriboga CA et al. *JPediatr.*146:402-407, 2005.
2. Wren TA et al. *JPediatrOrthop.*25:79-83, 2005.
3. Rodda JM et al. *JBoneJointSurgBr.*86:251-258, 2004.

Rapid Perturbation in Viremia Levels Drives Increases in Functional Avidity of HIV-Specific CD8 T cells

S. Vigano, V. Bellutti-Enders, A.L. Savoye, V. Rozot, M. Perreau, G. Pantaleo, A. Harari

Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Objectives: Functional avidity (i.e. antigen sensitivity) is a key feature of T cells. High functional avidity T cells are associated to virus clearance but their relevance in chronic persistent viral infections is unclear. Also, factors associated to the induction of low or high functional avidity T cells are poorly understood.

Methods: The functional avidity of HIV-specific CD8 T cells was investigated in different cohorts of HIV-infected subjects (n=56) including early acute and chronic (progressive and nonprogressive) infection. The functional avidity was determined for 157 HIV- and 63 CMV-, EBV-, Ad5- and Flu-specific CD8 T-cell responses. The relationships between functional avidity, T-cell differentiation, functional profile and CDR3 repertoire were analyzed in steady-state conditions and after virus rebound.

Results: Virus-specific CD8 T cells from patients with acute HIV infection had significantly lower functional avidity as compared to patients with progressive and nonprogressive chronic HIV infection (both $P<0.0001$) as well as to EBV/CMV-specific CD8 T cells ($P=0.02$) but were not different from Flu/Ad5-specific CD8 T cells or from HIV-specific CD8 T cells induced following immunization. The functional avidity of HIV-specific CD8 T cells was not different between progressors (n=40) and nonprogressors (n=27). The functional avidity correlated negatively with the co-expression of CD27/CD28 ($P<0.021$) and positively with the co-expression of PD1/CD160/2B4 ($P=0.0001$). Of note, 8 patients treated during HIV acute infection underwent treatment interruption and experienced a virus rebound. This induced an increase in functional avidity of HIV-specific CD8 T cells ($P=0.020$) associated to a loss of functionality ($P<0.0001$) and an increased expression of PD1/CD160/2B4 ($P=0.024$). Statistical modeling indicated a lack of interaction between functional avidity and time but a positive interaction ($P=0.01$) between avidity and virus rebound. Finally, the extent of increase in functional avidity of HIV-specific CD8 T cells directly correlated ($P=0.03$) to the level of CDR3 renewal.

Conclusions: The functional avidity of HIV-specific CD8 T cells is low during primary immune responses and increases following antigen exposure. Furthermore, increased TCR renewal provided a potential mechanistic basis for the accumulation of high functional avidity T-cell clones. These results advance our understanding of the factors influencing the functional avidity of CD8 T cells.

The Advantage of Viral Load in Prevention from Mother to Child Transmission

V. Ouk[1], K. Chhim[1], R. Thoun[1], B. Ban[1], S. Pao[1], S. Kaingseng[1], C.H. Nam[1], O. Segeral[3], J.F. Delfraissy[3-2]

[1]Infectious diseases unit, Esther Calmette, Phnom Penh, Cambodia [2]ANRS [3]Kremlin Bicêtre, France

Objective: To identify the interest of Viral Load (VL) in Prevention from Mother to Child Transmission (PMTCT) under HAART at least 3 months before delivery.

Methodology: Descriptive analysis of 26 dossiers of PMTCT patients follow up from first January 2012 to 31th Décember 2012 at Esther/Calmette Hospital, Cambodia. We provide the VL (real time RT PCR, Biocentric) to pregnant women at least 3 months under HAART before delivery with antiretroviral treatment : AZT + 3TC + EFV/NVP as main regiment. Adherence is considered.

Result: There are 19 VL provided. Two patients are not provided. One patient is loss of follow up. Two abortions: 1 spontaneous and 1 induced. Two are under HAART less than 3 months. 18 VL are seen undetectable and one VL detectable. Two of 18 patients didn't deliver yet. 16 new borns are undetectable. We provided a good adherence to all patients.

Conclusion: The monitoring by VL during PMTCT seems interesting to make sure that the contamination during pregnancy, especially for the HIV positive pregnant women who need baby, is insignificant.

HBV resistance pattern and liver fibrosis in patients coinfected with HIV and HBV. Case series.

O. Streinu-Cercel[1-2], A.M. Tudor[1-2], A. Streinu-Cercel[1-2], M. Paraschiv[2], D. Vlad[2], G. Ceapraga[2], A. Streinu-Cercel[1-2]

[1]Carol Davila University of Medicine and Pharmacy [2]National Institute for Infectious Diseases "Prof.Dr. Matei Bals", Bucharest, Romania

Objective: We assessed a particular group of patients, coinfected with HIV+HBV in their early years of life, during the late '80s, early '90s. They started ART usually with AZT and then with bitherapy, mainly ddC and 3TC, the drugs available at the time. During the period under survey 3TC was the only available option for coinfected patients in Romania.

Methods: We performed genotyping tests through RT-PCR in June 2010 and retrospectively studied: HIV viral load (VL) HBV-DNA, liver fibrosis, necroinflammation (FibroMax), treatment history.

Results: We assessed 15 coinfected patients, (8:7 male:female), with median ages (±SD) 20±2.6 years. At the time of the study, 10/15 had undetectable HIV VL; 5/15 had values ranging from 463 to 2,000,000 copies/mL. The median fibrosis was 0.32±0.24 (F1-F2), the median activity 0.23±0.21 (A0-A1).

All had received 3TC for 102±44 months (77.4±34.2% of the total ART duration). We identified the following HBV reverse-transcriptase resistance mutations: 173L (3/15), 180M (11/15), 204V (10/15), 204I (2/15). Three were still susceptible to 3TC (treatment duration: 13, 13, 24 months), entecavir and telbivudine. The other 12 were resistant to 3TC (median duration of 3TC: 109±25 months, min: 63 months, max: 145 months).

None had received entecavir; however, only 3/15 had strains still susceptible to entecavir; 12/15 were potentially resistant (PR) (180M+204V, 10/12; 180M+204I, 1/12; 204I; 1/12).

No resistance was recorded for adefovir or tenofovir; for telbivudine 3/15 were still susceptible, 2/15 resistant (204I), and 10/15 PR (204V).

At the time of mutation assessment, 9/15 were still receiving regimens with 3TC; all displayed mutations (180M+204V, 6/9; 173L+180M+204V, 2/9; 204I, 1/9). Interestingly, this subgroup presented a wide variation in HBV-DNA (10 million IU/mL, 5/9), although all were considered resistant to 3TC and were not under other antiviral agents active on HBV, making it apparent that other factors might play an important role.

Conclusion: In HIV+HBV coinfected patients with prior 3TC therapy, no resistance to adefovir and tenofovir was recorded, compared to a high rate of PR to entecavir and telbivudine. However, resistance testing is not sufficient to guide clinical decision-making in these patients, and various cofactors need to be evaluated.

Yang AIDS patient with lymphoma hospitalized in infectious disease clinic

S. Dragas, B. Dupanovic, B. Andric, D. Terzic, D. Lazovic

Infection desease clinic, Clinical center, Podgorica, Serbia-Montenegro

Objective: HIV infection results in impaired cellular immunity, and predispose developing neoplasm's. HIV-associated lymphoma is most commonly diagnosed in patients with advanced HIV, a low CD4 count (often <100/microL), high HIV viral load, and a prior diagnosis of AIDS. Sometimes, people are diagnosed with AIDS and AIDS-relatedlymphoma at the same time, and they are usually aggressive. There are three main types of AIDS-related lymphoma: Hodgkin, Non Hodgkin and Burkett lymphoma. The treatment options in these patients have unique challenges, and although the prognosis it still poor.

Methods: In this retrospective study we analyzed clinico-pathogenical features with potencial therapeutic implications in late presented AIDS patients with lymphoma hospitalized in Infectious disease Clinic from 2011-2013 year. The diagnosis of lymphoma was werified on patohistological examination.

Results: For male patients aged from 26-32 were hospitalized, and no one was on HAART (high active retroviral therapy) before admittion. All had serological positivity on Epstein Barr virus (EBV). Patient 1 had Large cell lymphoma, non T non B expresio, localized on paranasal sinus and epifarings. In addition he had chronic HBV infectio with presented ascites and lymphedema of extremitas inferior. Patient: 2 had large B cell lymphoma, localized also on paranasal sinus. Patient: 3 had Burkitt lymphoma, localized on central nervous systema, retroperitoneal and mediastinal region. In addition he had hydronephrosis and plased sonde Patient 4, 32 years old had Hodgkin lymphoma with mediastinal and retroperitoneal presentation. In addition he had disseminated mycoplasma tuberculosis infection.

All of patients had low CD4 Ty cont ranged from 24- 148 on admmition and high viral load (> 500.000 cop/ml). In all patients we started with recomended HAART therapy: lamivudine, abacavir, efavirenz, and with recomended chemotherapy: CHOP (Cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone) and in patient 4 with ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) protocol. Three of them died witheen 3 months months after addmition on clinic, and patient 1 is still on chemoterapy (first cycle).

Conclusion: All threated patients were yang late presented ADIS patients, and were not on HAART therapy before. Despite recent advances in the management of AIDS and its associated lymphomas, prognosis was poor.

Primary HIV drug resistance mutations associated with first-line ARV treatment failure in treatment-naïve adults in Vietnam

X. Vo[2], N. Vo Thi Tuyet[1], M.Q. Vo[2], V.C. Nguyen Van [2], T.C. Nguyen[2]

[1]HAIVN in Ho Chi Minh City [2]Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

Background: In Vietnam, genotyping test is not conducted for HIV patients before initiating ARV. Choosing ARV regimens is mainly following National Guideline on HIV/AIDS Care and Treatment for all patients. We assessed prevalence of primary HIV drug resistance at baseline and identified association between mutations with treatment outcomes in treatment-naive adults initiated with first-line ART in Vietnam.

Methods: 140 treatment-naive adults with age >=18 were enrolled in a longitudinal study from October 2008 to December 2010. All were commenced D4T/AZT plus 3TC and EFV/NVP. Genotyping was done at baseline at the Institut Pasteur in Ho Chi Minh City using an in-house sequencing assay. Treatment failure is defined by one of the three criteria: clinical (new or recurrent WHO stage 4 or certain stage 3 conditions); immunology (CD4 decrease to pre-therapy baseline or below, 50% fall from the on-treatment peak value, or persistent CD4 levels 250 copies/ml) at 12 months.

Results: Overall, 53% patients had at least one mutation. Prevalence of HIV drug resistance for NRTIs, NNRTIs and PIs were 3.6%, 5.7% and 0%, respectively. G190ACGR mutation was found in 50% patients with NNRTI drug resistance. Similarly, 40% and 20% patients with NRTI drug resistance had D67N and L210FLMSP mutations. After 12 months starting ARV, 27% patients had treatment failure including 10% for clinical, 10.7% for immunological and 16.3% for virological failures. Mortality was 10.8% patients. 60.5% those with treatment failure had at least one mutation at baseline. In multivariable analysis, HIV drug mutations were independently associated with immulogical failure (OR=6.8, 95% CI: 1.2-39.0), and not correlated to clinical (OR=2.6, 95% CI: 0.8-8.7) or virological failures (OR=1.1, 95% CI: 0.4-3.1) or death (OR=1.6, 95% CI: 0.5-5.1).

Conclusion: Prevalence of HIV drug resistance in treatment-naive adults in Vietnam was still low. However, patients with mutations found at baseline had a higher risk of immunological failure if starting the same ARV regimen as recommended for all patients in developing countries.

Targeting the RNase H of HIV to drive HIV into suicide - a novel microbicide

K. Moelling[4-1-3], F. Broecker[4-1], J. Hauber[3], P. Deuflhard[2], K. Andrae[2], K. Fackeldey[2]

[1]Max Planck Institue of Molecular Genetics [2]Zuse Institute Berlin (ZIB), Berlin [3]Heinrich Pette Institute, Hamburg, Germany [4]University of Zurich, Zurich, Switzerland

Objective: Sexual transmission of HIV is the major cause of spread of HIV in Africa and the Third World and an unmet medical problem. Recently microbicides have attracted attention because they allow females to protect themselves and their offsprings from infection during sexual intercourse. Several approaches have been analyzed but no satisfactory results have been obtained. We developed a method to drive HIV particles into suicide by activating its virion-associated RNase H with a highly specific oligodeoxynucleotide. The RNase H recognize a local RNA-DNA hybrid and will then cleave the viral RNA and destroy the infectivity of the virus before infection. On this basis we want to deveop a compound against HIV as microbicide to be applied to the vagina of females.

Methods: We have developed and described an oligodeoxynucleotide which can enter virus particles and destroy the viral RNA and infectivity of the virus. We applied this compound to primary viruse isolated from a swiss Hospita, to isolates from Uganda, to three animal models. One model is a caner model in mice based on the Spleen Focus Forming Retrovirus (SFFV), which causes cancer, another one was a mouse vagina model with a recombinant retrovirus vector to destroy the virus, and a third model was a Humanized SCID Mouse model with HIV treatment.

Results: The compound reduced viral infectivity of primary HIV isolates up to thousandfold. Then the compound reduced tumor formtion, increased survival and even prevented infection in SFFV-infections of mice. The virus load in the vagina was statistically signficantly reduced. Furthermore in HIV-treated humanized SCID mice we were able to prevent infection in more than 95%. No toxicity was observed. We published these resupts in several papers including Nature Biotech, 2007. A review was published: Broecker et al, ARHR **28,** 1397 -1403 (2012).

Conclusion: We developed and characterized an oligodeoxynucleotide to destroy HIV infectivity in vitro and in three different animal models. We demonstrated significant reduction of viral load and even prevented infection in two animal models. We are stabilizing the enzyme and want to develop it into a microbicide to prevent viral infection during sexaul transmission.

Regulatory T cells represent an important fraction of HIV-specific T cells

V. Brezar[1], N. Ruffin[1-2], J.D. Lelievre[1-2], Y. Levy[1-2], N. Seddiki[1-2]

[1]U955, Equipe 16, INSERM [2]Université Paris-Est Créteil/Vaccine Research Institute (VRI), Créteil, France

Introduction: Regulatory T-cells (Tregs) play a dual role in HIV infection. Decrease of immune activation is considered a positive aspect of Treg action since activated T-cells present a major source of active replication of the virus. On the other hand, Tregs are shown to block anti-HIV-specific immune responses. Although the importance of Tregs in the context of HIV-infection has been highlighted by numerous studies, the measurement of frequency and phenotype of HIV-specific Tregs is largely untaken.

Methods: We employed a novel assay in which co-expression of CD25 and CD134 (OX40) on antigen-stimulated CD4+ T-cells identifies antigen-specific T cells. We used PBMCs from both healthy and HIV-infected patients to measure frequencies of antigen-specific Tregs by performing a co-staining with FoxP3 and CD39 along with intracellular cytokine detection. We stimulated the cells with peptide-pools originated from different HIV-antigens (gag, pol, nef) as well as CMV lysats.

Results: Our results confirm that cells co-expressing CD25 and CD134 upon antigen-stimulation are antigen-specific as previously shown. We also show that these cells co-express CD154, another marker of antigen-specific cells. A large fraction of CD25+CD134+ cells, co-expressing FoxP3 and CD39 do not secrete cytokines (IFNg, IL2, TNFa) upon antigen stimulation. In HIV-infected patients, 50-80% of Gag p24-specific cells are FoxP3+CD39+ which could partially explain the low IFNg-response observed in these patients. By sorting 3 populations of CD4+ T cells based on CD25 high, intermediate or low expression, we show that CD25+CD134+CD39+FoxP3+ antigen-specific Tregs originate from FoxP3+CD25+ Tregs that upregulate CD134 upon stimulation with the cognate antigen. Whereas, antigen-specific cytokine-producing non-Tregs (CD25+CD134+CD39-FoxP3-) originate from CD25neg fraction. Depletion of CD25+ cells from PBMCs prior to stimulation, lead to almost complete absence of FoxP3+CD39+ HIV-specific cells which confirms the above observations.

Conclusions: Measuring the frequency of HIV-specific Tregs is very important especially in the context of HIV-vaccines. Standard assays measuring antigen-specificity by the production of different cytokines omit Treg responses. Thus the ability to measure the inducibility of Tregs upon stimulation with different antigens can open new venues for more efficient antigen-incorporation in future vaccines.

Detection of Cryptosporidium spp. in HIV/AIDS patients

W. Saksirisampant[2], P. Saksirisampant, J. Prownebon

[1]Parasitology, Department of Parasitology, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand., Bangkok [2]Parasitology, Chulalingkorn University, Pathumwan, Thailand

Objective: Cryptosporidiosis had been recognized as opportunistic infection in patients with AIDS. To determine *Cryptosporidium* spp. in fecal samples among Thai HIV/AIDS patients (from King Chulalongkorn Memorial hospital and from an AIDS care center) by various techniques.

Methods: During 2008-2012, of all the 135 fecal samples were determined by microscopy, PCR, ELISA and ICT.

Results: Modified Acid - Fast stain showed 24 (17.78%) positive, PCR and PCR-RFLP showed 34 (25.19%) positive with 27 *C. hominis,* 4 *C.meliagradis* and 3 *C.parvum.* The infected stools with *C.hominis* was significantly associated with abnormal stool consistency and with high oocyst intensity (50-100 - > 100/HPF (Fisher test, P value = 0.019). The ELISA of polyclonal Ab specific to *C.parvum* could detect Cryptospordim spp. Ag(s) in 28 (20.74%) samples without false positive. The ELISA of monoclonal Ab specific to *C.parvum* could detect (29, 21.48%), however, false positive with other protozoan and normal control specimens occurred. The ELISA could detect 1 oocyst/smear stool. Specificity and sensitivity of ELISA-polyclonal Ab (97.6% and 91.7%**)** have higher than the ELISA-monoclonal Ab (91.3% and 79.2%), respectively. Immunochromatography (ICT) detected 19 (14.07%) positive. The ICT technique had 100 % specificity but showed low sensitivity (79.2%).

Conclusions: The ELISA can be the aids of laboratory diagnosis and suitable for the epidemiological study. Both of polyclonal and monoclonal Abs specific to *C.parvum* with had been used for diagnose the disease in dairy cattle can apply to detect other Cryptosporidium species in human fecal specimens. These might due to the common antigenic epitope among the species.Microscopy needs expertise scientist. In this study, the ELISA, that detects the Ag(s) by the polyclonal Ab may helpfull for evaluation of the infection intensity and the effectiveness of the therapy since there was correlation between the ELISA-Ag level and the oocyst intensity.

Immune responses to measles vaccine in early treated HIV-infected children are comparable to those of HIV uninfected infants: the observational ANRS–PEDIACAM cohort in Cameroon

M. Tejiokem[6], A. Kfutwah[7], I. Penda[2-3], S. Tetang Ndiang[4], F. Ateba Ndongo[5], J. Warszawski[8-9-13], M. Fevrier[11], D. Scott-Algara[12], P. Tchendjou Tankam [6], A. Faye[10-14], L. Baril[1], F. Tangy[11]

[1]GlaxoSmithKline Vaccines, Brussels, Belgium [2]Unité de Jour, Hôpital Laquintinie [3]Faculté de Médecine et des sciences pharmaceutiques, Université de Douala, Douala [4]Service de Pédiatrie, Centre Hospitalier d'Essos [5]Unité de Jour, Centre Mère et Enfant de la Fondation Chantal Biya [6]Service d'Epidémiologie et de Santé Publique [7]Service de Virologie, Centre Pasteur du Cameroun, Yaoundé, Cameroon [8]Service d'Epidémiologie et de Santé Publique, Assistance Publique des Hôpitaux de Paris, Hôpital de Bicêtre [9]Equipe 4 (VIH et IST) - INSERM U1018 (CESP), Kremlin Bicêtre [10]Service de Pédiatrie Générale, Assistance Publique des Hôpitaux de Paris, Hôpital Robert Debré [11]Laboratoire de Génomique Virale et Vaccination [12]Unité de Régulation des infections Rétrovirales, Institut Pasteur de Paris [13]Université de Paris Sud [14]Université Paris Diderot, Paris, France

Objective: Measles, a vaccine-preventable disease remains a leading cause of death in children. Variations in antibody responses to measles vaccination in relation to age and HIV status have been reported. Here, we compared measles antibody responses in early treated-HIV infected infants to those of two control groups (HIV-exposed and HIV-unexposed) after routine vaccination following national recommendations.

Methods: PEDIACAM is an ongoing prospective observational cohort that includes as mentioned above, three infant groups. PEDIACAM offers systematic cART to HIV-infected infants, five years follow-up and routine childhood vaccination to each infant in the study. The measles vaccine (MV) doses were planned as follows; 6 and 9 months for HIV-exposed infants, 9 months for HIV-unexposed infants and a measles-mumps-rubella (MMR) dose at 15 months to all infants. Measles antibody titres were measured before and three months after each dose of MV by ELISA (Enzygnost, Dade Behring). Results were interpreted as negative, equivocal, and positive defined as antibody level ≥330mIU/mL.

Results: Overall, 547 infants were tested for measles antibodies at least once, 179 were HIV infected, 191 HIV-exposed uninfected and 177 HIV-unexposed. HIV-infected infants started cART at a median age of 4.2 months (IQR: 3.2-5.6) and 71% had low CD4+ T lymphocytes (<25%). Of the 443 infants tested prior to vaccination at a median age of 6.1 months (IQR: 5.6-6.8), 7% were seropositive. For those who followed the recommendations, comparable proportions of measles seropositive infants were observed with 93% for both HIV infected (39/42) and HIV exposed-uninfected (85/91) and 96% (128/134) for HIV unexposed infants at a median age of 18.4 months (IQR: 18.1-19.9). At a median delay of 3.0 months (IQR: 2.5-3.5) after one dose of MV, the proportions of measles seropositive infants were 75% (89/118), 74% (55/74), and 74% (108/146) respectively among HIV infected, HIV exposed-uninfected and HIV unexposed.

Conclusion: Early follow up and cART of HIV-infected infants results in good initial measles antibody response. It could be important to monitor the persistence of measles antibody titres in the different groups up to 5 years in order to evaluate herd immunity required for measles elimination.

