



Poster abstracts

Effect of CCR and HERV Polymorphism on Susceptibility to HIV-1 Infection in Polish Population

Katarzyna Zwolińska¹, Egbert Piasecki¹, Katarzyna Rybka¹, Brygida Knysz², Jacek Gašiorowski²,
Andrzej Gładysz²

¹*Laboratory of Virology, Institute of Immunology and Experimental Therapy, Wrocław, Poland*

²*Department of Infectious Diseases, Wrocław Medical University, Wrocław, Poland*

Polymorphism at many loci plays an important role in susceptibility to HIV-1 infection. The allelic forms of chemokine receptor CCR5 and CCR2 genes are known to be of importance. However, the significance of a particular allele in different populations or groups of patients has not been yet established. We determined the frequency of CCR5-delta32 and CCR2-64I alleles in HIV-1-positive patients from Lower Silesia Region of Poland using PCR method. In the case of CCR2 gene two pairs of primers and PCR-RFLP method were used. The frequency of CCR5-delta32 and CCR2-64I alleles in Polish population (healthy individuals, not exposed to HIV) was found to be 11.9% and 12.0%, respectively. In contrast to the control group, the frequency of CCR5-delta32 allele proved to be reduced in HIV-1-infected patients (8.2%). On the other hand, higher frequency of the allele was found in the group of exposed, uninfected individuals (16.7%). No effect was observed in the case of CCR2-64I allele (frequency of 11.5% in HIV-1-positive individuals). Mode of infection was found to be significant in allele frequency analysis. The frequency of CCR5-delta32 mutation was similar in homosexual and drug using HIV patients (9.6% and 9.2%, respectively). However, in the case of heterosexually-transmitted infection lower frequency of the allele was detected (4.4%) suggesting the protective effect in this mode of infection. It was in agreement with the data obtained for heterosexually-exposed uninfected individuals (16.7%). Regardless of CCR5 or CCR2 genotype no effect on progression of HIV-1 infection was found. Some of human endogenous retrovirus (HERV) sequences reveal structural and/or functional similarity with HIV sequences. Hence possible interactions with HIV life cycle resulting in influence on the course of the virus infection should be taken into account. We analyzed polymorphic HERV-K113 and HERV-K115 sequences encoding protein within the env gene, named Rec. This protein is a functional counterpart to the Rev protein of HIV that exports unspliced HIV transcripts from the nucleus. The genotype was determined by PCR method with three reactions for HERV-K113 and four for HERV-K115. Frequency of HERV-K113 and HERV-K115 in Polish population was found to be 12.7% and 7.7%, respectively. No significant difference was found between infected and uninfected patients. It suggested that the sequences had no effect on HIV-1 infection progression.

P-MO-2

Polymorphisms of HIV-1 Co-receptors and Their Ligands in Chinese Population and the Effects on HIV-1 Infection and the Disease Progression

Bo-Jian Zheng¹, Xiu-Ying Zhao¹, Shui-Shan Lee², Ka-Hing Wong², Kenny C. W. Chan², Fai Ng¹, Wing-Cheong Yam¹, Kwok-Yung Yuen¹, Mun-Hon Ng¹.

Department of Microbiology, the University of Hong Kong¹; Integrated Treatment Centre, Department of Health²; Hong Kong, China.

Background: Current knowledge concerning the role of HIV co-receptors CCR5 & CXCR4 and their natural ligands, RANTES and SDF-1, in pathogenesis of HIV infection varies with geographical location of study subject.

Method: Polymorphisms in CCR5, CXCR4, RANTES and SDF-1 genes from a study cohort of 1099 Chinese were identified and compared between uninfected and HIV infected individuals and between HIV patients with slow disease progression and fast disease progression. Selected mutants were further characterized phenotypically.

Results: The CCR5 gene was the most polymorphic, with 17 mutations being identified in promoter and ORF region, followed by RANTES gene, with 3 mutations being identified in promoter region. CXCR4 and SDF-1 genes were relatively conserved, with 1 synonymous mutation being identified in the former and 2 in the latter.

Six nonsynonymous mutations in CCR5 coding gene were characterized *in vitro* studies. Mutants 118delF, G106R caused a reduction in reactivity for N-terminus-specific antibody and abrogated the reactivity for the ECL-2-specific antibody. Functional studies further showed that HIV co-receptor activity was reduced by G106R and abrogated by 118delF.