Effects of early antiretroviral therapy (ART) on Natural killer (NK) cell phenotypes in HIV infected infants in Cameroon

A. Kfutwah[2], C. Devret[2], C. Didier[4], E. Ngo Malabo[2-3], M. Tejiokem[1], D. Scott-Algara[4]

[1]Epidemiology and Public Health Service [2]Virology Service, Centre Pasteur of Cameroon [3]University of Yaounde 1, Yaounde, Cameroon [4]Unité de régulations des infections Rétrovirales, Institut Pasteur, Paris, France

Background: NK cells are a key component of the innate immune response to tumours, viruses, fungi, parasites and bacteria. As part of the innate immunity, NK cells have an important role in host defense against HIV infection (resistance or progression), as well as the control of HIV replication *in vivo*. The integration of signals that NK cells receive through various inhibitory and activating cell surface receptors controls their activation and ability to kill target cells and produce cytokines. We evaluated NK cell repertoire in a population of infants, born to HIV infected or uninfected mothers.

Methods: Three groups of infants participating in the prospective ANRS-Pediacam cohort were considered; HIV infected infants born to HIV infected mothers and immediately put on ART after diagnosis (group 1i), HIV negative infants born to HIV positive mothers (group 1ni) and HIV negative infants born to HIV negative mothers (group 2ni). Blood samples were drawn at 6 (n=40, n=56), 9 (n=40, n=56) and 12 months (n=34, n=39) from infants of group 1i and 1ni respectively and at 6 (n=41) and 9 months (n=41) from group 2ni. Twelve different NK inhibiting and activating receptors were studied.

Results: The median age of the infants at inclusion was 6, 7 and 8 months for groups 1i, 1ni and 2ni respectively.Of the 12 receptors studied in all three groups during their first visits, 42% (5/12) were highly expressed (CD161, NKp30, NKp46, NKG2A, NKG2D), 17 % (2/12) were least expressed (NKp44, CD107a). In group 1i, an increase in 2 activating receptors (NKp46, NKG2D) and a decrease in 2 inhibiting receptors (CD161, NKG2A) was observed with time. In the non-infected groups (1ni and 2ni) one inhibiting and one activating receptor (NKG2A, NKG2D) increased while another inhibiting and activating receptor (CD161, NKp46) decreased with time.

Conclusions: We observed several modifications of NK cell repertoire in HIV infected and uninfected infants. The role of NK cell in infants born to HIV infected mothers will be further evaluated in larger cohorts.

HIV Disease Progression in Adult Cohort: Observational Database in Thailand

S. Uttayamakul[3], S. Likanonsakul[3], W. Klinbuayaem[2], J. Ananworanich[1], A. Avihingsanon[1], S. Keadpudsa[1], W. Prasithsirikul[3]

[1]The HIV Netherlands Australia Thailand Research Collaboration, the Thai Red Cross AIDS Research Center, Bangkok [2]Sanpatong Hospital, Chiangmai [3]Bamrasnaradura Infectious Diseases Institute, Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand

Background: Thailand was one of the first developing countries to implement a national antiretroviral therapy (ART) program in 1995. The progression to AIDS and its related mortality has fallen dramatically however the available data regarding natural history of HIV in long term outcome are limited.

Objective: To describe HIV disease progression in Thai adult patients treated with ART.

Methods: A multicenter, observational prospective cohort study has been conducted in HIV-infected Thai adult since 2008. The data were collected prospectively and analyzed until 31 Dec 2012. All HIV-infected patients currently receiving ART from 4 collaborative referral sites in Thailand were assessed for HIV disease progression to AIDS related illness or death. The baseline characteristics, ART regimens, CD4 cell count, HIV-1 viral load testing were measured for main outcomes.

Results: A total of 6,021 HIV-infected patients were enrolled. Of these, there are 5,878 (58.1% male, 41.9% female) has continued in the study with median age of 43.6 year (IQR 38.7-49.2) and range of age is 19.4-88 years. Late presenter patients with AIDS related illness stage B and C were found in 19.1 and 50.1%, respectively. After access to ART, majority of patients (93.4%) achieved undetectable viral load 500 cells/ul), respectively. The aging group with >50 years old was higher than 2010 and increased to 20%. The metabolism profiles such as lipid, sugar and kidney functions test were more abnormal in the aging group. The quality of life was found in average level. Most of ARV regimen changes (36%) were due to lipoatrophy/lipodystrophy/lipohypertrophy. The death was found only in 2.4% with the majority of death (69.9%) from non AIDS conditions.

Conclusion: HIV-infected patients in Thailand who had access to ART, appropriate care and laboratory monitoring can live longer and had progression rate comparable to those in developed countries. Non-AIDS conditions are increasing and need to be carefully monitored in the long-term.

HIV controllers patients with distinct viral load cut-off levels have distinct virologic and immunologic profiles

F. Cortes[1], C. Passaes[2-1], G. Bello[1], S. Teixeira [1], C. Vorsatz[3], D. Babic[4], M. Sharkey[4], B. Grinsztejn[3], V. Veloso[3], M. Stevenson[4], M. Morgado[1]

[1]Laboratório de AIDS e Imunologia Molecular, Fundação Oswaldo Cruz, Rio De Janeiro, Brazil [2]Service d'immuno-virologie, Commissariat à l'Energie Atomique et aux énergies alternatives, Fontenay Aux Roses [3]Instituto de Pesquisa Clínica Evandro Chagas - IPEC, Fundação Oswaldo Cruz, Rio De Janeiro, France [4]Department of Medicine, Miller School of Medicine, University of Miami, Miami, United States

Objective: To assess the relevance of distinct RNA viral load (VL) cut-off levels on the overall profile of the group of HIV controllers.

Methods: We performed a cross-sectional analysis of virologic (total, integrated and 2-LTR DNA), genetic (HLA-B and CCR5) and immunologic (T cell populations, chronic immune activation, Gag/Nef IFN-γ ELISpot and BED-CEIAanti-HIV-1 IgG) parameters of HIV-1 infected patients who naturally control viral replication at distinct levels: 1) Elite Controllers (EC, n=7) with VL

Results: The median time of HIV suppression for our cohort of HIV controllers was nine years. This cohort was enriched in the protective HLA-B alleles B*27 and B*57, but not significant differences were observed among distinct groups of controllers. Total and integrated HIV DNA for EC were significantly lower ($p < 0.05$) in comparison to NC and HAART groups,and for EEC in comparison to NC ($p < 0.05$). 2-LTR circles were not detected in EC patients, but were detected in three out of five EEC patients and six out of seven VC patients. While EC and EEC maintain normal T cell counts over time; some VC displayed negative $CD4^+$ T cells slopes and $CD4^+/CD8^+$ T cell ratios < 1. Both VC and EEC showed higher percentage of activated $CD8^+$ T cells ($p < 0.005$) and sCD14 levels in plasma ($p < 0.005$ only for EEC) than HIV-1 negative controls. EC displayed a weaker Gag/Nef IFN-γ T cell response and a significantly ($p < 0.05$) lower proportion of anti-HIV IgG antibodies than EEC, VC and NC groups.

Conclusion: EEC and VC appear to mount a stronger HIV-specific immune response, but experience increases in the level of immune activation, microbial translocation and size of the HIV DNA reservoir compared to EC. These results highlight the heterogeneity of the HIV controller population and reinforce the needs to a precise classification of patients with different levels of viral suppression.

A colorimetric, HIV-1 viral load assay based on reverse transcription loop-mediated isothermal amplification (RT LAMP) for disease progression monitoring in resource-limited settings

A. Papadopoulos[3], C. Penny[3], L. Mcnamara[1], D. Evans[2]

[1]Clinical HIV Research Unit, Department of Internal Medicine, Faculty of Health Sciences [2]Health Economics and Epidemiology Research Office, Department of Internal Medicine, Faculty of Health Sciences [3]Medical Oncology Research Laboratory, Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Objective: Monitoring of HIV-infected individuals on anti-retroviral treatment (ART) requires periodic viral load measurements to monitor response to treatment. Routine viral load monitoring, by real-time RT PCR, is neither affordable nor available in most resource-limited settings. Reverse transcription loop-mediated isothermal amplification is a rapid, low-cost method of nucleic acid amplification. We aimed to develop an RT-LAMP based viral load assay, using hydroxy-naphthol blue (HNB) dye. The proposed assay is interpreted by an HNB-induced colour change in the reaction that distinguishes between samples having above or below a clinically relevant, threshold viral load value.

Methods: RNA was extracted from HIV-1 sub-type C culture supernatant and also from uninfected plasma for a negative control. RNA was added to the RT-LAMP reaction consisting of Bst DNA polymerase, Thermoscript RT, Betaine, Hydroxy-naphthol blue, magnesium sulphate, and 6 primers targeting the integrase region of the HIV-1 Pol gene. The reaction, alongside a negative control, no RT control and no template control, was incubated in a heating block at 59°C for 1 hour, followed by inactivation at 85°C for 5 minutes. The amplification product was analysed by agarose gel electrophoresis. Restriction digestion was performed with *Bse*XI, which has a recognition site in the 217 bp target sequence, to confirm specificity of the reaction.

Results: The LAMP reaction produces a characteristic ladder-like banding pattern on a gel. This was observed in the reactions containing RNA from HIV-1 culture supernatant but absent in the negative controls. The reaction colour correlated with what was observed on the gel, whereby positive reactions turned from purple to pale blue. Successful restriction digestion of the product by *Bse*XI, confirmed the presence of the integrase-target in the reaction.

Conclusion: The results demonstrate the initial steps for development of the assay. Further work is required to determine the minimum incubation time at which a colour change from violet to blue will represent a sample of 400 or 1000 RNA copies per ml. This must be followed by validation against routine viral load testing to determine positive and negative predictive value. The assay can potentially be developed into a low cost, point of care test.

High proportion of CXCR4-using viruses in vertically HIV-1-infected infants in Thailand

T. Samleerat[2], P. Phiayura[2], S. Hongjaisee[2], W. Sirirungsi[2], N. Ngo-Giang-Huong[2-3-4], F. Barin[1]

[1]INSERM U966, Université François Rabelais and CHU Bretonneau, Tours, France [2]Faculty of Associated Medical Sciences, Chiang Mai University [3]Institut de Recherche pour le Développement (IRD) UMI 174-Programs for HIV Prevention and Treatment (PHPT), Chiang Mai, Thailand [4]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Ma, United States

Objective: Previous studies evaluating HIV-1 coreceptor usage in pediatric patients have been performed in newborns and children infected with subtype B, C or D. However, the coreceptor use by CRF01_AE subtype, especially in vertically infected infants remains poorly characterized. In this study, we aimed to evaluate the HIV-1 coreceptor usage in infants with vertically-acquired HIV-1 infection in Thailand, where the predominant circulating HIV-1 strains are CRF01_AE and the minority are subtype B.

Methods: A total of 234 HIV-1 infected infants (106 male and 128 female) participating in the National AIDS Program of the National Health Security Office (NHSO) of Thailand in 2007-2012 were included in this study. All children were received ARV prophylaxis according to the Thai national guidelines. The median age was 75 days (range: 12-266). C2-V3-C3 gp120 was amplified in a triplicate nested-PCR and sequenced. Coreceptor usage was predicted using the Geno2pheno [coreceptor] algorithm and analyzed with a false positive rate (FRP) of 10%. HIV *env* subtype was determined by phylogenetic methods. CCR5-Δ32 and CCR5 -m303 mutation genotypes was performed by PCR and nested-PCR restriction enzyme analysis, respectively.

Results: Two hundred eleven infants (90.17%) were infected with CRF01_AE strain, 22 (9.4%) were subtype B and 1 (0.43%) were subtype C. Concordance in tropism prediction for the triplicates was observed in all samples. CCR5 coreceptor-using strains were found in 127 infants (54.27%) and CXCR4 coreceptor-using strains were found in 107 infants (45.73%). No significant difference in age and clinical signs of AIDS (p=1.000) were observed between these populations. CCR5-Δ32 and CCR5-m303 mutations that may contribute to a selective pressure of viruses to alternatively use CXCR4 as a coreceptor were not found.

Conclusion: A high proportion of HIV-1 CXCR4-using variants was found among HIV-1 vertically infected infants with mainly CRF01_AE strain in Thailand. These observations may have implications for clinical and therapeutic aspects, especially in the early stage of HIV-1 infection in infants and may benefits for using of CCR5-antagonists in this population. However, previous studies reported that CRF01_AE is associated with high viral load and fast HIV-progression, therefore further studies are needed to elucidate this issue in our pediatric population.

What can be learnt about broad ADCC immunity to HIV from Influenza?

S. Kent, S. Jegaskanda, L. Wren, V. Madhavi, I. Stratov

Microbiology and Immunology, University of Melbourne, University Of Melbourne, Australia

Objective: HIV and Influenza are both variable viruses and it is difficult for infected or vaccinated subjects to generate effective antibodies that neutralize the majority of field strains for both viruses. However, non-neutralizing antibodies with effector functions such as Antibody-Dependent Cellular Cytotoxicity (ADCC) may be able to recognize a broader array of viral strains and help reduce infection or control disease. We studied the breadth of recognition of HIV and Influenza strains by ADCC antibodies.

Methods: Serum samples from cART-naïve HIV+ subjects (n=79) and influenza-seropositive healthy adults (n=182) were studied for ADCC to multiple strains of HIV or Influenza using both killing-based ADCC assays (RFADCC for HIV and ADCVI for influenza) and antibody-dependent NK cell activation assays.

Results: Influenza-specific ADCC antibodies were found in serum from over 90% of adults and typically recognized multiple divergent strains including the hemagluttinin of seasonal H1N1 and H3N2 strains. A large portion of adults (54%) had ADCC antibodies to the swine-origin H1N1 strain PRIOR to the onset of the epidemic. H1N1pdm-specific ADCC antibodies pre-2009 were even more common in subjects >45 years of age (75%, $p < 0.01$), an age group that was relatively protected from disease. ADCC recognition of HIV was also common and broad in HIV+ subjects, with ADCC recognition of HIV also exceeding 90% of subjects and over three quarters of infected subjects recognizing multiple subtypes of HIV-1 Env. ADCC recognition of Vpu was rarer (11%) and only found in subjects with slow HIV progression.

Discussion: There are striking parallels in the prevalence and breadth of ADCC recognition of HIV and Influenza. The increased breadth of influenza ADCC antibodies in older subjects likely represents stimulation of these antibodies from multiple prior infections. The association of ADCC with reduced infection (for influenza) and disease progression (for HIV) suggests that ADCC-based vaccines or immunotherapies may hold promise for these infections.

Female participation in clinical studies in HIV

R. Johnston[2], M.J. Curno[1], H. Etya-Ale[1], I. Hodges-Mameletzis[1], M. Price[3], S. Heidari[1]

[1]International AIDS Society, Geneva, Switzerland [2]Research, amfAR - The Foundation for AIDS Research [3]International AIDS Vaccine Initiative, New York, United States

Objective: An important goal in HIV clinical research is ensuring that affected populations are appropriately represented. This analysis was undertaken to determine the proportion of women participating in clinical studies of antiretroviral therapy (ART), preventive vaccines (VACC) and curative strategies (CURE) and to identify factors associated with female participation in order to describe gaps that need to be addressed to achieve gender parity in clinical research.

Methods: Systematic searches in PubMed were conducted for each of the three areas of research. For ART studies, articles describing clinical trials published during three time periods between 1994-2011 were included. For VACC studies, articles published between 2000-2012 that reported primary results from trials were included. For CURE studies, papers describing clinical studies published through 2012 were included. Studies seeking to enroll participants of only one sex were excluded. Data were extracted on the number of women, sources of funding (e.g. public, private), study location and trial phase (where reported).

Results: In total, 395 ART, 63 VACC and 134 CURE studies met inclusion criteria. Despite allowing for enrolment of men and women, gender of study participants was not reported in 15.2% ART, 15.9% VACC and 21.6% CURE publications. In studies reporting participants' gender, women represented a mean of 22.9% ART, 38% VACC and 22.1% CURE of the trial populations. There was an increase in female participation over time for ART ($p < 0.001$) and VACC ($p=0.03$) studies, but no linear relationship between female participation and time for CURE studies ($p=0.88$). High income countries were associated with fewer women in ART studies ($p<0.001$) and CURE studies ($p=0.02$), and more women in VACC studies ($p=0.02$). Funding source was associated with female participation for ART studies ($p=0.02$) but not VACC ($p=0.61$) or CURE ($p=0.85$) studies.

Conclusion: Women continue to be underrepresented in HIV studies, which may lead to missed opportunities and unintended clinical consequences. Regulatory agencies, funders, civil society and care providers can all play a role to instate as routine the appropriate representation and analysis of women in clinical studies.

Making Hard Choices: The Role of Economics

A. Whiteside, J. Cohen, M. Strauss

Health Economics and HIV/AIDS Research Division, Caprisa, Durban, South Africa

Objective: With the world's largest treatment program and over 340,000 incident cases annually, the response to HIV in South Africa is hotly contested and there is sometimes a dissonance between activism, science and policy. [1] Too often, policy is prescribed by automated models, generated with good intention, but parameterized with only epidemiological data. Successful programs that will make a real impact hinge on much more. Fiscal, infrastructural, human resources for health, political, and socio-cultural factors drive and shape the epidemic and its response. We are seeing the potentially hazardous implications of the science and policy divide in relation to the implementation of a universal test and treat programme in South Africa.

Methods: Through an analysis of the financial, infrastructural, human resources for health and governance landscape in South Africa, we assess the feasibility and associated costs of implementing a universal test and treat programme.

Results: In light of the implementation of the National Health Insurance over the next 14 years, expanding HIV testing, prevention and treatment programmes, as well as the withdrawal of PEPFAR support, South Africa cannot afford to implement a universal test and treat programme. The health care system infrastructure is too weak and fraught with human resources for health shortages and imbalances to support the strain of an increase in treatment services on the scale of universal test and treat.

Conclusion: In situating universal test and treat within the governance, fiscal, human resources for health, and infrastructural landscape in South Africa, we conclude that a nuanced HIV treatment and prevention approach is more appropriate than a one-size-fits-all universal test and treat programme. Whilst South Africa continues to make progress in reducing the rate of new infections, reducing mother-to-child-transmission and AIDS-related mortality, and increasing treatment coverage, we cannot risk derailing this momentum with premature decisions. In order to curb the HIV epidemic, countries must make decisions based on their local context, resources, priorities and evidence.

Reference:

1. Pillay Y. Global AIDS Response Progress Report 2012. http://www.unaids.org/en/dataanalysis/knowyourresponse/countryprogressreports/2012countries/ce_ZA_Narrative_Report.pdf (accessed November 23, 2012)

Health Economics and HIV and AIDS Research Division

Mechanisms of M-DC8$^+$ ("slan-DC") monocyte and CD16$^+$ NK cell accumulation in the spleens from HIV-1-infected patients

S. Amraoui[2-1], S. Degrelle[2-1], C.A. Dutertre[2-1], J.P. Jourdain[2], K. Schaekel[4], B. Fabiani[3], L. Garderet[3], R. Cheynier[2], Y. Richard[2], A. Hosmalin[2]

[1]equivalent contribution [2]Department Infection, Immunity, Inflammation, Institut Cochin [3]Hôpital Saint-Antoine, Université Pierre et Marie Curie, Paris, France [4]Department of Dermatology, University Heidelberg, Heidelberg, Germany

Objective: Progressive HIV infection correlates with chronic immune hyperactivation, mediated by inflammatory cytokines like TNF. It is related to intestinal epithelial damage and microbial LPS translocation into the circulation. M-DC8$^+$ or slan (6-sulfo Lac NAc glysosylation of PSGL1) monocytes are known to be present in active lesions from patients suffering from evolutive Crohn´s disease or psoriasis. In the peripheral blood mononuclear cells (PBMC) from viremic HIV-1 infected patients, we found an accumulation of M-DC8$^+$ monocytes, which were mostly responsible for the exaggerated TNF response to LPS of the PBMC from these patients (Dutertre Blood 2012). We addressed whether M-DC8$^+$ monocytes were also in high numbers in the lymphoid organs from HIV-infected patients.

Methods: We studied mononuclear spleen cells from 10 patients (6 HIV$^+$ with idiopathic thrombocytopenic purpura, 4 HIV$^-$) and spleen cryosections from 17 patients (9 HIV$^+$ including 4 untreated by antiretrovirals, 8 HIV$^-$). We localized monocytes, NK cells and TNFα by 12-color flow cytometry and immunohistofluorescence, and performed mRNA microarray analysis.

Results: M-DC8$^+$ (DD2$^+$) and CD16$^+$ NK cells were present in higher numbers in HIV-infected than in uninfected patients. They expressed the CX3CR1 chemokine receptor, and fractalkine (CX3CL1) mRNA was overexpressed in spleens from HIV-infected compared to uninfected patients.

Conclusion: Following HIV chronic infection and active infection and destruction of CD4$^+$ T lymphocytes beneath the intestinal epithelial barrier, which becomes permeable to bacterial products, induced cytokines induce MDC8$^+$ monocyte differentiation, and chemokines attract them to lymphoid organs, where circulating bacterial products stimulate TNF-a secretion, hence immune system hyperactivation, further CD4$^+$ T cell activation and infection, direct intestinal epithelial damage. Therefore, M-DC8$^+$ monocyte depletion might interrupt this vicious cycle and restore homeostasis and viral control.

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Human immune deficiency virus (HIV) post exposure prophylaxes knowledge among medical doctors in khartoum teaching hospital (March-May 2012)

R. Abuelhassan

International Medocal Corps - Libya, Tripoli, Libya A thesis submitted in partial fulfillment to the University of Medical Science and Technology (UMST) of the requirements for the degree Master of Public and Tropical Health - Supervisor: Dr. Kamal Hanafi - Researcher: Dr. Rania Abdallah Abuelhassan, Khartoum, Sudan

Objective: This study was conducted to assess the knowledge, practice of HIV post exposure prophylaxis use among Medical Doctors in Khartoum teaching hospital.

Method: A cross-sectional study conducted from February to May 2012 in Khartoum teaching hospital, Seventy Nine Medical Doctor participated in this quantitative study. In addition, more than 20 medical doctors have been participating in focus group discussions and key informative interviews.

Results: Among the total 79 participants, 24% had adequate knowledge about post exposure prophylaxis of HIV and 68.5% had been exposed (according to their knowledge) to HIV risk conditions.

The FGDs related the limited utilization of PEP services to the inadequate knowledge about PEP itself and insufficient awareness about PEP, understanding the risk of the exposure and the existence of the Sudanese guidelines on PEP.

Among the adequate knowledge participants group; social dynamics analysis showed that 53% were in-between 35 to 44 age group and females were dominating this knowledge by 63%, More over in between the hospital departments Obstetrics staff presented 32% of the adequate knowledge population, and finally services years from 2 to 10 were presenting together 84%.

Conclusions: The knowledge of the Medical Doctors about post exposure prophylaxis against HIV is inadequate. Though many of the studied doctors had HIV risk exposure, no one ever used post exposure prophylaxis.

Behavioral intervention for reduction of HIV/STD transmission amongst sex workers in uganda

P. Batwala

Clinical Epidemiology, China Uganda Friendship Hospital Naguru, Naguru, Uganda

Background: The objectives of this intervention research were to increase knowledge and awareness among sex workers and their clients regarding STD's including HIV/AIDS, (1) to study the clinical –social aspects among STD patients.

Methods: Referred cases 100 between July 2011 and March 2012) intervention activities were conducted through health care education, intensive counseling, awareness and condom use. Patients attending STD clinic were screened for anti-HIV antibodies. 96 male STDs in the age range of 17-50 years (27.35, 7.69) and 32 female sex workers in 15-45 years (26.94, 6.82) were included in the study. Information regarding their STD/AIDS perceptions, condom use, and partner relationship was obtained on a specialty prepared pro-forma, Although, very few sex workers used F.P. method, 10% males reported a condom usage at the some time, despite reporting risky behavior with multiple sex partners.

Results: In the beginning of the study very few sex workers (60%) were aware of AIDS disease. through, some of them (10%) were aware on the usefulness of condoms but practically none of them were using the same. However, after providing health care education and intervention counseling to the sex workers, 32.03% of the sex workers ensured that all their clients used condoms but regularly. STD was detected in 36.4% male and 37.5% female sex workers through case histories and clinical examinations including laboratory investigations.

Conclusions: The prevalence of both HIV1 and HIV2 infection amongst the sex workers in Uganda indicates that there is a need for an intensive health case education, counseling and awareness programmes to affect behavior change and STD control amongst the high risk groups.

Knowledge and attitudes of the youth in relation to Human Immunodeficiency Virus Counselling and Testing at the primary health care services of Limpopo Province, South Africa

S. Maputle

Department of Nursing Science, University of Venda, Limpopo Province, South Africa

Introduction: The Human Immunodeficiency Virus testing is an integral part of HIV prevention, treatment, and care efforts and knowledge of one's status is important for preventing the spread of the infection. As indicated by the World Health Organisation, those clients who have learnt that they are HIV positive are able to modify their behaviour with the purpose of reducing the risk of HIV transmission. The objectives of this quantitative study have been to assess the knowledge of the youth about HIV counselling and testing and also to determine the attitudes of the youth about HIV counselling and testing in the Primary Health Care services of the Limpopo Province, in South Africa.

Method: The context of the study was four Primary Health Care facilities. The target population was the youth between the ages of 16 and 35 years. The sampling method was non-probability convenient sampling, while data were being gathered by using a structured questionnaire. The data was summarised by means of descriptive statistics. Validity and reliability were ensured by undertaking a literature review; and developing and pre-testing the questionnaire. Ethical considerations were adhered to, and when necessary, the participants had to obtain consent from a parent or guardian to participate in the study.

Results: Results indicated that youth has knowledge and aware of the availability of HCT services; however had negative attitudes on the utilization.

Conclusion: Recommendations were made for future research studies in the field of HIV and AIDS prevention, and establishment of Youth-friendly HCT services at Primary Health Care facilities.

Undiagnosed HIV infection among adolescents seeking primary health care in Ghana

A. Simpore

City of God Foundation, Accra, Ghana

Background: Mother-to-child transmission of human immunodeficiency virus (HIV) infection was extremely common in the wester region of Ghana during the 1990s, and a substantial minority of infected infants have survived to reach adolescence undiagnosed. Studies have shown a high prevalence of HIV infection in hospitalized adolescents who have features associated with long-standing HIV infection, including stunting and frequent minor illnesses. We therefore investigated the epidemiology of HIV infection at the primary care level.

Methods: Adolescents (aged 10-18 years) attending two primary care clinics underwent HIV and Herpes simplex virus-2 (HSV-2) serological testing, clinical examination, and anthropometry. All were offered routine HIV counseling and testing. Patients attending for acute primary care (APC) who were HIV infected were asked about their risk factors.

Results: Five hundred ninety-four participants were systematically recruited (97% participation), of whom 88 (15%) were attending for antenatal care. HIV infection prevalence was higher among APC attendees than among antenatal care attendees (17% vs 6%; P < .007), but for the prevalence of HSV-2 infection, a marker of sexually acquired HIV, the converse was true (4% vs 14%; P < .002). Seventy (81%) of 86 HIV-positive APC attendees were previously undiagnosed. They had a broad range of presenting complaints, with a median CD4 cell count of 329 cells/microL (interquartile range, 176-485 cells/microL) and a high prevalence of stunting, compared with the corresponding prevalence among HIV-negative attendees (40% vs 12%; P < .001). Maternal transmission was considered to be likely by 69 (80%) of the 86 HIV-positive APC attendees, only one of whom was HSV-2 positive.

Conclusions: Unrecognized HIV infection was a common cause of primary care attendance. Routine HIV counseling and testing implemented at the primary care level may provide a simple and effective way of identifying older long-term survivors of mother-to-child transmission before the onset of severe immunosuppression and irreversible complications.

Public Health and Economic Consequences of Point of Care CD4 testing in South Africa

T. Stander[2], H. Miller-Janson[2], M. Bergh[2], C. Marais[2], B. Williams[3], J. Hargrove[3], R. Wood[1]

[1]Desmond Tutu HIV Centre, Cape Town [2]HEXOR (Pty) Ltd, Gauteng [3]SACEMA, Stellenbosch, South Africa

Background: Loss to follow-up (LTFU) and retention in care along the HIV treatment cascade remains a barrier to reaching universal access to antiretroviral (ART) coverage in South Africa. We explored the potential impact of Point of Care (POC) CD4 testing on LTFU and retention in care.

Methods: A multistate dynamic Markov model was constructed to replicate the disease progression of an HIV infected individual from infection until death. Using this model, we compared the public health, economic and financial impacts of the current centralised laboratory CD4 testing strategy to those of a POC CD4 testing strategy.

Results: By modelling these impacts the results were estimated over a 100 year time horizon. In the base case scenario, the number of HIV infected individuals can be reduced by 35,549 in 5 years, 72,540 in 10 years and 168,047 in 20 years, by using POC CD4 technology. This equates to 1.27 million new HIV infections averted over 100 years. In addition, 1.45 million new TB cases can be averted, 32.68 million life years can be gained and R144 billion in direct health care costs saved.

Conclusion: POC CD4 testing has a significant impact on LTFU and retention in care in the HIV treatment cascade by confirming CD4 count at the time of HIV diagnosis, improving retention in care for patients who require ART immediately, and increasing the proportion of patients who achieve viral suppression. It is cost saving when compared to centralised laboratory CD4 testing. The results indicate that a POC CD4 testing strategy could potentially bring substantial public health, economic and financial gains if implemented in South Africa and significantly contribute toward universal access to ART.

HIV drug resistance to dolutegravir (DTG) simultaneously diminishes viral replication fitness and viral DNA integration into host cells: what does this mean for HIV reservoirs

T. Mesplede, P. Quashie, M. Oliveira, M. Wainberg

Jewish General Hospital, McGill University AIDS Centre, Montreal, Canada

Objective: We are studying the relationship between viral replicative fitness and drug resistance, in the hope of developing a curative strategy for HIV. No HIV-infected drug-naive patient has yet developed resistance against dolutegravir (DTG). To characterize the resistance profile of this drug, we selected for resistance in tissue culture.

Methods: HIV-1 of different subtypes was grown in both MT-2 cells and in peripheral blood mononuclear cells, with the concentration of DTG being incrementally increased from 0.05 nM, i.e. 4 times less than the EC50. After 6 months, a final drug concentration of 50-100nM was achieved, beyond which virus could no longer be grown. Viral DNA was sequenced and site-directed mutagenesis was performed to confirm the relevance of any mutations in the integrase gene.

Results: R263K or G118R followed by H51Y were the most frequent integrase resistance mutations to arise in subtypes B and C, respectively. R263K alone conferred an approximate 2-3-fold level of resistance to DTG and a 30% drop in levels of recombinant integrase strand transfer activity and viral replicative capacity. H51Y alone did not significantly affect either enzyme activity or DTG resistance, but the combination of R263K together with H51Y increased DTG resistance to about 12-fold accompanied by a ≈70% loss in each of:

- viral replication capacity
- integrase strand tranfer activity as measured in biochemical assays using purified integrase enzyme
- the ability of viral DNA to become integrated into host cell DNA. Over > 1 year, no additional possibly compensatory mutations were identified.

Conclusions: These data are in contrast to those obtained with other drugs, whereby secondary mutations increase overall levels of drug resistance and simultaneously increase viral replication and enzyme function. We have shown why primary resistance to DTG has not yet arisen in clinical studies. The use of animal models may both validate our results and ultimately suggest that DTG be used to help purge HIV cellular reservoirs, perhaps over several cycles of DTG treatment, if it can indeed be confirmed that resistance to DTG is not compensated by other mutations located either within the integrase gene or elsewhere in the HIV genome.

HIV/AIDS in Africa: Could the story have been different in Nigeria?

C. Williams

Department of Haematology, College of Medicine, University of Ibadan, Ibadan, Nigeria Fred Hutchinson Cancer Research Center/University of Washington Center for AIDS Research, Seattle, Washington, United States

The current high seroprevalence rate (SPR) for HIV in Subsaharan Africa stands in striking contrast with the low rates, even in individuals of high-risk lifestyle, observed in surveys of pre-pandemic period in certain locales. Human retroviral research in Nigeria dates back to 1983 with the description of the index case of adult T-cell leukaemia/lymphoma. Subsequent screening of normal blood donors, school children and patients with haematological disorders with first-generation ELISA and lymphocyte immunophenotyping techniques revealed HTLV-I SPR of 6.4%, 0.0% and 0.0%-13% respectively, as well as unexplained fatal cases of immunodeficiency and lymphadenopathy/dermatitis. A WHO sponsored survey of risk factors of retroviral infection carried out in parts of Nigeria from 1985 to 1986 involved assessment of lifestyle of members of 5 population groups by a detailed questionnaire. They included 237 normal blood donors (NBD), 46 female commercial sex workers (FCSW), 54 male-, 17 female celibates (MC/FC) and 42 sexually transmitted diseases (STD) clinic patients (STDCP), who were screened for HTLVs and HIVs by ELISA and Western blot (WB). HTLV-I SPR for NBD, FCSW, MC, FC and STDCP were 4.6%, 13%, 16.7%, 1.85% and 11.8%. Multivariate analysis revealed eastern Nigerian origin (ENO) (p=0.0000095), female sex (p=0.037) and female sex of ENO (p=0.0006) but not ethnicity (p=0.215) or polygamy (p=0.43) as risk factors for HTLV-I infection. Confirmation of HIV-1/2 SPR in the study group was not possible until the development in the 1990's of the recombinant enhanced "Singapore" HIV-1/2 WB, which identified 2 HIV-1, but 0 HIV-2 cases, thus yielding HIV-1 SPR of 0% for FCSW, MC, FC, STDCP, and 0.15% -1.0% in NBD nationally. Retrospective estimates indicates expected ≈2400-4800 AIDS deaths in 1985/6 at the University College Hospital, the main health care unit of the region, ≈5 of which were clinically diagnosed. In addition to the educational challenges resulting in the knowledge gap (vis-à-vis the situation in developed countries) about HIV/AIDS, cultural and infrastructural challenges probably also contributed to the failure of averting the disastrous progression of the pandemic in areas where timely intervention might have been effective. Senegal was another locale where retroviral research program was in place prior to the HIV/AIDS crisis. The low Senegalese HIV SPR probably resulted from early recognition of the dangers of the new disease and the prevailing cultural millieu, in contrast to other West African locales. Recent Ugandan experiences indicate that a reversal of the pandemic is possible in Subsaharan Africa. Lessons learnt from failure to control HIV/AIDS could help in confronting emerging new health challenges, including AIDS associated malignancies, breast and lung cancer as well as other diseases of "westernization" and changing lifestyles in Africa.

Characterization of integrase region polymorphisms in HIV-1 CRF35_AD viruses from treatment-naïve patients

A. Memarnejadian[1], F. Jahanbakhsh[1], S. Ibe[3], J. Hattori[3], L. Sadeghi[1], A. Mansouri[1], W. Sugiura[3-4], K. Azadmanesh[2]

[1]Hepatitis & AIDS Dept. [2]Virology Dept., Institut Pasteur of Iran, Tehran, Iran [3]Department of Infectious Diseases and Immunology, Nagoya Medical Center [4]Graduate School of Medicine, Nagoya University, Nagoya, Japan

Objective: Natural polymorphisms of HIV-1 are widely studied in PR/RT regions, however; integrase (IN) region of non-B subtypes, especially circulating recombinant forms (CRF) are not well-characterized. Herein, we analyzed the natural polymorphism of IN-coding region in HIV-1 CRF35_AD, the dominant type in Afghanistan, Iran and Pakistan.

Methods: IN-coding region (864 bps) from 90 antiretroviral-naïve Iranian patient samples, which were previously determined as CRF35_AD based on PR-RT regions, was amplified and sequenced. As reference, 13 CRF35_AD IN sequences from Afghanistan were also retrieved from Los Alamos database. Nucleotide (nt) and amino acid (aa) sequences of all 103 samples were aligned either solely or separately against the 2004 Los Alamos clade B and clades A consensus sequences. Reported IN strand transfer inhibitor (INSTI) resistance mutations based on IAS-USA 2011, Stanford 2012 and ANRS 2010 were defined as: H51Y, Q65K, T66I, V72I, L74M, E92Q, T97A, F121Y, E138K, G140S, Y143R/C/H, P145S, Q146P, S147G, Q148H/R/K, V151A/L, S153Y, N155H, E157Q, G163R/K, S230R and R263K.

Results: Within the 103 analyzed sequences, mean intra-subtype nt and aa distances were 2.3% (range 0.8-9.1%) and 2.8% (range 1.3-7.5%), respectively. Of 288 aa positions 205 (71.2%) were conserved, in 58 (20.1%) positions the predominant residue was spread to more than 95% of sequences and in 25 (8.7%) positions the most predominant aa was spread in fewer than 95% of sequences that were considered as polymorphic. Mean inter-subtype nt and aa distances were respectively 7.1% and 5.3% (against clade B consensus), 3.3% and 2.9% (against clade A1 consensus), 6% and 5.3% (against clade A2 consensus). All catalytic residues at functional positions (DDE, HHCC) were absolutely conserved, however; among extended active residues, I141F/V (2.9%) was observed. Among INSTI-resistance associated mutations outside of the extended active sites L74M/V/I/L (3.8%), T97A/S (2.9%), E138K/D (1.9%), G140R (0.9%), E157Q/K (1.9%), G163V (0.9%) and R263K (1.9%) were detected. Several mutations including I60M (91%), I113V (93%), T125A (92%), R127K (96%), K136Q (90%) and V201I (99%) occurred as typical signatures for CRF35_AD.

Conclusions: CRF35_AD is rich in IN region natural polymorphisms. Further studies need to verify the effect of such natural resistance mutations/polymorphisms on the treatment of HIV/AIDS with INSTIs.

Importance of completing a diagnostic algorithm in HIV mother-to-child transmission

R. Toro[2], P. Mayon[2], M. Valle[2], M. Corazza[2], M. García[1], J. Morales[1], M. Agosti [1]

[1]Servicio de Enfermedades Infecciosas "Prof. Dr. Emilio Cecchini", Hospital de Niños "Sor María Ludovica" [2]Centro de Referencia de VIH/SIDA y Hepatitis Virales, Instituto Biológico, La Plata, Argentina

Aim: To report a case of HIV mother-to-child transmission with four non detectable RNA samples, which later had a serological positive HIV diagnosis at 20 months age.

Materials and Methods: Four samples from an HIV positive mother's child were analyzed by plasmatic RNA. HIV 1/2 antibody analysis was performed in a sample at 20 months age. For the diagnosis of mother-to-child transmission, we used the algorithm proposed by the Ministry of Health of the Buenos Aires Province, in which a child under 18 months age is considered HIV positive when two positive samples analyzed by virological methods, render positive. The first and second RNA determinations were performed by Cobas Amplicor (Roche), and the third and fourth by Cobas TaqMan HIV-1 test (Roche). The HIV 1/2 antibody analysis was performed with a Vironostika Ag/Ac HIV 1/2 kit (BioMerieux) and a HIV BLOT 2.2 kit (MP Diagnostics).

Results: The maternal diagnosis was made at birth, which was delivered vaginally. The child was admitted to a treatment and monitoring protocol. AZT was indicated from birth until six weeks old and also breastfeeding restriction was recommended. Quantitative RNA was performed at 17 days, 1, 3 and 4 months of age. In these 4 samples, viral RNA was not detected. Serology at 20 months of age was reactive by ELISA, with a positive banding pattern by western blot (CDC criteria), and viral load was 343,926 copies/ml (5.54 log).

Conclusions: The diagnosis of HIV in this child was performed by serological methods, at 20 months of age after 4 undetectable RNA samples. This case highlights the importance of prenatal prevention which allows early diagnosis, the implementation of the recommended conduct during pregnancy and childbirth and also avoids reaching childbirth without HIV diagnosis. It also stresses the importance of completing the diagnosis algorithm after 18 months of age even in patients with 3 undetectable RNA samples, to ensure maternal antibodies seroreversion or the detection of a late infection.

Design of nanocarriers for the intracellular delivery of triphosphate nucleotide analogues

G. Giacalone, H. Hillaireau, E. Fattal

Institut Galien Paris-Sud, UMR CNRS 8612, Faculté de Pharmacie, Université Paris-Sud, Chatenay-Malabry, France

Objective: Nucleotide analogues such as Azidothymidine-triphosphate (AZT-TP), the active form of Zidovudine, display important pharmacological activity for the treatment of HIV. The administration of these nucleotide analogues would bypass the intracellular phosphorylation which can be a metabolic bottleneck. This possibility is however limited by their instability in physiological conditions, and furthermore their hydrophilicity restricts their access to the target cells.

To address these issues, several nanocarriers have been proposed so far for the encapsulation of these molecules, but their applications are limited due to the low drug loading achieved.

Our strategy proposes the use of chitosan, a biocompatible and hydrophilic polysaccharide which is known to form nanoparticles through complexation with TPP; in contrast with previous methods consisting in loading preformed particles, in our case the drug itself will be the driving force for the formation of nanoparticles [1].

Methods: Different molar ratios between chitosan and AZT-TP (or ATP, used here as a model drug) have been tested in order to study the formation of nanoparticles; some selected ratios have been then evaluated for their size, surface properties, drug encapsulation and loading. In vitro cell uptake experiments were performed on a cell line of mouse macrophages (J774.A1), using the free molecule as a negative control. The intracellular distribution of the delivered molecules was further investigated using confocal laser microscopy.

Results: Colloidal suspensions have been obtained from chitosan and AZT-TP at N/P ≥ 1; nanoparticles present a minimal size about 200 nm with a zeta potential above +20 mV. An encapsulation efficiency of 70% can be reached, allowing loading rates as high as 44% w/w. A cell viability of 80% has been found for particle concentrations up to 0.6 mg/mL. The cellular uptake is at least 2-fold higher when molecules are delivered as nanoparticles, compared to the free molecules.

Conclusion: An original method is proposed to design nanoparticles, which allows high loading rates; this lowers the amounts of excipients needed, thus limiting the toxicity concerns. These nanosystems allow an efficient in vitro intracellular delivery of nucleotide analogues, namely AZT-TP. Further in vivo studies will investigate the potential of these nanocarriers.

Reference:

1. Giacalone G., Bochot A., Fattal E., Hillaireau H. *Biomacromolecules*.

Evaluation of HIV mother-to-child transmission for 5 years in public hospitals of Buenos Aires province, Argentina

R. Toro, P. Mayon, M. Valle, C. Gatti, M. Corazza

Centro Provincial de Referencia de VIH/SIDA y hepatitis virales, Instituto Biológico, La Plata, Argentina

Aim: To evaluate the HIV mother-to-child transmission (MTCT) in children born between 2008 and 2012 in public hospitals in Buenos Aires Province, Argentina.

Materials and Methods: We performed a retrospective study of 1345 children born to infected mothers between 2008 and 2012, monitored by this laboratory to detect possible MTCT. We applied the diagnostic algorithm proposed by the Ministry of Health of Buenos Aires Province, considering HIV positive all children born to infected mothers with two positive samples from virological methods. The plasmatic RNA was detected by Cobas Amplicor (Roche) until 2011 and Cobas TaqMan HIV-1 (Roche) in 2012. Maternal data such us time of HIV diagnosis, antiretroviral treatment, breastfeeding, mode of delivery were recorded.

Results: Plasma RNA analysis was performed in 1345 children born to HIV infected mothers. HIV was diagnosed in 63 children (4.68%). The positive rate was: 4.80% in 2008 (10/208), 3.50% (10/286) in 2009, 5.36% (15/280) in 2010, 3.62% (11/304) in 2011, 6,37% (17/267) in 2012. HIV diagnosis in mothers was made before or during pregnancy in 1133 women (84.24%) and at the time of delivery or postpartum in 196 cases (14.57%). Considering only the cases of infected children, these numbers are 35 (56.45%) and 27 (43.54%) respectively. There were no available data for 16 mothers (1.19%).

Conclusions: The percentage of children infected by MTCT was 4.68%. This rate has remained stable (between 3 and 6%) through the study years. The diagnosis before or during pregnancy was performed in a high percentage (84.24%), but when considering infected children this percentage decreases significantly (56.45%). Noteworthy, the Argentinean public health system guarantees access to diagnosis, treatment, and monitoring of HIV-positive patients. Social factors should be considered along with the scientific and technical advances, in the implementation of strategies for removing vertically acquired infections and to contribute to the goal of zero new infections by 2015.

Novel 2-styryl-8-hydroxyquinoline inhibitors (8SQs) demonstrate anti-integrase and protease HIV-1 activity in cell culture

K. Stanoeva[1], A. Hinkov[3], S. Raleva[2], A. Pavlov[4], R. Argirova[2]

[1]Medical University - Sofia [2]Dept. of Virology, National Center of Infectious and Parasitic Diseases [3]Lab. of Virology, Faculty of Biology, Sofia University, Sofia [4]Dept. of Chemistry and Pharmacology, Trakia University, Stara Zagora, Bulgaria

Objectives: Some SQs were found earlier to be integrase (IN) inhibitors (Bonnenfant, S. et al. 2004) This stimulated us to synthesize and evaluate new 2-Styryl-8-hydroxyquinolines (8SQs) for anti-HIV activity in cell culture.17 novel 8SQs were synthesized in Trakia University, Stara Zagora, Bulgaria and studied for cytotoxicity and anti-HIV activity in cell culture.