There was marked linkage disequilibrium between the alleles located near the promoter (-28G/A and -403G/A) and intron (In1.1C/T) of the RANTES genes, which resulted in 4 haplotypes. The haplotype-IN-I correlated to the lowest level of RANTES expression and was also more prevalent among HIV patients than in healthy donors.

The two SDF-1 mutants neither associated with HIV infection/pathogenesis, nor associated with variation in its transcription. However, up-regulated SDF-1 transcription was correlated with advanced disease progression.

Conclusion: The results are in general agreement with previous findings. They also suggest that the conformational change in CCR5 may directly affect HIV infection. Moreover, it was found that RANTES and SDF-1 expression appeared to correlate with HIV infection. This implicates CCR5 and CXCR4 and their natural ligands in HIV pathogenesis.



P-MO-3

HIV-1 Exposed Uninfected Individuals: Molecular Analysis of CCR5, CD45, and APOBEC3G Genes

Enrico M Treccarichi¹, Carmela Calbi¹, Enrica Tamburrini¹, Roberto Cauda¹, Christina Brahe²,
Mario Tumbarello¹ and Francesco D Tiziano²

¹*Department of Infectious Diseases, Catholic University, Rome, Italy*

²*Department of Medical Genetics, Catholic University, Rome, Italy*

Background: Despite multiple sexual exposure to human immunodeficiency virus type 1 (HIV-1) virus, some individuals remain HIV-1 seronegative (exposed seronegative, ESN). The mechanisms underlying this resistance remain still unclear, although a multifactorial model can be hypothesized. Although several genetic factors have been related to HIV-1 resistance, the homozygosity for a common mutation in *CCR5* gene (a 32 bp deletion, i.e. CCR5-Delta32 allele) is presently considered the most relevant one. More recently, other loci have been related to HIV-1 infection susceptibility: *CD45* and *APOBEC3G* genes. In particular, a relatively rare variant of *CD45* (C77G) has been found to be more frequent in HIV-1 infected individuals, whereas an *in vitro* mutation of *APOBEC3G* (D128K) has been demonstrated to inhibit HIV-1 replication. We report on the study of the genotype at *CCR5*, *CD45*, and *APOBEC3G* loci of 30 Italian ESN individuals (case group) who referred multiple unprotected heterosexual intercourse with HIV-1 seropositive partner(s), for at least two years.

Results: One hundred and twenty HIV-1 infected patients and a similar number of individuals representative of the general population were included as control groups. None of the analysed individuals had CCR5-Delta 32 homozygous genotype. Twenty percent of ESN subjects had heterozygous CCR5-Delta 32 genotype, compared to 7.5% of HIV-1 seropositive and 10% of individuals from the general population, respectively. Sequence analysis of the entire open reading frame of *CCR5* was performed in all ESN subjects and no polymorphisms or mutations were identified.

Subsequently, we have sequenced *APOBEC3G* exon 3, containing codon 128, and found only one sequence variant, the T357C (Phe119Phe) polymorphism, which was previously described. The distribution of this polymorphism was similar in the three population analysed.

Also the frequency of the C77G allele of *CD45* gene showed no differences between ESN subjects and the two control groups.

Conclusions: Our data show a significantly higher frequency of CCR5-Delta 32 heterozygous genotype ($p=0.04$) among the Italian heterosexual ESN individuals compared to HIV-1 seropositive patients, suggesting a partial protective role of CCR5-Delta 32 heterozygosity in our cohort.

P-MO-4

Antibodies Recognizing a Peptide Derived from the Second Conserved Region of HIV-1 gp120 are Associated with Non-progressive HIV infection

Ana Djordjevic^{*1}, Milena Veljkovic^{*2}, Sascha Antoni³, Maria Sakarellos-Daitsiotis⁴, Sanja Glisic¹, Ursula Dietrich³, Nevena Veljkovic¹

¹ Centre for Multidisciplinary Research, Institute of Nuclear Sciences VINCA, Belgrade, Serbia

² Laboratory for Radioisotopes, Institute of Nuclear Sciences VINCA, Belgrade, Serbia

³ Georg-Speyer-Haus, Institute for Biomedical Research, Frankfurt, Germany

⁴ Department of Chemistry, Section of Organic Chemistry and Biochemistry, University of Ioannina, Ioannina, Greece

* These two authors equally contributed

Background: Previous studies have demonstrated that antibodies with specific affinity to a 23 amino acids long peptide derived from the second conserved region of the C terminus of HIV-1 gp120, denoted as NTM, may be involved in the control of HIV disease progression.