Methods: MTT uptake assay, reverse transcriptase (RT) activity (Cavidi, Sweden) in supernatants and directly on recombinant RT were checked on MT-4 cells acutely infected with HIV-1 IIIB strain. Protease (PR) as a target was studied by modified screening method by direct spectrophotometric reading of specific substrate utilization by native HIV-1 PR in absence/presence of the studied substances.Biologically active compounds not targeting RT or PR (two out of seven), were evaluated for IN-activity by selection of viral mutants after continuous exposure to increasing concentrations of them during 30-33 passages. Replicative kinetics and dynamics of emergence of resistant mutants were documented and IN domain was sequenced. Mitochondrial toxicity of anti-IN inhibitors was studied by Real-time PCR.

Results: Seven novel 8SQs inhibitors of HIV-1 IIIB replication were found – three out of them were RT-inhibitors, two others showed anti-PR-activity and two – anti-IN activity - all with IC50 in nanoM concentrations. Both novel IN inhibitors – 105B and 241demonstrated also weak PR activity (21% for 105B and 25% for 241). Double mutants were selected after passaging with them: one mutation was localized in NTD domain (N17S and E10D for 105B and 241 respectively) and the other – identical for both mutants (D231I) - in CTD region. The identical mutation is probably due to partially common chemical structure – different substitutes at C2 in the phenol ring. Accordingly, 100 – 1000 fold increase of IC50 was observed for those mutants. One of them (105B) showed mitochondrial toxicity.

Conclusions: Despite considerable progress in development of antiretroviral therapy, problems of toxicity, adherence and resistance still cause a therapy failure. Therefore, there is an urgent need for new drugs with low cytotoxicity and high activity. Here we describe 7 new 8SQs inhibitors of HIV-1 replication in MT-4 cells – two out of them – IN inhibitors with weak anti-PR activity.

The role of operational research to understand HIV/AIDS regional healthcare system in Brazil and its importance to scaling-up antiretroviral therapy

G. cortes Fernandes[2-1], M. Cavaliere De Almeida[3-1], E. Massae Yokoo[3], S. Hiromi Tuboi[3]

[1]Centro de Epidemiologia, Estatística e Pesquisa, Santa Casa de Misericórdia de Juiz de Fora, Juiz De Fora [2]Faculdade de Medicina, Universidade Federal de Juiz de Fora, Juiz de Fora [3]Instituo de Saúde da Comunidade, Universidade Federal Fluminense, Niterói, Brazil

Objective: Transition between scientific evidence and its implementation is difficult. Despite improved care resulting from antiretroviral scale-up, morbidity related to inadequancies in care delivery still exists. Lost of follow-up may range from 15% to 55%. As a result, retention in care has been increasingly recognized as an important program indicator. The aim of this study is to help local care delivery programs to better utilize quality improvement methods by detecting usefull indicators, understanding their weakness and providing strategies to address their own performance and to redesign processes.

Methods: Using a cohort study we applied standard quality improvement concepts focusing on retention in care and clinical outcomes, redesigned local processes, collected data and highlighted critical steps in process to identify possible performance indicators. Patients over 18 years-old under antiretroviral therapy at a regional reference center for HIV/AIDS care in Brazil who attended medical appointments from July to August/2011 were included in the study. Lost to follow up was defined as those who hadn't returned to medical appointment within 90 days after the 6th month of treatment. In order to evaluate the relation between individual factors and the results, the variables with < 0,25 in unvarying analyses were included in a logistic regression model, in which, a level of significance of 0,05 was adopted.

Results: Among 250 patients included in the study 44 (17,6%) were lost, 1 (0,4%) died, and 2 (0,8%) were transferred to another institution. Age (OR 2,30 p=0,04), time of diagnose, time of admission to the program and the assistant physician (OR 5,90 p=0,00) were statistically related to lost to follow up.

Conclusion: The estimated loss to follow up allied to the identification of factors associated to the record of death allow us to conclude that a better understanding of the process is needed and this cohort provided the first step for process understanding and optimization. Although we have in Brazil a scenarium of well stablished infrastructure for HIV care (multidiscilinary staff, free access to medication and laboratory tests), processes and outcomes are not ordinarily optmized or monitored. Local cohort studies could help local programs to understand barriers to retention and to define more efficient tools and strategies to optimize and monitor their process.

Accumulation of deleterious mutations: a new strategy to limit HIV-1 proliferation?

V. Vivet-Boudou[2], C. Isel[2], Y. El Safadi[2], R. Smyth[1], G. Laumond[3], C. Moog[3], J. Mak[1], J.C. Paillart[2], R. Marquet[2]

[1]Centre for Virology, Burnet Institute, Melbourne, Australia [2]IBMC, UPR 9002, Architecture et Réactivité de l'ARN, CNRS [3]Interaction virus-hôte et maladies hépatiques, INSERM-Université de Strasbourg, Strasbourg, France

The development of new drugs targeting HIV-1 is imperative due to the high mutation and recombination rates of this virus which favour the emergence of resistant strains. The need for new drugs will intensify with the increasing number of HIV-1 positive persons who have access to antiviral therapy. In this context identifying new HIV-1 inhibitor targets would minimize the risk of emergence of mutations conferring cross-resistance to a large number of drugs.

With the goal of limiting HIV-1 proliferation by increasing the mutation rate of the viral genome, we synthesized a series of pyrimidine nucleoside analogues modified in position 5 of the aglycone moiety but unmodified on the sugar part. The synthetic strategies allow us to prepare the targeted compounds directly from commercially available nucleosides. All compounds were tested for their ability to reduce HIV-1 proliferation in cell culture. Two of them displayed a moderate antiviral activity in single passage experiments. The same two compounds plus two additional ones were potent inhibitors of HIV-1 RT activity in serial passage assays, in which they induced a progressive loss of HIV-1 replication. In addition, viruses collected after seven passages in the presence of these compounds replicated very poorly after withdrawal of these compounds, consistent with the accumulation of deleterious mutations in the HIV-1 genome. To confirm our results we peformed thermal denaturation of short DNA duplexes modified by our compounds and extension of a primer annealed to a modified template. We also determined the mutation rate and spectrum of the HIV-1 genome upon treatment with the two most interesting compounds in cell culture and compare them to the *in vitro* data.

A more detailed analysis of the anti-HIV-1 activity in cell culture and in vitro as well as the results of the biophysical evaluations which resulted in the identification of two inhibitors compounds will be presented.

Strategies for HIV- and TB-co-infected patients in the Ukraine

R. Otto-Knapp[2], Y. Lesiuk[4], A. Frieder[3], C. Fléchet[2], A.K. Starzacher[2], T. Bauer[2], H. Karcher[1]

[1]Department of Infectiology, Charité-Universitätsmedizin [2]Helios Klinikum Emil von Behring-Pneumologie, Berlin [3]Connect plus e.V, Würzbug, Germany [4]Medical Director, AIDS Centre, Chernivtsi, Ukraine

Objective: HIV and TB services currently exist in the Ukraine as independently operating structures with the consequence of suboptimal care of co-infected patients. TB care is performed in centralized structures facing the problem of rising numbers of multiresistant TB (MDR-TB). In 2007 16% of new cases were MDR-TB [1]. HIV is a growing problem in the Ukraine (estimated prevalence: 1,6% [2]). With the help of international donors HIV centers have been established in every Oblast in the recent years. This project aims to improve the cooperation between HIV and TB services.

Methods: In September 2009 a workshop was held in Chernivtsi, Western Ukraine. Participants were the medical directors of the TB and HIV services of 4 Oblasts, 2 representatives of the German non-governmental organisation (NGO) Connect plus e.V., a German HIV and a TB specialist. Routine procedures and problems arising in the daily work with co-infected patients were presented and analyzed. The Workshop results and future strategies were discussed thereafter with representatives of WHO Ukraine.

Results: Following main problems were identified:

- Case finding: the transfer of HIV patients to TB-facilities for TB-testing leads to a loss of patients and reports.
- Diagnostic: modern culture methods and rapid drug susceptibility testing are not available. X-Ray equipment is often of low quality and maintenance is not assured.
- Treatment: TB medication can exclusively be prescribed by TB specialists. A surveillance system for co-infected patients does not exist.
- Prevention: Isolation with face masks is missing. MDR patients are often isolated in groups. Instructions for postexposure prophylaxis are missing.

Conclusion: Following strategies were set up:

- A physician in every TB clinic at Oblast level has the task to identify co-infected patients, integrate them into HIV care and develop appropriate treatment strategies.
- The outpatient long-term care of co-infected patients will take place in the AIDS-centres. HIV-clinicians will receive a permission to prescribe TB drugs.
- A regional HIV/TB coordinator will supervise the project progress on site. A local surveillance system will be set up with electronic data collection according to recommendations of the International Union Against Tuberculosis and Lung Disease [3].

Literature:

1. WHO, TB country profile, Ukraine, 2007
2. Epidemiological fact sheet 2008, UNAIDS/WHO Working Group on Global HIV/AIDS
3. Williams G, Alarcon E, Jittimanee S, Walasimbi M, Sebek M, Berga E, Scatena Villa T. Best practice for the care of patients with tuberculosis. A guide for low-income countries. International Union Against Tuberculosis and Lung Disease, Paris: 2007

SIVagm infection of rhesus macaques: a model of functional cure with persistent reservoirs of replication-competent virus

C. Apetrei[2], D. Ma[2], A. Cillo[1], C. Xu[2], J. Kristoff[2], J.W. Mellors[1], I. Pandrea[2]

[1]School of Medicine, University of Pittsburgh [2]Center for Vaccine Research, University of Pittsburgh, Pittsburgh, United States

Objectives: SIVagm infection of rhesus macaques (RMs) is functionall-cured: initial high level viremia (10^8 copies/ml) and massive mucosal $CD4^+$ T cell depletion are followed by durable control of SIVagm replication, complete recovery of $CD4^+$ T cells, normalization of T-cell activation and seroreversion. The advantage of this new model is that the functional cure occurs in all SIVagm-infected RMs. Immune control of SIVagm replication can be temporary reversed by experimental CD8-cell depletion. Our objectives were to further characterize the RM/SIVagm model of functional cure and test activation strategies in vitro.

Methods: Complete control of SIVagm persistent chronic SIVagm, viremia was confirmed by using qPCR with single copy sensitivity (SCA). Replication competence of the controlled virus was determined by inoculation of uninfected RMs with plasma from CD8-depleted controllers. Diversity of the rebounding virus was assessed using single genome amplification (SGS). *In vitro* activation of the virus from the persistent reservoirs was performed with HDAC inhibitors (SAHA and Romidepsin).

Results: SCA revealed low viremia, averaging 20 copies/ml (range: 10-30), in RMs in the initial stages of the virus control (9 months after undetectable viremia by conventional qPCR). In the long-term controllers (4 years after infection) the levels of plasma RNA ranged from 0.5-2 copies/ml). RM inoculation with plasma collected during virus rebound after CD8 cell depletion resulted in peak viremia of 10^8-10^9 SIVagm RNA copies/ml, followed by control of viremia with kinetics similar to that following infection with high titer SIVagm stock virus. SGA of rebound plasma virus after CD8 cell depletion revealed sequence homogeneity consistent with clonality. Rebound virus was genetically similar to the original SIVagm stock, suggesting that the viral reservoirs that were the source of the rebounding virus were seeded early after infection.

Conclusions: Our results further validate SIVagm-infected RMs as a model of functional cure of replication-competent retrovirus infection. Deciphering the mechanisms of control may identify new strategies to achieve functional cure. This model is well suited to “dissect” activation strategies aimed to deplete viral reservoirs without the complexity of multidrug antiretroviral therapy and the need to boost cellular immune responses (which effectively control the virus in our model).

Success of MMT in HIV positive opioid dependent patients in Georgia

K. Todadze

Research Institute on Addiction, Centre for prevention of mental health and drug addiction, Tbilisi, Georgia

Background: The main route of HIV transmission is injective use of opioids (55-60%) in Georgia. Although prevalence of HIV among drug users is only 2-3%, the high number of IDUs and high prevalence of hepatitis C in this population could be the predictor of HIV increase. Methadone maintenance treatment (MMT) has been implementing throughout the country since 2005 as one of the important harm reduction strategies.

Method: 42 randomly selected HIV positive drug users undergoing MMT with intensive psychological counseling have been studied for 3 years. They received ARV therapy before inclusion in MMT at least 6 month. Quality of life, level of depression, anxiety and other data were measured before starting MMT and after 3, 6, 12, 18 months. The illegal use of psychotropic-narcotics was checked through random urine-testing 3 times per patient per month.

Results: The study showed significant improvement of patients' status. The remarkable decrease of depression and anxiety was observed (dynamic of average scores of depression - 24, 14, 14, 13, 14 and anxiety-46, 40, 40, 41, 39). Life quality increased in comparison with the starting data (76, 85, 86, 88, 93). The positive answers on psychotropic-narcotics were observed in 6,7% on average.

Conclusions: The analyses of data showed that combination of MMT, ARV and psychological counseling significantly improves the physical and psycho-social status of HIV positive IDUs, improves life quality and treatment adherence, dramatically decreases use of illegal psychotropic-narcotic drugs and decreases the risk of HIV transmission among injecting population.

Overcoming raltegravir resistance, place of elvitegravir and second generation integrase strand transfer inhibitors (INSTIs)

Y. Pommier[2], M. Metifiot[2], C. Marchand[2], X.Z. Zhao[1], S. Hughes[3], T. Burke[1]

[1]Laboratory of Chemical Biology [2]Laboratory of Molecular Pharmacology, Bethesda [3]HIV Drug Resistance Program, National Cancer Institute, Frederick, United States

Anti-HIV integrase (IN) small molecules are being actively pursued following the FDA approval of raltegravir (RAL; Isentress, Merck) and elvitegravir (EVG; "Quad pill", Stribild, Gilead), and the promising activity of dolutegravir (DTG; ViiV Healthcare). These drugs are highly targeted to the IN active site and are often referred to as INSTIs (Integrase Strand Transfer Inhibitors). The INSTIs act by chelating two metal ions at the interface of the enzyme and the viral DNA, thereby inhibiting integration by blocking the binding of the target/chromosomal DNA and acting as interfacial inhibitors [1,2]. As a consequence of the high affinity targeting of IN, point mutations of IN are a major mechanism of resistance to raltegravir. Our biochemical studies contributed to establish the 3 main IN mutation pathways driving RAL resistance: Y143, Q148 and N155 with additional G140 mutations acting to restore IN fitness due to Q148 mutations. We will describe DLG's resistance profile, show that DTG overcomes not only the Y143 pathway (like EVG) but also infringes on the 2 other RAL-resistance pathways: Q148 and N155. Finally, we will present co-crystal, biochemical and antiviral data on a novel series of INSTIs being developed at the National Cancer Institute [3].

1. Pommier Y, Marchand C. Interfacial inhibitors: targeting macromolecular complexes. Nat Rev Drug Discov. 2012;11:25-36.
2. Pommier Y, Johnson A, Marchand C. Integrase inhibitors to treat HIV/AIDS. Nat Rev Drug Discov. 2005;4:236-48.
3. Metifiot M, Maddali K, Johnson BC, Hare S, Smith SJ, Zhao XZ, et al. Activities, Crystal Structures, and Molecular Dynamics of Dihydro-1H-isoindole Derivatives, Inhibitors of HIV-1 Integrase. ACS Chem Biol. 2013;8:209-17.

Social constraints related to children born of HIV positive mothers at Bamako district in Mali

K. Koné[1], O. Ouédraogo[1], N. Diallo[1], F. Gankpé[1], C. Mésenge[1], S. Doa[2]

[1]Santé, Université Senghor, Alexandrie, Egypt [2]SEREFO laboratory, Université de Bamako, Bamako, Mali

Objective: The objective of this survey was to analyze the social constraints related to food of children from 0 to 18 months born to HIV mothers in the district of Bamako (Mali) from 2010 to 2012.

Method: It was a prospective study which was done from June to September 2012. The study population was of HIV positive mothers with children from 0 to18 months followed in the PMTCT pediatric center of Gabriel Touré hospital in Bamako. Data were collected with ai(d of a on a questionnaire both during face to face interview and focus group discussion.

Results: Sixty women were interviewed in total, with an average age of 30 years. Uneducated mothers (48%), married monogamous (65%) and living in an extended family (65%) were the most represented. 23% of women with HIV positive did inform their spouses of their HIV status. Among informed fathers 24% did accept to be screened, 37% were HIV negative and 39% were HIV positive There were 44% of mothers who have diversified food early pressure with their surroundings (0,015), and 17% who diversified due to the unavailability of milk (P0,000). Some fathers did not agree with the method of feeding chosen by their spouse (18%), the involvement of inform fathers in the management of child feeding was notified in 73% cases. There was no statistical variation between the serological status of the father and his involvement in infant feeding (P=0,624). There was 41% of fathers did not participate in PMTCT.

Conclusion: This study reveals that the feeding of children born to mothers with HIV is complex and deserves a closer attractive look from all stakeholders on PMTCT. The support and involvement of partners of women in PTME programs irrespective of their HIV status is essential for the effective success of programs.

Keywords: Breastfeeding, HIV, Mother and Child, Mali

Antibody and T cell response to the protease cleavage sites drive extensive mutations and correlated with protection against higher dose of SIVmac239 challenge and disease progression in *Cynomolgus macaques*

M. Luo[1-2], D. Tang[1], R. Capina[1], X.Y. Yuan[1], J. Pinto[3], C. Prego[3], M. Alonso[3], C. Barry[1], R. Pilon[1], D. La[1], C. Daniuk[1], J. Tuff[1], S. Pillet[1], T. Bielawny[1], C. Czarnecki[1], P. Lacap[1], G. Wong[1], S. Tyler[1], B. Liang[1-2], T. Ball[1-2], P. Sandstrom[1], G. Kobinger[1-2], F. Plummer[1-2]

[1]National Microbiology Lab, Manitoba [2]Medical Microbiology, University of Manitoba, Winnipeg, Canada [3]Pharmacy and Pharmaceutical Technology, University of Santiago de Compostela, Santiago, Spain

Objective: An estimated 2.7 million people are newly infected by HIV-1 every year. For every 2 persons starting treatment, 5 become newly infected. A safe and effective preventative HIV-1 vaccine is the only way to stop the new infections and control the pandemic. The protease of HIV-1 is a small 99-amino acid aspartic enzyme that mediates the cleavage of Gag, Gag-Pol and Nef precursor polyproteins. The process is highly specific, temporally regulated and essential for the production of infectious viral particles. A total of twelve proteolytic reactions are required to generate a viable virion. Therefore, a vaccine targeting the 12 protease cleavage sites of HIV-1 could be effective. We conducted a proof of concept study with Cynomolgus macaques and SIVmac239 as a model.

Method: A cocktail of peptides overlapping the 12 protease cleavage sites (PCS) of SIVmac239 was used as immunogens and deliver the peptides with a modified vesicular stomatitis virus (VSV) vector and with a nanostructure mucosal delivery system. The recombinant VSV-peptides were used to immunize cynomolgus macaques and nanopackaged peptides were used to boost the immune response to the PCS peptides of SIVmac239. The immunized macaques and controls were cumulatively challenged intrarectally with increased dosage of SIVmac239. T cell and antibody responses to the PCS peptides, response to the infection, CD4+ and CD8+ T cell responses and viral load after infection were monitored throughout the study period.

Results: Antibody and T cell responses to the 12 protease cleavage site can protect macaques against higher dosage of SIVmac239 challenge ($p=0.005$, $R=0.42$) and the vaccine group maintains significantly higher CD4+ counts ($p=0.0002$) than the controls weeks after being infected. Viral sequence analysis detected extensive mutations in the PCS and the flanking region. Extensive amino acid mutations in PCS and the flanking region are correlated with lower viral load ($p<0.0001$).

Conclusion: A HIV vaccine targeting protease cleavage sites is very promising and can be used for prevention and treatment.

Design, development and clinical evaluation of a new lentiviral based anti-HIV therapeutic vaccine

E. Sarry, M. Rodriguez, A. Bejanariu, L. Casaban, E. Sabbah Petrover, C. Bauche

Theravectys, Villejuif, France

Theravectys, a spin-off the Institut Pasteur founded in 2005, develops a new generation of prophylactic and therapeutic vaccines using optimized lentiviral vectors. It's most advanced product, a therapeutic anti-HIV vaccine treatment, has entered clinical Phase I/II. This vaccination should allow seropositive patients to gain an immunological status identical to the so-called "Functional Cured" patients who develop an efficient immunological response capable of controlling the infection without therapy.

Vaccine candidates are integrative and self-inactivated live-recombinant lentiviral vectors. They encode an HIV antigen, under the regulation of a patented promoter that is preferentially induced in APC (generating of a strong, specific and long lasting T-cell immune response), and showing a basal level expression in all cells (allowing their elimination by the settled immune response).Furthermore, Theravectys developed a vaccination regimen based on iterative immunizations with lentivectors encoding the same HIV transgene, relying on different VSV-G serotypes for pseudotyping without generating cross-neutralizing antibodies. These vaccine candidates are classified as "Live recombinant vectored vaccines" (EMA, 2011).

Theravectys set up an innovative manufacturing process combining high production yields, impurity profiles compatible with direct injections into humans and high immunogenicity. Pilot and GMP batches have been manufactured and GLP preclinical studies (amongst which biodistribution, shedding and toxicity) performed, that showed the restricted diffusion of the vaccine candidates after injection and their fast disappearance within few weeks, correlated with an absence of macroscopic and microscopic toxicity.

These data allowed the settlement of an anti-HIV therapeutic Phase I/II clinical trial that has received the authorizations of the French and Belgium regulatory agencies in 2012. This trial will be held in France and Belgium and is enrolling of 36 HIV-1 infected patients. Theravectys' anti-HIV vaccine treatment will be assessed at three doses and safety, tolerability and immunogenicity compared to a placebo group. Results are expected by 2014 with intermediary analyses in November 2013.

Mapping the conformational epitope of the 12G5 anti-CXCR4 HIV neutralizing antibody

A. Fischer[2], V. Fievez[2], M. Counson[2], J.C. Schmit[2-1], C. Seguin-Devaux[2], S. Deroo[2], A. Chevigne[2]

[1]Service national des maladies infectieuses, Centre hospitalier de Luxembourg [2]Laboratoire de rétrovirologie, CRP Santé, Luxembourg, Luxembourg

Objective: The antibody 12G5 is a mouse monoclonal antibody recognizing a conformational epitope on CXCR4 extracellular domains and is commonly used to assess expression and correct folding of CXCR4 at the cell surface. Moreover this antibody presents neutralizing properties towards HIV-1 infection. The aim of our study was to perform the mapping of the epitope recognized by the antibody 12G5 to identify mimotopes able to elicit a neutralizing immune response blocking T-tropic HIV-1 entry.

Methods: Two different phage libraries displaying 12-mer and 20-mer randomized peptides were screened on immobilized 12G5. Positive clones were sequenced and the corresponding peptides were analyzed for their neutralizing properties towards CXCL12 binding and HIV-1 entry. In parallel, chimeric X4/X7 receptors and synthetic peptides corresponding to CXCR4 extracellular loops were used to investigate the implication of each CXCR4 extracellular domain.

Results: A unique sequence and 11 different sequences were selected with the 20-mer and the 12-mer library, respectively. Sequence alignment revealed that 83% of the selected clones started with the Y-XXX-EE/D sequence among which 60% presented a Ser or Thr at second position (YS/T-XX-EE/D). Interestingly CXCR4 N-terminus presents two YT combinations at positions 7 and 12 suggesting that these residues may be part of the epitope. Binding of the 12G5 mAb to chimeric X4/X7 receptors confirmed the importance of the N-terminus and showed that the extracellular loop 1 (ECL1) but not ECL3 is required for strong interaction with the Mab. Studies with peptides demonstrated that only peptide ECL2 neutralized the binding of 12G5 antibody. Moreover peptide corresponding to the 20-mer mimotope showed inhibitory activity towards CXCL12 binding (IC_{50}= 130 µM) and confered partial protection to MT4 cells against HIV-1 infection by X4-tropic virus (III_B) and to a lesser extent by R5-tropic virus (BaL).

Conclusion: The 12G5 epitope is complex and involves multiple determinants mainly located in the N-term, ECL1 and ECL2 of CXCR4. The selected 20-mer mimotope efficiently mimics the CXCR4 extracellular surface as demonstrated by its ligand neutralizing properties and represents interesting candidate to elicite a neutralizing immune response or to develop HIV-1 entry inhibitors targeting the co-receptor binding site of gp120.

Th2-Th1 shift with the multiantigenic formulation TERAVAC-HIV-1 in Balb/c mice

D. García-Díaz, I. Rodríguez, Y. Santisteban, G. Márquez, Y. Terrero, E. Brown, E. Iglesias

AIDS Vaccine Project, Center for Genetic Engineering and Biotechnology, Havana, Cuba

Objective: In chronic HIV infection a progressive Th1 to Th2/Th0 cytokine-profile shift is related to disease progression. TERAVAC-HIV-1 is a multiantigenic vaccine candidate comprising the recombinant protein CR3 that contains T cell epitopes and the surface (S) and nucleocapsid (C) antigens of Hepatitis B Virus (HBV). Previous studies showed that such virus-like particles of the HBV provide a Th1 adjuvant effect. We investigated whether TERAVAC would elicit a Th1 response after subcutaneous or simultaneous nasal (IN)-subcutaneous (SC) inoculation in the presence of an ongoing HIV-specific Th2-type response.

Methods: In a first set of immunizations to induce a Th2 response, Balb/c mice were immunized subcutaneously with: groups 1 and 2, Placebo; 3-4, mixture of C and S (C+S); 5-6, CR3; 7-8, HIV-1_{IIIB} viral lysate and 9, TERAVAC (positive control of Th1 response). Antigens were adjuvated in alum. In the second phase to assess the induction of a Th1 profile, the same animals were inoculated with: group 1, C+S simultaneous IN and SC administration (C+S (SC+IN)); 2, C+S via SC (C+S (SC)); groups 3, 5 and 7 TERAVAC (SC+IN); and groups 4, 6 and 8 TERAVAC (SC). Group 9 was excluded in the second phase. Total antigen dose was the same per mouse. ELISAs were used to quantitate the secretion of IFN-γ, IL-4 and IL-10 in culture supernatant fluids of splenocytes stimulated with CR3, and whole Ag-specific IgG titers and isotypes in sera.

Results: The HIV (CR3)-specific Th2-type response was verified by induction of IL-4 and IL-10 secretion of ex vivo stimulated splenocytes without secretion of IFN-γ or IgG2a antibodies in serum. Further subcutaneous or simultaneous nasal-subcutaneous immunizations of the same mice with TERAVAC promoted IFN-γ secretion and production of IgG2a antibodies in accordance with a Th1-type response.

Conclusion: Our findings evidenced that in an HIV-specific Th2 environment, subcutaneous and simultaneous nasal-subcutaneous administrations of TERAVAC-HIV-1 generate a Th1-cell-type response. It supports the use of this vaccine candidate in the therapeutic scenario.

HIV-1 Antibody epitope prediction based on neutralization of diverse viral strains

G. Chuang, M. Louder, T. Zhou, Y. Kwon, M. Pancera, J. Mascola, P. Kwong, I. Georgiev

Vaccine Research Center, NIAID/NIH, Bethesda, United States

Objective: Identification of the precise epitopes targeted by HIV-1 broadly neutralizing antibodies is a key step to the development of epitope-based HIV-1 vaccines. Ideally, epitope residues are determined in the context of antibody-antigen complex structures, though structure determination may in many cases be infeasible. Variation of antigen sequence variation within an antibody epitope is more likely to affect the antibody neutralization potency than variation outside of the epitope. Hence, neutralization data on diverse viral strains may encode substantial information about an antibody epitope. The goal of this research is to develop a bioinformatics tool to identify antibody epitope residues by analyzing the neutralization activity of a given antibody against a set of viral strains.

Methods: The algorithm ranks antigen residues based on the mutual information score between the amino acid type of each residue position and neutralization potency for each strain. The prediction accuracy of the algorithm is enhanced by incorporating structural information of the unbound antigen. Specifically, antigen structure information was used to filter out: (1) surface inaccessible residues and (2) top-ranking (mutual information) residues that were not in the vicinity of other residues with high mutual information scores.

Results: The algorithm was evaluated on a neutralization panel of 181 HIV-1 strains and 19 antibodies, for which antibody-antigen structures were available. When ranking each residue by mutual information alone, the method gave an average of 4.2-fold enrichment of accurately predicted epitope residues at a 5% false positive rate, compared to random selection ($p<0.01$). Incorporating structural information of the unbound antigen, the method gave an average of 7.7-fold enrichment of accurately predicted epitope residues at a 5% false positive rate compared to random selection ($p<0.001$) and performed significantly better than ranking with mutual information alone ($p<0.001$).

Conclusion: Since neutralization assays are a standard step in the evaluation of antibodies against viruses such as HIV-1 and influenza, the method described here could serve as an efficient screening tool to predict antibody epitope residues.

Induction of HIV mucosal immunity after encapsulation of Nod1 & Nod2 ligands in biodegradable nanocarriers coated with Gag antigen and oral or parenteral administration

V. Pavot[1], N. Rochereau[2], C. Primard[1], V. Lahaye[1], C. Genin[2], E. Perouzel[3], T. Lioux[3], S. Paul[2], B. Verrier[1]

[1]UMR 5305 vaccinology, CNRS, Lyon [2]Gimap, Saint Etienne [3]InvivoGen, Toulouse, France

The use of TLR ligands as mucosal adjuvant for vaccine administration is already largely described whereas the use of Nod-like receptor ligands is still investigated. As activation of intracytoplasmic Nod like-receptors is able to induce production of pro-inflammatory molecules, we have evaluated if their co-delivery into nanoparticles carrying HIV-Gag antigens could amplify the mucosal immune responses in mice at vaginal and intestinal levels.

We used Poly(Lactic Acid) (PLA) nanoparticles (NPs) (~200 nm) for co-delivery of p24 and Nod ligands. As Nod like-receptors are mainly expressed by antigen presenting cells, we first assessed the capacity of free or encapsulated ligands to induce monocyte derived dendritic cells (MoDCs) maturation *in vitro*. Then, we compared oral and subcutaneous immunizations of BALB/c mice with encapsulated Nod ligands co-delivered with HIV-1 Gag antigen into the same or different NPs. To assess adjuvant effect, p24-specific cellular and humoral responses were analyzed on splenocytes and in vaginal lavages, faeces and sera.

The state of MoDCs maturation was characterized by the expression of CD80, CD83 and CD86. We showed that encapsulation of Nod1 or Nod2 ligand increases significantly their expression and the secretion of pro-inflammatory cytokines, compared to the effect of free ligands, probably due to a better uptake of encapsulated ligands.

By analyzing humoral immune responses, we observed that oral co-administration of p24 and Nod2 ligand by two different NPs was the most efficient formulation to induce anti-p24 IgG and IgA responses in faeces. By contrast co-formulation of p24 and Nod1 ligand into the same NP induced better CD8 IFNγ responses. Subcutaneous immunizations with Nod encapsulated NPs were able to induce high systemic and mucosal IgG responses. Moreover, co-delivery of p24 and Nod2 ligand into the same NP was the only formulation to induce high specific CD8 IFNγ responses.

Encapsulation of Nod ligands into PLA nanoparticles seems to favour their action on DCs maturation as well as induction of mucosal immune responses after oral or subcutaneous co-administration with PLA-p24 NPs. Use of those ligands as mucosal adjuvant deserves further experiments and we are investigating the mechanisms involved and the synergistic effect of combining both immunization routes.

T cells responses in macaques immunized with a single dose of a novel Non-Integrative One Cycle SHIV-based lentivirus genome as DNA vaccine

G. Arrode-Bruses[2], M. Moussa[2], A. Malogolovkin[2], M. Bacard-Longere[1], Y. Chebloune[2]

[1]Laboratoire de Virologie, Institut de Biologie, CHU de Grenoble [2]UMR5163 CNRS-UJF, University Joseph Fourier, Grenoble, France

Objective: Innovative vectors and immunization strategies are hardly needed to generate safe and efficacious HIV vaccines. Many types of vectors have been used in non-human primate model of HIV vaccine but they fail to recapitulate the efficacy of Live-attenuated vaccines. In this work we developed novel lentiviral-based vectors that are non-integrating, replication-limited (single cycle) and driven by lentiviral LTRs that have constitutive promoters. These vectors synergize the properties, without the inconvenient, of both DNA and Live-attenuated vaccines.

Methods: We immunized, by injection and electroporation, six macaques with a single dose of CAL-SHIV-IN$^-$ DNA and performed a longitudinal characterization of induced T cell responses during 47 weeks post-immunization (PI). We used conventional INF-g ELISPOT assay to measure antigen specific cells with immediate effector functions. We performed 6-day and 12 day- *in vitro* stimulation assays of PBMCs cultured under antigenic and/or homeostatic driven proliferation conditions to evaluate the functional recall capacity of the vaccine-induced T cells at different time points of their development. We used multiparametric FACS based assays to determine their memory phenotype and functions (proliferation, cytokine expression, lytic content).

Results: We found that all immunized macaques developed and maintained peripheral CD8+and CD4+ T cells responses mainly against Gag and Nef antigens. During the primaryexpansion phase, immediate effector cells as well as progressively proliferating cells with limited effector functions were detected. Both cells expressed markers associated with effector and central memory phenotypes. These responses contracted and then lately reemerged (W20-30 PI) in absence of any antigen boost. From W30 to 44 PI, these circulating responses decreased in the 6 day assay but underwent massive expansion in the 12 day assay. Cell analyses indicated that after initial re-exposure to Ag for 6 days and in presence of homeostatic cytokines for additional 6 days, vaccine specific CM and EM T cells have expanded and acquired immediate effector functions.

Conclusion: A single dose delivery of our DNA vaccine allowed the generation and persistence of vaccine specific memory T cells with immediate as well as recallable effector functions. Whether these responses are associated with control of viral replication after challenge is under investigation.

Type I IFN counteracts the induction of antigen-specific immune responses by lipid-based delivery of mRNA vaccines

C. Pollard[1-3], J. Rejman[4], W. De Haes[1], B. Verrier[5], E. Van Gulck[1], L. Heyndrickx[1], T. Naessens[3], S. Desmedt[4], S. De Koker[3], J. Grooten[3], G. Vanham[1-2]

[1]Virology unit, Institute of Tropical Medicine [2]Department of Biomedical Sciences, University of Antwerp, Antwerp [3]Lab of Molecular Immunology, Ghent [4]Laboratory of General Biochemistry and Physical Pharmacy, Ghent University, Ghent, Belgium [5]Institut de Biologie et Chimie des Protéines, University of Lyon, Lyon, France

Objective: The use of DNA and viral vector-based vaccines for the induction of cellular immune responses is increasingly gaining interest. However, concerns have been raised regarding the safety of these immunization strategies. Due to their lack of genome integration, mRNA-based vaccines have emerged as a promising alternative. Moreover, the use of mRNA vaccines could be beneficial in the context of therapeutic HIVvaccination as it enables the delivery of multiple HIV genes and their mutations, thus allowing the approximate patient-specific representation of the quasi species of the virus. In this study, we evaluated the potency of HIV-1 Gag encoding mRNA complexed with the cationic lipid DOTAP/DOPE as a novel vaccination approach.

Methods: C57BL/6 mice were vaccinated subcutaneously with mRNA encoding the HIV-1 antigen Gag or the model antigen Ovalbumine complexed with DOTAP/DOPE. Induction of immune responses was assessed in wild-type and IFNaR-/- mice by ELISPOT, serum ELISA and by performing an *in vivo* killing assay.

Results: We demonstrate that immunization of mice with mRNA encoding Gag complexed with DOTAP/DOPE elicits antigen-specific, functional T cell responses resulting in specific killing of Gag peptide-pulsed cells and the induction of humoral responses. In addition, we show that DOTAP/DOPE complexed antigen-encoding mRNA displays immune-activating properties characterized by secretion of type I IFN and the recruitment of pro-inflammatory monocytes to the draining lymph nodes. Finally, we demonstrate that type I IFN inhibit the expression of DOTAP/DOPE complexed antigen-encoding mRNA and the subsequent induction of antigen-specific immune responses, ultimately resulting in decreased killing of antigen-pulsed cells.

Conclusion: We have demonstrated the feasibility of using antigen-encoding mRNA complexed with the cationic lipid DOTAP/DOPE as a novel immunization strategy capable of evoking functional T cell responses. Furthermore, we have gained surprising insights regarding the negative role of type I interferons in modulating the mRNA based immune response. These findings are of high relevance for the further design and development of RNA based vaccines, and will pave the way towards improved mRNA vaccination approaches.

Design and functional analysis of a novel DNA construct as model of HIV-1 vaccine

M. Moussa, G. Arrode-Brusés, A. Smaoune, A. Malogolovkin, Y. Cheblounе

UMR5163 CNRS-UJF, University Joseph Fourier, Grenoble, France

Objective: Design of safe and efficacious vaccine against HIV-1, capable of inducing protective immunity in human, remains a tough challenge in the fight against HIV-1 in human. Live-attenuated viruses induce protective immunity against pathogenic viruses in Non-human primate model but they were associated with reversion to pathogenic in infants and some adults. Here we designed and studied the functional properties of a chimeric non-integrating, one cycle SHIV-KU2- based genome driven by the LTRs of the naturally attenuated caprine arthritis encephalitis lentivirus (CAEV) having constitutive promoters.

Methods: The construct was transfected into HEK-293 cells and supernatants were harvested and tested for infectivity in human T cell lines. BALB/c and humanized NOD/SCID/b2 mice were immunized intramuscularly by a single dose of our DNA vaccine. Splenocytes were isolated at week 2 and 4 post-immunization, and specific immune responses (cytokine production and proliferative capacity) to viral antigens were examined by INF-g ELISPOT and multi-parametric flow cytometry assays.

Results: We found that our DNA construct produces viral particles that are able to undergo a single cycle of replication in cultured target cells. Immunization of BALB/c mice induced T cell immune responses similar to those induced by our previous replication and integration defective SHIV-KU2-based vector. However, in contrast antigen-specific T cell responses were significantly higher in humanized NOD/SCID/b2 mice immunized with our new integration defective one-cycle replication vector CAL-SHIV-IN- compared to those immunized with our former D4SHIV-KU2 DNA. These results suggest that the additional cycle of replication in human target cells has substantially enhanced the antigenicity of the novel vector.

Conclusion: This proof of the concept in the mouse model helped us to initiate the immunogenicity studies of this vaccine in non-human primate model (see G. Arrode-Bruses presentation). The ongoing work will establish whether induced immune responses are associated with the control of pathogenic viruses used for challenge. Overall the data of the present study will provide valuable information about the potential use of chimeric lentivirale genomes as prophylactic or therapeutic vaccine against HIV-1.

Glucopyranosyl lipid A aqueous adjuvant significantly enhances HIV CN54gp140 antigen-specific T and B cell immune responses elicited by a DNA, MVA and recombinant protein vaccine regimen

P.F. Mckay, A.V. Cope, J.F. Mann, J. Swales, S. Joseph, M. Esteban, R. Tatoud, D. Carter, J. Weber, R.J. Shattock

Imperial College, London, United Kingdom

Objective: Using a unique vaccine-antigen matched and single HIV Clade C approach we have assessed the immunogenicity of a DNA-poxvirus-protein strategy in mice, administering MVA and protein immunizations sequentially or simultaneously in the presence or absence of a novel TLR4 adjuvant (GLA-AF).

Methods: Groups of 10 BALB/c mice were vaccinated with combinations of HIV *env*/*gag-pol-nef* plasmid DNA followed by MVA-C (HIV *env*/*gag-pol-nef*) with GLA-AF adjuvanted or unadjuvanted HIV CN54gp140 protein that was either co-administered in different muscles of the same animal with MVA-C or given sequentially at 3 week intervals. Mice were sampled (serum and vaginal wash) prior to each vaccination and three weeks post final immunization. Antigen-specific immunoglobulin production was assessed in the both the systemic and mucosal compartments by semi-quantitative ELISA. Splenocytes were harvested at necropsy and assessed by IFN-gamma ELISpot and intracellular cytokine assays for antigen-specific T cell responses to Env and Gag peptide pools.

Results: The DNA prime established a population of B cells that were able to mount a statistically significant anamnestic response to the boost vaccines. The greatest antigen-specific systemic antibody response was observed in animals that received all vaccine components. Moreover, a high proportion of the total mucosal IgG (30 - 50%) present in the vaginal vault of these vaccinated animals was vaccine antigen-specific. The potent elicitation of antigen-specific immune responses to this vaccine modality was also confirmed in rabbits. Importantly, co-administration of MVA-C with HIV CN54gp140 protein adjuvanted with GLA-AF significantly augmented the antigen-specific T cell responses to the Gag antigen, a transgene expressed by the MVA-C vector. Statistical significance was assessed in the various assays by one-way Anova and multiple comparisons or by student *t* test.

Conclusion: We have demonstrated that co-administration of MVA and GLA-AF adjuvanted HIV CN54gp140 protein was equally effective in the generation of humoral responses as the sequential regimen thus shortening and simplifying the immunization schedule. In addition, a significant benefit of the condensed vaccination regime was that T cell responses to proteins expressed by the MVA-C were potently enhanced, an effect that was likely due to enhanced immunostimulation in the presence of systemic GLA.

Experimental Evidence and Potential Immunotherapeutic Applications of Vaccine-Induced Antibodies Against 3S, a Highly Conserved Motif of gp41, in HIV-1-infected Patients Treated with Antiretroviral Therapy

C. Katlama[3-5], O. Launay[2], S. Gharakhanian[6], R. Ho Tsong Fang[1], B. Autran[4-5], V. Vieillard[4-5], J. Crouzet[1], R. Murphy[7], P. Debré[4-5]

[1]InnaVirVax, Génopole, Evry [2]AP-HP Cochin & Inserm CIC BT505 [3]Inserm U943, AP-HP Pitié Salpêtrière [4]Inserm UMRS945 [5]Université Pierre et Marie Curie, Paris, France [6]InnaVirVax, Cambridge Innovation Center, Cambridge, Ma [7]Northwestern University, Chicago, Il, United States

Background/Hypothesis: We hypothesize that 3S, a highly conserved motif of HIV-1 gp41, is an important immunotherapeutic target. This peptide binds to gC1qR, leading to expression on CD4 of NKp44L, the natural ligand of NKp44 on activated NK cells, thus provoking CD4 apoptosis/depletion. Anti-3S antibodies prevent NKp44L expression and ensuing cytotoxic events. Proof-of-concept in an AIDS primate model showed beneficial effects on CD4 count, immune activation, markers of inflammation. VAC-3S dose ranging, GLP toxicity assessment were performed in rats/mice. Anti-3S antibodies in 5 cohort studies (Total N=923) correlated with a lack of CD4 decrease and/or HIV disease progression. We have developed a novel immunotherapeutic vaccine (VAC-3S), comprised of 3S and commercially used carrier and adjuvant was developed.

Methods: This First-In-Human clinical trial was a prospective, randomized, placebo-controlled, double-blind dose-escalation study to assess safety, immunogenicity of 0.1, 1.0, 10 mg of VAC-3S with 3 IM immunizations, W0, W4, W8. Anti-3S antibodies were assessed by ELISA. Secondary endpoints include NKp44L expression, lymphocyte activation/differentiation.

Results: Twenty-five HIV-1-infected patients (23 men), CD4 counts ³200 cells/mm^3,ART-controlled wererandomized. Median (range), age was 47 years (32-54), CD4 710 c/mm^3 (311-1187), CD4 nadir 336 c/mm^3 (127-739), ART initiated 3.0 yrs (1.1-7.1), none had detectable HIV RNA. The primary endpoint, safety, was reached: no SAEs nor viral rebound noted. 76 % of patients had grade (gr) 1 or gr2 local reactions, 28 % had gr1 or gr2 systemic reactions, 2 patients had gr1/2 LFT increases, 2 gr3/gr4 events were in placebo group. At week 12 point, CD4 T lymphocytes counts, percentages, activation or differentiation markers remain stable. Increases in 3S antibody were statistically higher in the 10 µg dose.

Conclusions: VAC-3S vaccine has shown safety, evidence of immunogenicity at 10mg x3 injections IM q4 weeks. Higher doses need to be investigated. We hypothesize VAC-3S therapeutic applications could potentially include: *(a)* thereconstruction of physiologic immune homeostasis in immunological poor-responders to antiretroviral treatment;*(b)* thepotentialization of therapeutic vaccines generating CTL response against the HIV reservoirs by preserving CD4 helper function; *(c)* shielding the immune system during a “functional cure” multi-therapeutic approach.

α4β7, a surrogate marker to monitor specific mucosal immune response

M.L. Baron[3-4], P. Ordonnez-Rigato[1], N. Salabert[3-4], I. Méderlé-Mangeot[3-4], A. Cosma[3-4], N. Dereuddre-Bosquet[3-4], Y. Lévy[2], R. Le Grand[3-4]

[1]Laboratory of Medical Investigation, Sao Paulo, Brazil [2]Faculté de Médecine de Créteil, INSERM U955, Créteil [3]Institute for Emerging Disease and Innovative Therapies (iMETI), Division of Immuno-Virologie, CEA, Fontenay-Aux-Roses [4]UMR E1, Université de Paris Sud, Orsay, France

Background: An HIV vaccine may require the induction of a protective response at mucosal level. Our objective was to define a surrogate marker to follow specific mucosal responses in Non Human Primate (NHP) vaccinated with recombinant modified vaccinia virus Ankara expressing HIV LAI genes (HIV-rMVA).