Objective: To determine a short peptide derived from the NTM and to compare the reactivity of antibodies present in sera of HIV-1 infected long-term non-progressors and progressors with NTMshort (NTMs).

Methods: Informational spectrum analyses and computational peptide scanning was applied to identify peptide NTMs. The peptide (NTMs)₄-SOC₄ carrier conjugate was synthesized and used as antigenic target in ELISA. Two groups of serum samples were studied: 7 LTNPs and 5 HIV-1 infected progressors with matched CD4 counts and low viral load.

Results: The sequence FTDNAKTI was chosen based on peptide scanning results and spectral similarity to peptide NTM. In ELISA testing significantly higher levels of (NTMs)₄-SOC₄ reactive Abs was observed in sera of LTNPs compared to a control group of HIV progressors.

Conclusion: Thus, antibodies recognizing the second conserved region of the HIV-1 gp120 derived octapeptide NTMs (FTDNAKTI), seem to be associated with preventing disease progression.



P-MO-5

Innate Differences in the IL2 Regulation in CD4⁺ T Cells from Non-Human Primates as a Mechanism of SIV Disease Resistance

Pavel Bostik, Susan Stephenson, Erika Noble, Aftab A. Ansari

Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA 30322, USA

Gradual loss of antigen specific T cell responses associated with the impairment of the immune system is characteristic for progressive HIV infection of humans. Similar characteristics leading to the AIDS-like disease constitute hallmarks of pathogenic SIV infection in rhesus macaques (RM) in non-human primate (NHP) model of AIDS. In both cases this immune system dysfunction is detectable in both the CD4⁺ and CD8⁺ cells prior to any major signs of CD4⁺ T cell depletion. One of the hallmarks of this dysfunction and subsequent progression to AIDS is the dysregulation of IL2 production in CD4⁺ T cells. IL2 is a key factor in multiple processes involved in the survival of T lymphocytes and in the regulation of T cell responses. CD4⁺ T cells from HIV infected patients exhibit deficient IL2 autocrine function and the addition of exogenous IL2 is sufficient to correct cell cycle abnormalities characteristic for these cells. Interestingly, certain NHP species – such as sooty mangabeys (SM) - are natural hosts of SIV and are AIDS-like disease resistant despite high levels of virus replication comparable to those found in SIV disease susceptible species. One of the innate characteristics of the CD4⁺ T cells from the SIV disease resistant SM is their increased ability to produce IL2 upon TCR stimulation leading to the resistance of these cells to the induction of immunological unresponsiveness. Further studies of the molecular mechanisms of the IL2 regulation in CD4⁺ T cells and its correlation to the SIV disease outcome show, that T cells from SM exhibit significantly higher constitutive production of IL2 and spontaneously synthesize 2-3 fold higher levels of IL2 than corresponding cells from RM or humans. Sequence analysis of the IL2 promoter from humans, RM and SM showed high degree of identity between the human and RM sequences. However, single nucleotide variations in the in the –180 AP-1 like site and additional sequence upstream around nucleotide –200 were consistently found in IL2 promoter sequences from SM. Reporter assays using IL2 promoter driven GFP expression constructs in primary CD4⁺ T cells showed that the increased constitutive synthesis of IL2 in SM correlates with higher baseline activity of the IL2 promoter proximal fragment. Utilizing hybrid reporter constructs containing –180 and –200 sites from SM and RM spliced together or duplicated identified the –180 sequence as a negative and –200 sequence as a positive sites. Activity of both SM and RM derived constructs was upregulated by p300 and downregulated by CREB to a similar degree. However, chromatin immunoprecipitation analysis showed that the higher production of IL2 is accompanied by increased p300 and decreased CREB binding to the proximal promoter region in CD4⁺ T cells from SM *in vivo*. In addition the nucleotide substitution around position –200 increases the affinity of this site for the binding of transcription factor Oct-1. These results suggest that these unique innate regulatory mechanisms of the IL2 promoter in SM represent an underlying mechanism of the increased IL2 production in CD4⁺ T cells and as such may represent one of the innate mechanisms of the SIV disease resistance.