Methods: Two groups of four cynomolgus macaques were immunized at weeks 0 and 8 with $5x10^7$ plaque forming unit (pfu) of HIV-rMVA (ANRS, France) by subcutaneous route (SC). A long lasting boost was performed nine months or 2 years after the first immunization with $5x10^7$ pfu or $5x10^8$ pfu of the HIV-rMVA by SC. Among different candidate marker tested, α4β7, an integrin which promote homing of the T cells to intestinal sites has been chosen. Blood, Peripheral Blood Mononuclear Cells as well as Mucosal Mononuclear Cells from rectal biopsies were collected at different time points after each immunization. Phenotypic characterization as well as ELISpot and intracellular staining were performed to follow α4β7 expression in different T cell subtypes and in HIV- and MVA- specific T cells.

Results: α4β7 expression is increased in peripheral T cells after each HIV-rMVA immunization (from 11.5% ± 2.4 to 32.5% ± 13.8 in CD4+ T cells; from 9.7% ± 2.1 to 28.5% ± 10.5 in CD8+ T cells). Interestingly, proliferative MVA- or HIV-specific $CD4^+$ T cells from blood which produce IFNγ or IL-2 cytokine were also α4β7 expressing cells. Moreover, we found a correlation between systemic and mucosal anti-HIV specific response by ELISpot ($p=0.05$).

Conclusions: These data suggest that tracking α4β7 expression in T cells from blood could be a useful surrogate marker for monitoring intestinal mucosal specific response and could so allow avoiding the invasive procedure of rectal biopsies.

Retroviral restrictions and non-human primate models for the development of lentiviral vectors as vaccines

A. Boulay[3], M. Latil[2], P. Souque[3], P. Charneau[3], C. Gommet[1], F. Chrétien[2], A.S. Beignon[3]

[1]Central Animal Facilities [2]Human Histopathology and Animal Models, Infection & Epidemiology Departement [3]Molecular Virology and Vaccinology, Virology Department, Institut Pasteur, Paris, France

Objective: Lentiviral vectors (LVs) are promising vaccine vectors. However, because of species-specific retroviral restrictions, the magnitude and the quality of the antigen expression resulting from the injection of HIV-derived LV might differ between macaques and humans. Our goal was to define the best predictive non-human primate/LV model, which recapitulates the cellular tropism and the spatio-temporal expression of the antigen expected in humans immunized with a HIV-1 derived LV.

Methods: We evaluated macaques from two groups differing by their TRIM5a and TRIM-Cyp restrictions (cynomolgus and pigtailed macaques) and two LVs encoding eGFP differing by their capsids (HIV-1 and a chimeric HIV-1($_{CA}$SIVmac) derived LVs) for the *in vitro* transduction capacity of activated PBLs, monocytes-derived dendritic cells (DCs) and muscle cells. We also compared the immunogenicity of the HIV-1 and HIV-1($_{CA}$SIVmac)-derived LV encoding SIVmac239 GAG after intramuscular injection of cynomolgus and pigtailed macaques.

Results: First, we could evidence the absence of restriction of HIV-1 derived LV in cynomolgus macaques DCs in contrast to PBLs, confirming what was previously reported with DCs from rhesus macaques. Second, muscle cells (both myoblasts and myotubes cultured and differentiated *in vitro* from muscle biopsies) from cynomolgus macaques could be potently transduced with a HIV-1 derived LV. Finally, HIV-1 derived LV was not less immunogenic than HIV-1($_{CA}$SIVmac)-derived LV in cynomolgus or than in pigtailed macaques.

Conclusion: Altogether, our data validate the use of cynomolgus macaques immunized with HIV-1 derived LV as good pre-clinical model to assess the immunogenicity and protective efficacy of LVs.

Structural basis for diverse N-glycan recognition and enhanced HIV-1 neutralization by V1/V2-directed antibodies

M. Pancera[2], S. Shahzad-Ul-Hussan[2], A. Doria-Rose[2], J. Mclellan[2], K. Dai[2], S. Loesgen[2], R. Staupe[2], Y. Yang[2], B. Zhang, B. Bailer[2], M. Louder[2], R. Parks[3], J. Eudailey[3], K. Lyoyd[3], J. Blinn[3], S.M. Alam[3], B. Haynes[3], M. Amin[1], L.X. Wang[1], D. Burton[4], W. Koff[5], G. Nabel[2], J. Mascola[2], C. Bewley[2], P. Kwong[2]

[1]Institute of Human Virology and Department of Biochemistry & Molecular Biology, Baltimore [2]NIH/NIAID/VRC, Bethesda [3]Duke Human Vaccine Institute, Duke University Medical Center, Durham [4]Department of Immunology and Microbial Science and IAVI Neutralizing Antibody Center, The Scripps Research Institute, La Jolla [5]IAVI, New York, United States

Objective: 30 years after the discovery of HIV-1, no vaccine exists. However, passive immunizations of broadly neutralizing antibodies have shown protection from challenges in animals, indicating their potential role in protection. Recent years have seen an explosion of extremely broad and potent neutralizing antibodies isolated from infected donors. These antibodies target four major sites of vulnerability on the viral spike. PG9 and PG16 are somatically related antibodies which neutralize 70-80% of strains and target a glycopeptide epitope in the V1/V2 region of gp120. They are able to penetrate the glycan shield that HIV-1 uses to evade the host immune response but it is not clear if they are able to accommodate diverse glycans. Our objective is to understand antibodies like PG16 and to characterize their Env epitopes for vaccine design and to improve their neutralization for use in passive protection.

Methods: We used x-ray crystallography to visualize PG16 bound to its epitope, a V1/V2 scaffold; NMR to determine affinities of PG16 and PG9 to various glycans and neutralization assays to evaluate biologically relevance of glycan recognition.

Results: The structure of PG16 bound to HIV-1 scaffolded V1/V2 revealed an epitope comprising strand C of V1/V2 and both high mannose-type (N160) and complex-type (N156/N173) *N*-linked glycans. STD-NMR confirmed that PG16 bound complex-type glycans with mM affinity but not PG9. The structure showed that three residues in the light chain of PG16 were critical for sialic acid binding of complex-type glycans. We introduced these affinity matured residues into PG9 to create the chimeric antibody PG9/PG16-RSH. PG9/16-RSH show greater potency and breadth than PG9 or PG16, recapitulating properties of both parent antibodies.

Conclusion: Our results suggest that a complex-type *N*-linked glycans often exits at N156/173 of gp120. We also defined a mechanism for recognition of diverse glycan at N173 site by the somatically related antibodies PG9 and PG16 and note that for glycan-reactive antibodies, ~60-70% of affinity matured antibody residues involved in epitope recognition bind glycans. Vaccine design efforts for V1/V2-containing immunogens will need to take into account specific glycans at two different sites.

AUTHORS & CO-AUTHORS INDEX

M Author/Co-Author

PARTICIPANTS LIST

Wasim ABBAS
UNIVERSITE DE FRANCHE-COMTE
France
wazim_cemb@hotmail.com

Quarraisha ABDOOL KARIM
CAPRISA
Durban, South Africa
abdoolq2@ukzn.ac.za

Salim ABDOOL KARIM
CAPRISA
Durban, South Africa
karims1@ukzn.ac.za

Rania ABUELHASSAN
International Medical Corps
Tripoli, Libya
dr.raniaalahmer@yahoo.com

Priyamvada ACHARYA
NIH
Bethesda, United States
acharyap@mail.nih.gov

Bashir ADAM
MINISTRY OF HEALTH
Algeneina, Sudan
bashir.2kotch@yahoo.com

Lucille ADAM
CEA
Fontenay Aux Roses, France
lucille.adam@cea.fr

Swati AHIR
NATIONAL INSTITUTE FOR RESEARCH IN REPRODUCTIVE HEALTH
Mumbai, Maharashtra, India
swatiahir86@gmail.com

Andres ALCOVER
INSTITUT PASTEUR
Paris, France
andres.alcover@pasteur.fr

Miguel ALFONZO
UNIVERSIDAD CENTRAL DE VENEZUELA
Caracas, Venezuela
miguelacho1998@hotmail.com

Awatef ALLOUCH
INSTITUT PASTEUR
Paris, France
awatef.allouch@pasteur.fr

Melissa AMEUR
CNRS
Paris, France
ameur.melissa@yahoo.fr

Sonia AMRAOUI
INSERM U1016, INSTITUT COCHIN
Garges Les Gonesse, France
sonia.amraoui@inserm.fr

Jane ANDERSON
HOMERTON UNIVERSITY HOSPITAL
London, United Kingdom
janderson@nhs.net

Anne-Marie ANDERSSON
UNIVERSITY OF COPENHAGEN
Copenhagen K, Denmark
annema@sund.ku.dk

Marie-Line ANDRÉOLA
CNRS UMR5234
Bordeaux, France
marie-line.andreola@reger.u-bordeaux2.fr

Pervin ANKLESARIA
BILL & MELINDA GATES FOUNDATION
Seattle, United States
Pervin.Anklesaria@gatesfoundation.org

Svitlana ANTONYAK
GROMASHEVSKY RESEARCH INSTITUTE
Kyiv, Ukraine
dianashevchenko@ukr.net

Cristian APETREI
UNIVERSITY OF PITTSBURGH
Pittsburgh, United States
apetreic@pitt.edu

Victor APPAY
INSERM U945
Paris, France
victor.appay@upmc.fr

Fernando ARENZANA
INSTITUT PASTEUR
Paris, France
farenzan@pasteur.fr

Kevin ARIEN
INSTITUTE OF TROPICAL MEDICINE
Antwerpen, Belgium
karien@itg.be

Geraldine ARRODE-BRUSES
UMR 5163 CNRS-UJF
La Tronche, France
arrodebg@ujf-grenoble.fr

Birgitta ASJÖ
UNIVERSITY OF BERGEN
Bergen, Norway
birgitta.asjo@gades.uib.no

Bertrand AUDOIN
IAS
Geneva, Switzerland
bertrand.audoin@iasociety.org

Brigitte AUTRAN
HOPITAL PITIE-SALPETRIERE
Paris, France
brigitte.autran@psl.aphp.fr

Noemi BALAJTI
GLAXOSMITHKLINE KFT
Budapest, Hungary
noemi.h.balajti@gsk.com

Françoise BALEUX
INSTITUT PASTEUR
Paris, France
francoise.baleux@pasteur.fr

Franck BARBIER
AIDES

Francis BARIN
UNIVERSITE FRANÇOIS RABELAIS
Tours, France
fbarin@med.univ-tours.fr

Marie-Laurence BARON
CEA
Fontenay-Aux-Roses, France
marie-laurence.baron@cea.fr

Dan BAROUCH
BETH ISRAEL DEACONESS MEDICAL CENTER
Boston, United States
dbarouch@bidmc.harvard.edu

Françoise BARRÉ-SINOUSSI
INSTITUT PASTEUR
Paris, France
francoise.barre-sinoussi@pasteur.fr

Cécile BAUCHE
THERAVECTYS
Villejuif, France
cbauche@theravectys.com

Agnes BEBY-DEFAUX
CENTRE HOSPITALIER ET UNIVERSITAIRE DE POITIERS
Poitiers, France
agnes.beby-defaux@chu-poitiers.fr

Anne-Sophie BEIGNON
CEA
Fontenay-Aux-Roses, France
anne-sophie.beignon@cea.fr

Richard BENAROUS
MUTABILIS
Romainville, France
richard.benarous@mutabilis.fr

Daniela BENATI
INSTITUT PASTEUR
Paris, France
daniela.benati@pasteur.fr

Mohamed BENLEULMI
CNRS
Bordeaux, France
mohamed.benleulmi@u-bordeaux2.fr

Cyprien BERAUD
CRP SANTE
Luxembourg
cyprien.beraud@crp-sante.lu

Mara BIASIN
UNIVERSITY OF MILAN
Milan, Italy
mara.biasin@unimi.it

Francesca BISIO
UNIVERSITY OF GENOA
Genova, Italy
francesca.bisio.77@gmail.com

Chrystèle BLIN
INSTITUT PASTEUR
Paris, France
chrystele.blin@pasteur.fr

Marie-Lise BLONDOT
INSERM
Palaiseau, France
marie-lise.blondot@inserm.fr

Morgane BOMSEL
INSERM U1016
Paris, France
morgane.bomsel@inserm.fr

Veronica BORDONI
NATIONAL INSTITUTE FOR INFECTIOUS DISEASES L.SPALLANZANI
Rome, Italy
veronica.bordoni@inmi.it

Marie BORGGREN
STATENS SERUM INSTITUTE
Copenhagen, Denmark
mabo@ssi.dk

Persephone BORROW
UNIVERSITY OF OXFORD
Oxford, United Kingdom
persephone.borrow@ndm.ox.ac.uk

Jérôme BOUCHET
CNRS
Paris, France
jerome.bouchet@pasteur.fr

Francois BOUE
HOPITAL ANTOINE BECLERE UNIVERSITE PARIS SUD
Clamart, France
francois.boue@abc.aphp.fr

Thomas BOURLET
UNIVERSITE JEAN-MONNET
France
thomas.bourlet@univ-st-etienne.fr

Mélanie BOUVIN
INSERM U966
Tours, France
mel.bv@neuf.fr

Roxane BRACHET
ANRS
Paris, France
roxane.brachet@anrs.fr

Lotte BRACKE
INSTITUTE OF TROPICAL MEDICINE
Antwerpen, Belgium
lbracke@itg.be

Martine BRAIBANT
UNIVERSITE DE TOURS
Tours, France
braibant@med.univ-tours.fr

Anne BRELOT
INSTITUT PASTEUR
Paris, France
anne.brelot@pasteur.fr

Vedran BREZAR
INSERM
Créteil, France
vedran.brezar@inserm.fr

Gilles BRÜCKER
ORVACS
Paris, France
gilles.brucker@inserm.fr

Françoise BRUN-VEZINET
HOPITAL BICHAT-CLAUDE BERNARD
francoise.brun-vezinet@bch.ap-hop-paris.fr

Florence BUSEYNE
INSTITUT PASTEUR
Paris, France
florence.buseyne@pasteur.fr

Frederic BUSHMAN
UNIVERSITY OF PENNSYLVANIA, SCHOOL OF MEDICINE
Philadelphia, United States
bushman@mail.med.upenn.edu

Marina CAILLET
INSTITUT PASTEUR
Paris, France
marina.caillet@laposte.net

Brigitte CALLES
GILEAD SCIENCES
Boulogne Billancourt, France
Brigitte.Calles@gilead.com

Vincent CALVEZ
HOPITAL PITIE-SALPETRIERE
Paris, France
vincent.calvez@psl.ap-hop-paris.fr

Leonel CAMPOS
INSTITUTO EMILIO RIBAS
São Paulo, Brazil
leonelfcampos@gmail.com

Céline CAMUS
INSERM
Rennes, France
celine.camus@univ-rennes1.fr

Paula CANNON
UNIVERSITY OF SOUTHERN CALIFORNIA
Los Angeles, United States
pcannon@usc.edu

Guislaine CARCELAIN
HOPITAL PITIE SALPETRIERE
Paris, France
guislaine.carcelain@psl.aphp.fr

Sylvain CARDINAUD
INSERM
Paris, France
scardinaud@gmail.com

Ana Paola CARRANCO ARENAS
ERASMUS MC
Rotterdam, The Netherlands
acarranc@lcg.unam.mx

Rita CASETTI
INMI L. SPALLANZANI
Roma, Italy
rita.casetti@inmi.it

Kerry CASEY
MEDIMMUNE, LLC
Gaithersburg, Maryland, United States
caseyk@medimmune.com

Marina CAVAZZANA-CALVO
HÔPITAL NECKER ENFANTS-MALADES
m.cavazzana@nck.aphp.fr

Lisa CHAKRABARTI
INSTITUT PASTEUR
Paris, France
chakra@pasteur.fr

Nathalie CHAMOND
CNRS
Paris, France
nathalie.chamond@parisdescartes.fr

Christina CHANG
ALFRED HOSPITAL, MONASH UNIVERSITY
Victoria, Australia
christina.chang@burnet.edu.au

Pierre CHARNEAU
INSTITUT PASTEUR
Paris, France
pierre.charneau@pasteur.fr

Charlotte CHARPENTIER
HOPITAL BICHAT-CLAUDE BERNARD
Paris, France
charlotte.charpentier@bch.aphp.fr

Yahia CHEBLOUNE
UNIVERSITE JOSEPH FOURIER
Grenoble, France
ychebloune@lyon.inra.fr

Mathieu CHEVALIER
INSTITUT PASTEUR
Paris, France
mathieu.chevalier@pasteur.fr

Francesca CHIODI
KAROLINSKA INSTITUTET
Stockholm, Sweden
Francesca.Chiodi@ki.se

Christian CHRISTNER
ASSOCIATION ACTIONS TRAITEMENTS
Paris, France
cchristner@actions-traitements.org

Gwo-yu CHUANG
VRC/NIAID/NIH
Bethesda, United States
gwo-yu.chuang@nih.gov

Iryna CHUKHALOVA
DNEPROPETROVSK REGIONAL CENTER OF AIDS PREVENTION
Kyiv, Ukraine
avia@dinadis.ua

Mathieu CLAIREAUX
INSTITUT PASTEUR
Paris, France
mathieu.claireaux@pasteur.fr

Andrew CLARK
VIIV HEALTHCARE
Brentford, United Kingdom
andrew.8.clark@viivhealthcare.com

Isabelle CLERC
CNRS
Montpellier, France
isaclercfr@yahoo.fr

N. CLUMECK
CHU SAINT-PIERRE
Brussels, Belgium
nclumeck@stpierre-bru.be

Myron COHEN
UNIVERSITY OF NORTH CAROLINA
Chapel Hill, United States
myron_cohen@med.unc.edu

Isabelle COHEN CODAR
ABBVIE LABORATORY
Rungis, France
isabelle.cohen-codar@abbvie.com

Yves-Marie COÏC
INSTITUT PASTEUR
Paris, France
yves-marie.coic@pasteur.fr

Philippe COLIN
INSTITUT PASTEUR
Paris, France
philippe.colin@pasteur.fr

Guilherme CORTES FERNANDES
UNIVERSIDADE FEDERAL DE JUIZ DE FORA
Juiz de Fora, Brazil
cortesfernandes@gmail.com

Valerie COURGNAUD
IGMM
Montpellier, France
valerie.courgnaud@igmm.cnrs.fr

Thérèse CROUGHS
CYTHERIS
Issy Les Moulineaux, France
sdemoulin@cytheris.com

Judith CURRIER
UCLA
Los Angeles, United States
jscurrier@mednet.ucla.edu

Magdalena CZUBALA
CARDIFF UNIVERSITY
Cardiff, United Kingdom
CzubalaMA@cardiff.ac.uk

Júlia Maria DA SILVA VOORHAM
UNIVERSITY MEDICAL CENTER GRONINGEN
Groningen, The Netherlands
j.m.da.silva-voorham@umcg.nl

Patrizia D'ALESSIO
BIOPARK CAMPUS CANCER
Villejuif, France
endocell@wanadoo.fr

Alice DAUTRY
INSTITUT PASTEUR
Paris, France
charles.dauvergne@pasteur.fr

Annie DAVID
INSTITUT PASTEUR
Paris, France
annie.david@pasteur.fr

Marie-Pierre DE BETHUNE
JANSSEN INFECTIOUS DISEASES BVBA
Beerse, Belgium
mdbethun@its.jnj.com

Paola DE CARLI
SIDACTION
Paris, France
p.decarli@Sidaction.org

Amélie DE SAINT-JEAN
UNIVERSITE JEAN-MONNET
Saint-Etienne, France
amelie.desaintjean@free.fr

Patrice DEBRÉ
HOPITAL PITIE SALPETRIERE
Paris, France
patrice.debre@psl.aphp.fr

Zeger DEBYSER
KU LEUVEN
Leuven, Belgium
zeger.debysr@med.kuleuven.be

Laura DECHTER
NIH/OAR
Bethesda, United States
LDechter@s-3.com

Steve DEEKS
UCSF
San Francisco, United States
SDeeks@php.ucsf.edu

Jules DEFORGES
UNIVERSITE PARIS DESCARTES
Paris, France
jules.deforges@gmail.com

Nathalie DEJUCQ-RAINSFORD
IRSET-INSERM U1085
Rennes, France
nathalie.dejucq-rainsford@inserm.fr

Constance DELAUGERRE
HOPITAL SAINT-LOUIS / IUH
Paris, France
constance.delaugerre@sls.aphp.fr

Jean-François DELFRAISSY
ANRS
Paris, France
jf.delfraissy@anrs.fr

Blandine DENIS
UPMC-INSERM
Paris, France
blandine.denis@ccde.chups.jussieu.fr

Nathalie DEREUDDRE-BOSQUET
CEA
Fontenay-Aux-Roses, France
nathalie.bosquet@cea.fr

Chantal DESCHAMPS
INSERM, INSTITUT COCHIN
Paris, France
chantal.deschamps@inserm.fr

Delphine DESJARDINS
CEA
Fontenay-Aux-Roses, France
delphine.desjardins@cea.fr

Martin DEYMIER
EMORY UNIVERSITY
Decatur, United States
mdeymie@emory.edu

Cristina DI PRIMIO
SCUOLA NORMALE SUPERIORE DI PISA
Pisa, Italy
c.diprimio@sns.it

Céline DIDIER
INSTITUT PASTEUR
Paris, France
celine.didier@pasteur.fr

Carl DIEFFENBACH
NIAID, NIH
Bethesda, United States
CDieffenba@niaid.nih.gov

Song DING
EUROVACC FOUNDATION
Amsterdam, The Netherlands
song.ding@eurovacc.org

Catherine DOLLFUS
APHP-HOPITAL TROUSSEAU
Paris, France
catherine.dollfus@trs.aphp.fr

Daniel DOUEK
NIAID/NIH/DHHS
Bethesda, United States
ddouek@nih.gov

Snezana DRAGAS
KBC CRNE GORE
Podgorica, Serbia-Montenegro
snezana.dragas.congress@live.com

Ludovic DRYE
INSTITUT PASTEUR
Paris, France
ludovic.drye@pasteur.fr

Audrey DUMAS
COCHIN INSTITUT
Paris, France
audrey.dumas@inserm.fr

François DUPRÉ
SIDACTION
Paris, France
F.Dupre@sidaction.org

Laurence DUPUIS
CEA
Fontenay-Aux-Roses, France
laurence.dupuis@cea.fr

Francois DURAND
MERCK
Courbevoie, France
francois_durand@merck.com

Dominic DWYER
ICPMR
Westmead, Australia
dominic.dwyer@sydney.edu.au

Prakash EKAMBARANELLORE
INDUS BIOTECH PVT LTD
Taipei, Taiwan
prakash@indusbiotech.com

Hicham EL COSTA
INSTITUT PASTEUR
Paris, France
hicham.el-costa@pasteur.fr

Valérie ESCANDE
INSTITUT PASTEUR
Paris, France
valerie.escande@pasteur.fr

Edwin ESCOBAR
VENEZUELAN INSTITUTE FOR SCIENTIFIC RESEARCH-IVIC
Estado Miranda, Venezuela
edscobar@gmail.com

Alex ESTEVEZ
OAR/NIH
Bethesda, United States
Lx4mail@gmail.com

Jean-François ETARD
EPICENTRE
Paris, France
jean-francois.etard@epicentre.msf.org

Veronique FABRE-MERSSEMAN
UPMC
Paris, France
v.mersseman@voila.fr

Oliver FACKLER
UNIVERSITY HOSPITAL HEIDELBERG
Heidelberg, Germany
oliver.fackler@med.uni-heidelberg.de

Marylinda FAMIGLIETTI
SAN RAFFAELE SCIENTIFIC INSTITUTE
Milano, Italy
marylinda_famiglietti@yahoo.it

Anthony FAUCI
NIAID/NIH
Bethesda, United States
afauci@niaid.nih.gov

Benoit FAVIER
CEA
Fontenay-Aux-Roses, France
benoit.favier@gmail.com

Zsófia FEISZT
EGYESITETT SZENT ISTVAN ES SZENT LASZLO KORHAZ
Budapest, France
sfeiszt@yahoo.com

Eva Maria FENYÖ
LUND UNIVERSITY
Lund, Sweden
eva_maria.fenyo@med.lu.se

Cindra FEUER
AVAC
New York, United States
cindra@avac.org

Diana FINZI
NIH
Bethesda, United States
dfinzi@nih.gov

Aurelie FISCHER
CRP SANTE
Luxembourg, Luxembourg
aurelie.fischer@crp-sante.lu

Hugues FISHER
TRT-5
hugues.fischer@gmail.com

Cécile FLÉCHET
HELIOS KLINIKUM EMIL VON BEHRING-PNEUMOLOGIE
Berlin, Germany
cecileflechet@gmail.com

Eric FLEUTELOT
SIDACTION
Paris, France
e.fleutelot@Sidaction.org

Anders FOMSGAARD
STATENS SERUM INSTITUT
Copenhagen, Denmark
afo@ssi.dk

Richard FOX
UNIVERSITY OF WASHINGTON
Vashon, United States
richfox@uw.edu

Remi FROMENTIN
VACCINE AND GENE THERAPY INSTITUTE-FLORIDA
Port St Lucie, United States
rfromentin@vgtifl.org

Robert GALLO
UNIVERSITY OF MARYLAND
Baltimore, United States
rgallo@ihv.umaryland.edu

Moran GALPERIN
INSTITUT PASTEUR
Paris, France
morangal@pasteur.fr

Jeanne GAPIYA NIYONZIMA
ANSS
Kigobe, Burundi
nigapiya@gmail.com

Aude GARCEL
SPLICOS THERAPEUTICS
Montpellier, France
aude.garcel@igmm.cnrs.fr

Murray GARDNER
UNIVERSITY OF CALIFORNIA
Davis, United States

Pamela GASSE
INSTITUT PASTEUR
Paris, France
pamela.gasse@pasteur.fr

Patrice GAUDINEAU
SIDA INFO SERVICE
Paris, France
pgaudineau@sida-info-service.org

Anne GAUVRIT
INSTITUT PASTEUR
Paris, France
anne.gauvrit@pasteur.fr

Michael GERNER
NIAID
Bethesda, United States
gernermy@niaid.nih.gov

Shahin GHARAKHANIAN
INNAVIRVAX & SG MD CONSULTING LLC
Cambridge, United States
shahin.gharakhanian@gmail.com

Giovanna GIACALONE
UNIVERSITE PARIS SUD, FACULTE DE PHARMACIE
Chatenay-Malabry, France
giovanna.giacalone@u-psud.fr

Jean-Claude GLUCKMAN
UMR 7212 CNRS - UNIVERSITE PARIS DIDEROT
Paris, France
jean-claude.gluckman@univ-paris-diderot.fr

Peter GODFREY-FAUSSETT
UNAIDS
Geneve, Switzerland
godfreyp@unaids.org

Anne GOLDFELD
HARVARD MEDICAL SCHOOL
Boston, Ma, United States
goldfeld@idi.harvard.edu

Céline GOMMET
CEA
Fontenay Aux Roses, France
celine.gommet@cea.fr

Gregg GONSALVES
YALE LAW SCHOOL, NEW HAVEN
gregg.gonsalves@gmail.com
United States

Leslie GOSSE
CEA
Paris, France
leslie.gosse@cea.fr

Marie-Lise GOUGEON
INSTITUT PASTEUR
Paris, France
marie-lise.gougeon@pasteur.fr

Philip GOULDER
UNIVERSITY OF OXFORD
Oxford, United Kingdom
philip.goulder@paediatrics.ox.ac.uk

Mykola GRAZHDANOV
DONETSK CENTER AIDS PREVENTION
Kyiv, Ukraine
visa@dinadis.ua

Carmem Beatriz GRIPP
OSWALDO CRUZ FOUNDATION
Niterói, Brazil
carmembg@ioc.fiocruz.br

Johan GROOTEN
UNIVERSITY GHENT
Zwijnaarde, Belgium
johan.grooten@dmbr.ugent.be

Rob GRUTERS
ERASMUS MC
Rotterdam, The Netherlands
r.gruters@erasmusmc.nl

Carolin GUENZEL
INSTITUT COCHIN / INSERM
Paris, France
carolin.guenzel@inserm.fr

Ashley HAASE
UNIVERSITY OF MINNESOTA
Minneapolis, United States
haase001@umn.edu

Gunther HAASE
PIT
Clervaux, Luxembourg
immunobiology.g.h@pt.lu

Maria Blanca HADACEK
JANSSEN
Paris, France
mhadacek@its.jnj.com

Jean HALLAK
HOPITAL SANTA CABRINI
Laval Qc, Canada
hallak.jean@gmail.com

Chiraz HAMIMI
INSTITUT PASTEUR
Paris, France
chiraz.hamimi@pasteur.fr

Catherine HANKINS
AMSTERDAM INSTITUTE FOR GLOBAL HEALTH AND DEVELOPM
Amsterdam, The Netherlands
c.hankins@aighd.org

Alexandre HARARI
CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS
Lausanne, Switzerland
alexandre.harari@chuv.ch

Mark HARRINGTON
TREATMENT ACTION GROUP
New York, United States
markhar@gmail.com

Ayrin HARUNOVA-KÖK
INSERM U955
Créteil, France
ayrin.kok@inserm.fr

Assia HASSIMI
INSERM
Paris, France
assia.samri@upmc.fr

Thierry HEIDMANN
CNRS
Villejuif, France
heidmann@igr.fr

Franz HEINZ
MEDICAL UNIVERSITY VIENNA
Vienna, Austria
virologie@meduniwien.ac.at

Cécile HERATE
INSTITUT COCHIN / INSERM
Paris, France
cecile.herate@inserm.fr

Leo HEYNDRICKX
INSTITUTE OF TROPICAL MEDICINE
Antwerpen, Belgium
lheyndrickx@itg.be

Raphael HO TSONG FANG
INNAVIRVAX
Evry, France
raphaelfang@innavirvax.fr

Bruno HOEN
CHU SAINT-JACQUES
Besançon, France
bruno.hoen@univ-fcomte.fr

Ursula HOFER
NATURE REVIEWS MICROBIOLOGY
London, United Kingdom
ursula.hofer@nature.com

Thomas HOPE
Northwestern University, Evanston
thope@northwestern.edu

Anne HOSMALIN
INSTITUT COCHIN
Paris, France
anne.hosmalin@inserm.fr

Stéphane HUA
INSERM
Le Kremlin Bicetre, France
stephane.hua@u-psud.fr

Sophie HUE
VACCINE RESEARCH INSTITUT
Créteil, France
sophie.hue@hmn.aphp.fr

Helena HUERGA
EPICENTRE
Paris, France
helena.huerga@epicentre.msf.org

Eric HUNTER
EMORY UNIVERSITY
Atlanta, Ga, United States
ehunte4@emory.edu

Nicolas HUOT
CEA / DSV
Fontenay-Aux-Roses, France
huot@vms.cnrs-gif.fr

Enrique IGLESIAS
CENTER FOR GENETIC ENGINEERING AND BIOTECHNOLOGY
Havana, Cuba
enrique.iglesias@cigb.edu.cu

Andrea IMLE
UNIVERSITY HOSPITAL HEIDELBERG
Heidelberg, Germany
andrea.imle@med.uni-heidelberg.de

Catherine INIZAN
INSTITUT PASTEUR
Paris, France
catherine.inizan@pasteur.fr

Jacques IZOPET
CHU TOULOUSE
Toulouse, France
izopet.j@chu-toulouse.fr

Brooks JACKSON
JOHNS HOPKINS UNIVERSITY
Baltimore, United States
bjackso@jhmi.edu

Beatrice JACQUELIN
INSTITUT PASTEUR
Paris, France
beatrice.jacquelin@pasteur.fr

Anais JASPART
CNRS MFP UMR 5234
Bordeaux, France
anais.jaspart@etud.u-bordeaux2.fr

Jun JIN
INSTITUT PASTEUR
Paris, France
jun.jin@pasteur.fr

Simon JOCHEMS
INSTITUT PASTEUR
Paris, France
simon.jochems@pasteur.fr

Rowena JOHNSTON
AMFAR - THE FOUNDATION FOR AIDS RESEARCH
New York, United States
rowena.johnston@amfar.org

JUNIASTUTI
AIRLANGGA UNIVERSITY
Surabaya, Indonesia
juniastutisyafik@yahoo.com

Barbara JUNKER
PT-DLR PROJECT MANAGING AGENCY
Bonn, Germany
barbara.junker@dlr.de

Vladimir JURISIC
UNIVERSITY OF KRAGUJEVAC
Kragujevac, Serbia-Montenegro
vdvd@lycos.com

Nadine KAMANDE
Kinshasa, Congo (Democratic Republic)
nadine.kamande@vodacom.cd

Adeeba KAMARULZAMAN
UNIVERSITY OF MALAYA
Kuala Lumpur, Malaysia
adeeba@ummc.edu.my

Ingrid KARLSSON
STATENS SERUM INSTITUT
Copenhagen, Denmark
iks@ssi.dk

Christine KATLAMA
France
christine.katlama@psl.aphp.fr

Michael KATZE
UNIVERSITY OF WASHINGTON
Seattle, United States
honey@uw.edu

Michel KAZATCHKINE
ONU
Geneva, Switzerland
contact@michelkazatchkine.com

Stephen KENT
UNIVERSITY OF MELBOURNE
University Of Melbourne, Australia
skent@unimelb.edu.au

Michael KESSLER
michael.kessler@intoon-media.com

Anfumbom KFUTWAH
CENTRE PASTEUR DU CAMEROUN
Yaounde, Cameroon
kfutwah@pasteur-yaounde.org

Woottichai KHAMDUANG
IRD UMI 174 PHPT - FACULTY OF ASSOCIATED MEDICAL SCIENCES, CHIANG MAI UNIVERSITY
Muang, Chiang Mai, Thailand
kwoottichai@gmail.com

Iryna KOLTSOVA
ODESSA NATIONAL MEDICAL UNIVERSITY
Odessa, Ukraine
koltsova@eurocom.od.ua

Svitlana KOMAR
OKHMADET
Kyiv, Ukraine
travel@dinadis.ua

Kadidia KONE
UNIVERSITÉ SENGHOR
Alexandrie, Egypt
kdiedia@yahoo.fr

Lyudmila KOTLIK
ODESA REGIONAL SANITARY-EPIDEMIC STATION
Odessa, Ukraine
tatyanagridina@mail.ru

Edmundo KRAISELBURD
UNIVERSITY OF PUERTO RICO-MEDICAL SCIENCES CAMPUS
San Juan, Porto Rico
edmundo.kraiselburd@upr.edu

Guenter KRAUS
JANSSEN INFECTIOUS DISEASES BVBA
Beerse, Belgium
gkraus@its.jnj.com

Hans-Georg KRÄUSSLICH
UNIVERSITÄT HEIDELBERG
Heidelberg, Germany
hans-georg.kraeusslich@med.uni-heidelberg.de

Nevan J. KROGAN
UNIVERSITY OF CALIFORNIA AT SAN FRANCISCO
San Francisco, United States
nevan.krogan@ucsf.edu

Olena KULIBABA
THE UKRAINIAN I. I. MECHNIKOV ANTI-PLAGUE RESEARCH
Odessa, Ukraine
Lenuka.87@mail.ru

Jordan K. KYONGO
INSTITUTE OF TROPICAL MEDICINE
Antwerp, Belgium
jkyongo@itg.be

Galyna KYSELYOVA
SIMFEROPOL REGIONAL CENTER OF AIDS PREVENTION
Kyiv, Ukraine
avia3@dinadis.ua

Christine LACABARATZ
INSERM U955
Créteil, France
christine.lacabaratz@inserm.fr

Benoit LACOMBE
INSERM
Paris, France
benoit.lacombe@inserm.fr

Mireille LAFORGE
CNRS FRE3235
Paris, France
mirhaddad@gmail.com

Bernard LAGANE
Institut Pasteur
Paris, France
bernard.lagane@pasteur.fr

Hichem LAHOUASSA
INSTITUT COCHIN INSERM U1016-CNRS 8104
Paris, France
hichem.lahouassa@inserm.fr

Olivier LAMBOTTE
HOPITAL BICETRE
Le Kremlin Bicêtre, France
olivier.lambotte@bct.aphp.fr

Clifford LANE
NIH, DCR
clane@niaid.nih.gov

Joep LANGE
AIGHD/ACAD. MEDICAL CENTER, UNIVERS. OF AMSTERDAM
Amsterdam, The Netherlands
j.lange@aighd.org

Roger LE GRAND
CEA
Fontenay-aux-roses, France
roger.legrand@cea.fr

Muriel LE GUERN
MLGBIOSENS
Rueil Malmaison, France
mlgbiosens@gmail.com

Anna LE TORTOREC
INSERM U1085
Rennes, France
anna.letortorec@univ-rennes1.fr

Camille LÉCUROUX
INSERM
Le Kremlin-Bicêtre, France
camille.lecuroux@u-psud.fr

William LEE
GILEAD SCIENCES
Foster City, United States
William.Lee@gilead.com

Jacques LEIBOWITCH
HOPITAL RAYMOND POINCARÉ
Garches, France
jacques.leibowitch@rpc.aphp.fr

Jean-Daniel LELIEVRE
APHP, INSERM, UPEC
Créteil, France
jean-daniel.lelievre@hmn.aphp.fr

Nicolas LEVY
IGBMC
Illkirch, France
nlevy@igbmc.fr

Yves LEVY
HOPITAL HENRI MONDOR/ INSERM U955
Créteil, France
yves.levy@hmn.aphp.fr

Sharon LEWIN
Alfred Hospital
Melbourne, Australia
sharon.lewin@monash.edu

Sophie LHUILLIER
SIDACTION
Paris, France
s.Lhuillier@Sidaction.org

Jeffrey LIFSON
SAIC-FREDERICK
Bethesda, United States
lifsonj@mail.nih.gov

Isabelle LONJON
JANSSEN
Paris, France
domanec@numericable.fr

Cecilio LOPEZ-GALINDEZ
INSTITUTO DE SALUD CARLOS III
Madrid - Majadahonda, Spain
clopez@isciii.es

Hugues LORTAT-JACOB
CNRS
Hugues.Lortat-Jacob@ibs.fr

Jens LUNDGREN
UNIVERSITY OF COPENHAGEN
Copenhagen N, Denmark
jdl@cphiv.dk

Lingjie LUO
INSTITUT PASTEUR
Paris, France
lingjie.luo@pasteur.fr

Ma LUO
NATIONAL MICROBIOLOGY LAB
Manitoba, Canada
Ma.Luo@phac-aspc.gc.ca

Anthony MACHADO
HOPITAL SAINT-LOUIS
Paris, France
Anthony.machado@inserm.fr

Matias MACHADO
INSTITUT PASTEUR DE MONTEVIDEO
Montevideo, Uruguay
mmachado@pasteur.edu.uy

Catharina MAIJGREN STEFFENSSON
GILEAD SCIENCES
Solna, Sweden
catharina.maijgrensteffensson@gilead.com

Michael MALIM
KING'S COLLEGE LONDON SCHOOL OF MEDICINE
London, United Kingdom
michael.malim@kcl.ac.uk

Joao MAMEDE
IGMM CNRS UMR5535
Montpellier, France
jmamede@igmm.cnrs.fr

Anne-Genevieve MARCELIN
PITIE-SALPETRIERE HOSPITAL
Paris, France
anne-genevieve.marcelin@psl.aphp.fr

David MARGOLIS
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
Chapel Hill, United States
dmargo@med.unc.edu

Leonid MARGOLIS
NIH
Bethesda, United States
margolil@helix.nih.gov

Florence MARGOTTIN-GOGUET
INSTITUT COCHIN
Paris, France
florence.margottin-goguet@inserm.fr

Ivica MARIĆ
BLOOD TRANSFUSION CENTRE OF SLOVENIA
Ljubljana, Slovenia
ivica.maric@ztm.si

Gilles MARODON
INSERM
Paris, France
gilles.marodon@upmc.fr

Grégoire MARTIN
INSTITUT GUSTAVE ROUSSY
Villejuif, France
gregoire.martin@igr.fr

Loic MARTIN
CEA (COMMISSARIAT A L'ENERGIE ATOMIQUE)
Gif sur Yvette, France
loic.martin@cea.fr

Frédéric MARTINON
CEA
Fontenay Aux Roses, France
frederic.martinon@cea.fr

Preston MARX
TULANE UNIVERSITY
Louisiana, United States
pmarx@tulane.edu

John MASCOLA
NIAID, NIH
Bethesda, United States
jmascola@mail.nih.gov

Line MATTHIESSEN
EUROPEAN COMMISSION
Brussels, Belgium
tuija.jansson@ec.europa.eu

Giulia MATUSALI
INSERM
Rennes, France
giulia.matusali@univ-rennes1.fr

Julien MATYSIAK
UMR MICROBIOLOGIE FONDAMENTALE ET PATHOGENICITE
Bordeaux, France
julien.matysiak@wanadoo.fr

Aimé MBOYO
PNMLS
Kinshasa, République démocratique du Congo
dramboyo@gmail.com

Craig MCCLURE
UNICEF
New York, United States
cmcclure@unicef.org

Margaret MCCLUSKEY
UNITED STATES AGENCY FOR INTERNATIONAL DEVELOPMENT
Washington, Dc, United States
mmccluskey@usaid.gov

Regina MCENERY
IAVI REPORT & VAX
New York, United States
rmcenery@iavi.org

Paul MCKAY
IMPERIAL COLLEGE
London, United Kingdom
p.mckay@imperial.ac.uk

Arash MEMARNEJADIAN
INSTITUT PASTEUR OF IRAN
Teheran, Iran
memarnejad@pasteur.ac.ir

Myriam MEMMI
UNIVERSITE JEAN-MONNET
Saint-Etienne, France
mariakitsa@yahoo.fr

Thorsten MEMPEL
HARVARD MEDICAL SCHOOL
Boston, United States
tmempel@mgh.harvard.edu

Inna MENKOVA
INSTITUT MONDOR DE RECHERCHE BIOMEDICALE
Créteil, France
inna.menkova@gmail.com

Elisabeth MENU
INSTITUT PASTEUR
Paris, France
elisabeth.menu@pasteur.fr

Marie-Louise MICHEL
INSTITUT PASTEUR/INSERM
Paris, France
marie-louise.michel@pasteur.fr

Yves MICHIELS
FACULTE PHARMACIE GENEVE
Longvic, France
yves.michiels@u-bourgogne.fr

Donna MILDVAN
DIVISION OF INFECTIOUS DISEASES
New York, United States
dmildvan@chpnet.org

Gilles MIRAMBEAU
IDIBAPS
Barcelona, Spain
gilles.mirambeau@gmail.com

Marc MIRANDE
CNRS
Gif-sur-yvette, France
mirande@lebs.cnrs-gif.fr

Lamia MIRI
INSTITUT PASTEUR DU MAROC
Mohammedia, Morocco
lamia.miri1@gmail.com

Warren MITCHELL
AVAC: GLOBAL ADVOCACY FOR HIV PREVENTION
New York, United states
mitchell@avac.org

Christiane MOECKLINGHOFF
JANSSEN
Neuss, Germany
cmoeckli@its.jnj.com

Karin MOELLING
UNIVERSITY ZÜRICH
Zürich, Switzerland
moelling@imm.uzh.ch

Jean-Michel MOLINA
HOPITAL SAINT-LOUIS
Paris, France
jean-michel.molina@sls.ap-hop-paris.fr

Luc MONTAGNIER
FONDATION MONDIALE RECHECHE ET PREVENTION SIDA
Paris, France
fondation.sida@unesco.org

Christiane MOOG
INSERM
Strasbourg, France
c.moog@unistra.fr

Uriel MORENO NIEVES
INSTITUT PASTEUR
Paris, France
uriel.moreno-nieves@pasteur.fr

Marion MORGAND
FACULTE DE MEDECINE
Tours, France
marion.morgand@etu.univ-tours.fr

Hugo MOUQUET
INSTITUT PASTEUR
Paris, France
hmouquet@rockefeller.edu

Maha MOUSSA
UNIVERSITE JOSEPH-FOURIER
Saint Martin D'hères, France
mahamoussa@live.fr

Barbara MUELLER
UNIVERSITY HOSPITAL HEIDELBERG
Heidelberg, Germany
barbara_mueller@med.uni-heidelberg.de

Jackson K MUKONZO
MAKERERE UNIVERSITY, COLLEGE OF HEALTH SCIENCES
Kampala, Uganda
mukojack@yahoo.co.uk

Michaela MÜLLER-TRUTWIN
INSTITUT PASTEUR
Paris, France
michaela.muller-trutwin@pasteur.fr

Shannon MURRAY
UPMC/INSERM
Paris, France
smurray1@uw.edu

Gary NABEL
SANOFI
Cambridge, United States
gary.nabel@sanofi.com

Vivek NARANBHAI
WELLCOME TRUST CENTRE FOR HUMAN GENETICS (OXFORD) AND CAPRISA (SOUTH AFRICA)
Oxford, United Kingdom
vivekn@well.ox.ac.uk

Dani NAZZAL
INSTITUT PASTEUR/INSERM
Paris, France
dani.nazzal@pasteur.fr

Pacifique NDISHIMYE
INSTITUT NATIONAL D'HYGIENE
Salé, Morocco
ndipac@yahoo.fr

Ibra NDOYE
NATIONAL AIDS COUNCIL
Dakar, Senegal
indoye@cnls-senegal.org

Philippe NGO VAN
ABBVIE
Rungis, France
philippe.ngovan@abbvie.com

Florence NIEDERGANG
INSERM CNRS UNIVERSITE PARIS DESCARTES
Paris, France
florence.niedergang@inserm.fr

Takeshi NISHIJIMA
AIDS CLINICAL CENTER, NATIONAL CENTER FOR GLOBAL HEALTH AND MEDICINE
Tokyo, Japan
tnishiji@acc.ncgm.go.jp

Nicolas NOEL
INSERM U1012
Le Kremlin Bicêtre, France
nicolas_noel1@yahoo.fr

Marie-Thérèse NUGEYRE
INSTITUT PASTEUR
Paris, France
marie-therese.nugeyre@pasteur.fr

Michel NUSSENZWEIG
THE ROCKEFELLER UNIVERSITY
New York, United states
nussen@rockefeller.edu

Patrycja NZOUNZA
INSTITUT GUSTAVE ROUSSY
Villejuif, France
patrycja.nzounza@gmail.com

Oksana OBERTYNSKA
NATIONAL MEDICAL ACADEMY OF POSTGRADUATE EDUCATION
Kiev, Ukraine
steptavit@rambler.ru

Shinichi OKA
NATIONAL CENTER FOR GLOBAL HEALTH AND MEDICINE
Tokyo, Japan
oka@acc.ncgm.go.jp

Vara OUK
ESTHER CALMETTE
Phnom Penh, Cambodia
oukvara@hotmail.com

Julie OVERBAUGH
FRED HUTCHINSON CANCER RESEARCH CENTER
Seattle, United States
joverbau@fhcrc.org

Mirko PAIARDINI
EMORY UNIVERSITY
Atlanta, United States
mirko.paiardini@emory.edu

Karolina PALUCKA
BAYLOR RESEARCH INSTITUTE
Dallas, United States
karolinp@baylorhealth.edu

Marie PANCERA
NIH/NIAID/VRC
Bathesda, United States
mpancera@mail.nih.gov

Gianfranco PANCINO
INSTITUT PASTEUR
Paris, France
gianfranco.pancino@pasteur.fr

Ivona PANDREA
UNIVERSITY OF PITTSBURGH
Pittsburgh, United States
pandrea@pitt.edu

Giuseppe PANTALEO
UNIVERSITY OF LAUSANNE
Lausanne, Switzerland
Giuseppe.Pantaleo@chuv.ch

Andrea PAPADOPOULOS
UNIVERSITY OF THE WITWATERSRAND
Johannesburg, South Africa
andreaolga88@gmail.com

Laura PAPAGNO
INSERM U945
Paris, France
laura.papagno@upmc.fr

Matthew PARSONS
UNIVERSITY OF MELBOURNE
Parkville, Australia
parsonsms@gmail.com

Caroline PASSAES
INSTITUT PASTEUR
Paris, France
caroline.passaes@cea.fr

Laura PATTACINI
FRED HUTCHNSON CANCER RESEARCH CENTER
Seattle, United States
lpattaci@fhcrc.org

Livia PEDROZA MARTINS
ANRS
Paris, France
livia.pedroza@anrs.fr

Vincent PELLETIER
COALITION PLUS
Paris, France
vpelletier@aides.org

Cecile PELTEKIAN
VACCINE RESEARCH INSTITUTE -VRI- ANRS
Créteil, France
cecile.peltekian@inserm.fr

Caroline PEREIRA BITTENCOURT
INSTITUT PASTEUR
Paris, France

Alan PERELSON
LOS ALAMOS NATIONAL LABORATORY
Los Alamos, Nm, United States
asp@lanl.gov

Maryse PERESSIN
INSERM U1110
Strasbourg, France
maryse.peressin@unistra.fr

Danielle PEREZ BERCOFF
CRP-SANTE, LUXEMBOURG
Luxembourg
danielle.perezbercoff@crp-sante.lu

Matthieu PERREAU
LAUSANNE UNIVERSITY HOSPTITAL
Lausanne, Switzerland
Matthieu.Perreau@chuv.ch

Renaud PERSIAUX
REMAIDES (AIDES)
Paris, France
rpersiaux@aides.org

Nicolas PETIT
INSERM
Paris, France
nicolas.petit1984@gmail.com

Gilles PEYTAVIN
HOPITAL BICHAT CLAUDE BERNARD
Paris, France
gilles.peytavin@bch.ap-hop-paris.fr

Thi Thu Huong PHAN
VAAC
Hochiminh, Vietnam
huongphanmoh@gmail.com

Gilles PIALOUX
HOPITAL TENON
Paris, France
gilles.pialoux@tnn.aphp.fr

Louis PICKER
OREGON HEALTH & SCIENCE UNIVERSITY
Beaverton, United States
pickerl@ohsu.edu

Jose Henrique PILOTTO
FUNDAÇÃO OSWALDO CRUZ
Rio De Janeiro, Brazil
pilotto@uninet.com.br

Fréderic PIO
SIMON FRASER UNIVERSITY
Bunraby, Canada
fpio@sfu.ca

Sylvia PIRITYI
GALXOSMITHKLINE KFT
Budapest, Hungary
sylvia.h.pirityi@gsk.com

Punnee PITISUTTITHUM
MAHIDOL UNIVERSITY
Bangkok, Thailand
punnee.pit@mahidol.ac.th

Louis PIZARRO
SOLTHIS
Paris, France
contact@solthis.org;isabelle.loureiro@solthis.org

Mickael PLOQUIN
INSTITUT PASTEUR
Paris, France
mickael.ploquin@pasteur.fr

Béatrice POIRIER-BEAUDOUIN
INSTITUT PASTEUR
Paris, France
beatrice.poirier-beaudouin@pasteur.fr

Guido POLI
UNIVERSITÀ VITA-SALUTE SAN RAFFAELE
Milano, Italy
poli.guido@hsr.it

Charlotte POLLARD
INSTITUTE OF TROPICAL MEDICINE
Antwerp, Belgium
Charlotte.Pollard@dmbr.ugent.be

Hélène POLLARD
TRT-5
Paris, France
helene@pollard.fr

Yves POMMIER
NATIONAL CANCER INSTITUTE
Bethesda, United States
pommier@nih.gov

Jeyasingh PONNUSWAMI
CHRISTIAN MISSION HOSPITAL
Tamilnadu, India
jeyasingh08@gmail.com

Wilfried POSCH
INNSBRUCK MEDICAL UNIVERSITY
Innsbruck, Austria
wilfried.posch@i-med.ac.at

Candice POUX
CEA
Fontenay Aux Roses, France
candice.poux@cea.fr

Bruno POZZETTO
UNIVERSITE JEAN-MONNET
Saint-Etienne, France
bruno.pozzetto@univ-st-etienne.fr

Nicoel PRADA
INSTITUT PASTEUR
Paris, France
nicole.prada@pasteur.fr

Bénédicte PUISSANT-LUBRANO
CHU DE TOULOUSE
Toulouse, France
puissant.b@chu-toulouse.fr

Elodie PYSSON
INSTITUT PASTEUR
Paris, France
elodie.pysson@pasteur.fr

Valentina QUERCIOLI
Scuola Normale Superiore di Pisa
Pisa, Italy
valentina.quercioli@sns.it

Héloïse QUILLAY
INSTITUT PASTEUR
Paris, France
hquillay@pasteur.fr

Anne QUIST
KAROLINSKA UNIV. HOSPITAL/UNIV LAB.
Stockholm, Sweden
anne.quist@karolinska.se

Cecilia RAMIREZ
INSERM
Paris, France
cecilia.ramirez@inserm.fr

Vadrevu RAVI
SAI SUDHA HOSPITAL
Kakinada, India
ravi58v@yahoo.com

R. Keith REEVES
HARVARD MEDICAL SCHOOL
Southborough, United States
roger_reeves@hms.harvard.edu

Peter REISS
AMC
Amsterdam, The Netherlands
p.reiss@amc.uva.nl

Olivier RESCANIÈRE
INSTITUT PASTEUR
Paris, France
olivier.rescaniere@pasteur.fr

Felix REY
INSTITUT PASTEUR
Paris, France
felix.rey@pasteur.fr

Marie-Anne REY-CUILLE
ANRS
Paris, France
marie-anne.rey-cuille@anrs.fr

Jacques REYNES
HOPITAL SAINT-ELOI
Montpellier, France
j-reynes@chu-montpellier.fr

Gaèle RIGAULT
MILTENYI BIOTEC
Paris, France
gaele@miltenyibiotec.fr

Pierre ROSENBAUM
CEA
Fontenay Aux Roses, France
pierre.rosenbaum@cea.fr

Eric ROSENTHAL
HOPITAL DE L'ARCHET
Nice, France
rosenthal.e@chu-nice.fr

Anna Laura ROSS
ANRS
Paris, France
anna-laura.ross@anrs.fr

Laurent ROSSIGNOL
TRT-5
Paris, France
rossignol@trt-5.org

Christine ROUZIOUX
UNIVERSITE PARIS DESCARTES
Paris, France
christine.rouzioux@nck.aphp.fr

Willy ROZENBAUM
HOPITAL SAINT-LOUIS
Paris, France
willy.rozenbaum@sls.aphp.fr

Nicolas RUFFIN
INSERM
Créteil, France
nicolas.ruffin@inserm.fr

Elisa SABA
UNIVERSITA VITA-SALUTE SAN RAFFAELE
Milan, Italy
saba.elisa@hsr.it

Michela SABBATUCCI
ISTITUTO SUPERIORE DI SANITA'
Rome, Italy
michela.sabbatucci@iss.it

Farideh SABRI
KAROLINSKA INSTITUTE
Stockholm, Sweden
farideh.sabri@ki.se

Alessandra SACCHI
INMI L. SPALLANZANI
Roma, Italy
alessandra.sacchi@inmi.it

Asier SAEZ-CIRION
INSTITUT PASTEUR
Paris, France
asier.saez-cirion@pasteur.fr

Héla SAÏDI
INSTITUT PASTEUR
Paris, France
hela.saidi@pasteur.fr

Wilai SAKSIRISAMPANT
CHULALONGKORN UNIVERSITY
Bangkok, Thailand
fmedwss@yahoo.com

Nina SALABERT
CEA
Fontenay Aux Roses, France
nina.salabert@cea.fr

Jerome SALMON
INSTITUT GUSTAVE ROUSSY
Villejuif, France
jerome.salmon@igr.fr

Tanawan SAMLEERAT
FACULTY OF ASSOCIATED MEDICAL SCIENCES, CHIANG MAI UNIVERSITY
Chiang Mai, Thailand
tsamleerat@gmail.com

Eveline SANTOS DA SILVA
CRP-SANTE
Luxembourg
eveline.santosdasilva@crp-sante.lu

Joanna SANTOS-OLIVEIRA
FIOCRUZ
Rio De Janeiro, Brazil
joanna_reis@yahoo.com.br

Bruno SARGUEIL
CNRS

Gabriella SCARLATTI
SAN RAFFAELE SCIENTIFIC INSTITUTE
Milano, Italy
scarlatti.gabriella@hsr.it

Birgit SCHRAMM
EPICENTRE
Paris, France
birgit.shramm@epicentre.msf.org

Olivier SCHWARTZ
INSTITUT PASTEUR
Paris, France
olivier.schwartz@pasteur.fr

Christine SCHWIMMER
INSERM
Bordeaux, France
christine.schwimmer@isped.u-bordeaux2.fr

Nabila SEDDIKI
UNIVERSITE PARIS-EST CRETEIL
Créteil, France
nabila.seddiki@inserm.fr

Robert SEDER
VRC, NIAID, NIH
Bethesda, United States
abramt@niaid.nih.gov

Rafick SÉKALY
VACCINE AND GENE THERAPY INSTITUTE
Port St. Lucie, United States
rpsekaly@vgtifl.org

Prakash SELLAPPA GK
NAVAL AIDS RESEARCH CENTER
Namakkal, India
nimr@bsnl.in

Barbara SHACKLETT
UNIVERSITY OF CALIFORNIA, DAVIS
Davis, United States
blshacklett@ucdavis.edu

Victoria SHARP
ST. LUKE'S ROOSEVELT HOSPITAL
New York, United States
Vsharp@chpnet.org

Hong SHEN
UNIVERSITY OF WASHINGTON
Seattle, United States
hs24@uw.edu

Abubakarr SIDIBAY
YOUTH CRIME WATCH S.L
Freetown, Sierra Leone
ets_et_frere@yahoo.co.uk

Guido SILVESTRI
YERKES NATIONAL PRIMATE RESEARCH CENTER
Atlanta, United States
gsilves@emory.edu

Marie-Christine SIMON
ANRS
Paris, France

Martine SINET
INSERM
Le Kremlin-Bicêtre, France
martine.sinet@u-psud.fr

Kasha SINGH
MONASH UNIVERSITY/UCL
Melbourne, Australia
kpsin2@student.monash.edu.au

Dona SLEIMAN
LCRB
Paris, France
donasleiman@hotmail.com

Nikaïa SMITH
UNIVERSITE PARIS DESCARTES - CNRS UMR8601
Paris, France
nikaia.smith@etu.parisdescartes.fr

Maja SOMMERFELT
BIONOR PHARMA ASA
Skien, Norway
maja.sommerfelt@bionorimmuno.com

Joseph SONNABEND
New York, United States
benson@panix.com

Anders SÖNNERBORG
KAROLINSKA INSTITUTET
Stockholm, Sweden
anders.sonnerborg@ki.se

Adèle SOURISCE
INSERM U1016
Paris, France
adele.sourisce@inserm.fr

Christiane STAHL-HENNIG
GERMAN PRIMATE CENTER
Goettingen, Germany
stahlh@dpz.eu

Tienie STANDER
HEXOR (PTY) LTD
Gauteng, South Africa
tienies@hexor.co.za

Kamelia STANOEVA
MEDICAL UNIVERSITY - SOFIA
Sofia, Bulgaria
kamelia.stanoeva@gmail.com

Isabelle STAROPOLI
INSTITUT PASTEUR
Paris, France
istaro@pasteur.fr

Anna Katharina STARZACHER
HELIOS E. V. BEHRING KH BERLIN
Berlin, Germany
akstarzacher@googlemail.com

Tetiana STEPCHENKOVA
NATIONAL MEDICAL ACADEMY OF POST-GRADUAT EDUCATION
Kiev, Ukraine
steptavit@yahoo.com

Oana STREINU-CERCEL
NATIONAL INSTITUTE FOR INFECTIOUS DISEASES
Bucharest, Romania
oana_st_c@yahoo.com

Maryna SYROVATSKA
ABBOTT LABORATORIES
Kyiv, Ukraine
diana@dinadis.ua

Kévin TARTOUR
U1111 ENS LYON
Lyon, France
kevin.tartour@ens-lyon.fr

Roger TATOUD
IMPERIAL COLLEGE LONDON
London, United Kingdom
r.tatoud@imperial.ac.uk

Ran TAUBE
BEN GURION UNIVERSITY OF THE NEGEV
Beer Sheva, Israel
rantaube@bgu.ac.il

Serge TCHAMGOUE
CENTRE HOSPITALIER DE LIBOURNE
Libourne, France
serge.tchamgoue@ch-libourne.fr

Patrice Yves TCHENDJOU TANKAM
CENTRE PASTEUR DU CAMEROUN
Yaoundé, Cameroon
tchendjou@pasteur-yaounde.org

Mathurin Cyrille TEJIOKEM
CENTRE PASTEUR DU CAMEROUN
Yaoundé, Cameroon
tejiokem@pasteur-yaounde.org

Clare THOMAS
NATURE PUBLISHING GROUP
London, United Kingdom
c.thomas@nature.com

Maria-Isabel THOULOUZE
INSTITUT PASTEUR
Paris, France
thoulouz@pasteur.fr

Branislav TIODOROVIC
FACULTY OF MEDECINE,INSTITUTE OF PUBLIC HEALTH
NIS, Serbia-Montenegro
tiodorovic.branislav@gmail.com

Branislav TIODOROVIC
FACULTY OF MEDICINE, NIS
Nis, Serbia-Montenegro
travel@abba.rs

Carine TISNÉ
CNRS
carine.tisne@parisdescartes.fr

Biliana TODOROVA
SIV/iMETI/DSV/CEA
Fontenay-Aux-Roses, France
biliana.todorova@cea.fr

Rosana TORO
FACULTAD DE CIENCIAS EXACTAS- UNLP
Gonnet, Argentina
rosanat26@hotmail.com

Thi Xuan Lien TRUONG
INSTITUT PASTEUR DE HO CHI MINH VILLE
Hochiminh, Vietnam
truongxuanlien@gmail.com

Naho TSUCHIYA
LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
London, United Kingdom
naho.tsuchiya@lsthm.ac.uk

Nathalie ULRYCK
CNRS
Paris, France
nathalie.ulryck@parisdescartes.fr

Silvio URCUQUI INCHIMA
UNIVERSIDAD DE ANTIOQUIA
Medellín, Colombia
silviourcuqui@gmail.com

Sumonmal UTTAYAMAKUL
BAMRASNARADURA INFECTIOUS DISEASES INSTITUTE
Nonthaburi, Thailand
sumonmal@health.moph.go.th

Rajshekhar UZGARE
AIDS CLINICS
Mumbai, India
druzgare47@gmail.com

Renaud VAILLANT
THERAVECTYS
Villejuif, France
rvaillant@theravectys.com

Alejandro VALLEJO
HOSPITAL RAMON Y CAJAL
Madrid, Spain
alejandro.vallejo@salud.madrid.org

Philippe VAN DE PERRE
INSERM - UNIVERSITY MONTPELLIER 1
Montpellier, France
p-van_de_perre@chu-montpellier.fr

Carine VAN LINT
UNIVERSITY OF BRUSSELS (ULB)
Gosselies, Belgium
cvlint@ulb.ac.be

Jacqueline VAN TONGEREN
AMSTERDAM INSTITUTE FOR GLOBAL HEALTH (AIGHD)
Amsterdam, The Netherlands
j.vantongeren@aighd.org

Gudio VANHAM
INSTITUTE OF TROPICAL MEDICINE
Antwerpen, Belgium
gvanham@itg.be

Jean-Pierre VARTANIAN
INSTITUT PASTEUR
Paris, France
jean-pierre.vartanian@pasteur.fr

Stefano VELLA
ISTITUTO SUPERIORE DI SANITÀ – ISS
Rome, Italy
stefano.vella@iss.it

Marie VENDEVILLE
INSTITUT PASTEUR
Paris, France
marie.vendeville@pasteur.fr

Fred VERDULT
VOLLE MAAN
Amsterdam, The Netherlands
fred@volle-maan.nl

Christel VÉROLLET
IPBS-CNRS
Toulouse, France
Christel.Verollet@ipbs.fr

Bernard VERRIER
CNRS
Lyon, France
bernard.verrier@ibcp.fr

Francois VILLINGER
EMORY UNIVERSITY
Atlanta, Ga, United States
fvillin@emory.edu

Domenico VIOLA
INMI L. SPALLANZANI
Roma, Italy
domenico.viola@inmi.it

Valérie VIVET-BOUDOU
CNRS
Strasbourg, France
v.vivet@ibmc-cnrs.unistra.fr

Alain VOLNY-ANNE
Paris, France
volnyanne_alain@hotmail.com

Mark WAINBERG
MCGILL UNIVERSITY AIDS CENTRE
Montreal, Canada
mark.wainberg@mcgill.ca

Simon WAIN-HOBSON
INSTITUT PASTEUR
Paris, France
simon.wain-hobson@pasteur.fr

Yongjin WANG
INSTITUT PASTEUR
Paris, France
yongjin.wang@pasteur.fr

Ajeng Pratiwi Fitri WARDANI
PADJADJARAN UNIVERSITY
Bandung, Indonesia
dr.ajengpratiwi@gmail.com

Laurence WEISS
INSTITUT PASTEUR
Paris, France
laurence.weiss@pasteur.fr

Robin WEISS
UNIVERSITY COLLEGE LONDON
London, United Kingdom
r.weiss@ucl.ac.uk

Winfried WEISSENHORN
UNIVERSITÉ JOSEPH FOURIER
Grenoble, France
weissenhorn@embl.fr

Wendy WERTHEIMER
NIH OFFICE OF AIDS RESEARCH
United States
wendyw@nih.gov

Jack WHITESCARVER
NIH
Bethesda, United States
botchwar@od.nih.gov

Alan WHITESIDE
UNIVERSITY OF KWAZULU NATAL
Durban, South Africa
whitesid@ukzn.ac.za

Aurelie WIEDEMANN
INSERM
Créteil, France
aurelie.wiedemann@inserm.fr

Doris WILFLINGSEDER
INNSBRUCK MEDICAL UNIVERSITY
Innsbruck, Austria
doris.wilflingseder@i-med.ac.at

Christopher WILLIAMS
FRED HUTCHINSON CANCER RESEARCH CENTER/UNIVERSITY OF WASHINGTON CENTER FOR AIDS RESEARCH
Port Angeles, United States
cwilliam@nisa.net

Cecile WINTER
HOPITAL ANDRÉ GRÉGOIRE
Paris, France
cecile.winter@chi-andre-gregoire.fr

Steven WOLINSKY
NORTHWESTERN UNIVERSITY
Chicago, United States
s-wolinsky@northwestern.edu

Johan XAVIER
ENS LYON / CNRS
Lyon, France
johan.xavier@ens-lyon.fr

Yazdan YAZDANPANAH
HÔPITAL BICHAT-CLAUDE BERNARD
Paris, France
yazdan.yazdanpanah@bch.aphp.fr

Yasuko YOKOTA
NATIONAL INSTITUTE OF INFECTIOUS DISEASES
Tokyo, Japan
yyokota@nih.go.jp

Michelle YONG
MONASH UNIVERSITY
Melbourne, Australia
michyong@yahoo.com

Jamila YOUNES
CEA
Fontenay-Aux-Roses, France
jamila.younes@cea.fr

Oleg ZLATA
SIMFEROPOL REGIONAL CENTER OF AIDS PREVENTION
Kyiv, Ukraine
avia2@dinadis.ua

Susan ZOLLA-PAZNER
NEW YORK UNIVERSITY SCHOOL OF MEDICINE
New York, United States
susan.zolla-pazner@nyumc.org