This work was supported by the NIH RO1 AI65362.

P-MO-6

Phage-displayed Chemokines as Tools for Studying CCR5-mediated Interactions

Lejczak C.¹, Delhalle S.¹, Deroo S.¹, Plessier J.-M.¹, Beaupain N.¹, Schmit J.-C.^{1,2}

¹CRP-Sante, Laboratoire de Retrovirologie, Luxembourg, Luxembourg

²Service National des Maladies Infectieuses, CHL, Luxembourg, Luxembourg

Background: We aim to identify peptide sequences interacting with the chemokine receptor CCR5 by using phage-displayed peptide libraries. In order to assess the efficiency of the selected phage, we constructed positive control phage displaying the natural CCR5 ligands, Macrophage Inflammatory Protein (MIP)1 α , MIP1 β and Regulated on Activation, Normal T-cells Expressed and Secreted (RANTES).

Methods: Total RNA was extracted from peripheral blood mononuclear cells (PBMC) from a healthy donor and reverse-transcribed. cDNA coding for the MIP1 α , MIP1 β and RANTES chemokines were then amplified by PCR, ligated in the fUSE5 phage vector (generous gift of Pr G.Smith) and electroporated into *E.coli* cells to obtain phage expressing the chemokines.

Results: Analysis of the bacterial clones confirmed the correct sequence of the chemokines inserted in the phage vector. Expression of chemokines was assessed in ELISA by immobilising chemokine-specific antibodies and detecting the interaction of the phage with the antibodies. Phage expressed-chemokines were specifically recognised by their respective anti-chemokines antibodies. Competition assays where phage mixed to increasing amounts of recombinant protein were added to immobilised anti-chemokines antibodies and detected with peroxidase-coupled anti-M13 antibody were performed. The purified chemokine-expressing phage were able to compete specifically with their respective recombinant chemokine for binding to the anti-chemokine antibodies, with IC50 of 3, 30 and 10 ng for MIP1 α , MIP1 β and RANTES, respectively, while equimolar recombinant proteins did not compete.

The biological activity of the phage-displayed chemokines on the CCR5 receptor was analysed by monitoring the calcium flux upon binding of the phage chemokines. The calcium flux was monitored after addition of recombinant RANTES or phage-displayed RANTES on wild-type as well as on CCR5-expressing U87 glioma cells.

Results showed that RANTES-expressing phage elicited a calcium response in U87CCR5 cells.

Conclusions: We describe the successful construction of phage expressing the natural ligands of the chemokine receptor CCR5. We have shown that these chemokines are present at the surface of the phage and are recognised by specific antibodies. Moreover, preliminary results indicate that the RANTES-expressing phage acts as an agonist of CCR5. These positive control phage are valuable tools for the screening of CCR5 with phage-displayed peptide libraries. Phage chemokines are a low-cost alternative to purified recombinant proteins to monitor protein-receptor interactions.

A Study to Correlate the Type of STD Vis-a-vis CD 4 Counts from India

Kabir Sardana

Maulana Azad Medical College, Delhi, India

Background: HIV infection is primarily a sexually transmitted disease (STD), In India. A number of prospective studies of STD findings in HIV-I infected patients have been published, almost all from countries where homosexuality is the main risk factor. We have analyzed the incidence of STD in relation to the CD 4 counts and analyzed the concomitant skin conditions seen.

Methods: We prospectively examined 3000 STD cases from 2002 to 2006 .Out of this 164 were HIV positive and most had multiple partners (65%)and in majority heterosexuality was a risk factor (89%). The majority were of stage II (WHO) (70%), while stage IV was less frequent (19%). The mean CD4 cell count was 280/mm³. A detailed history was followed by examination and VDRL, HIV-ELISA,TPHA ,HIV-ELISA, and smears and biopsies were undertaken were needed. CD4 cells/mm³ counts were measured at first visit and at 6 monthly intervals.

Results: Out of 3000 STD cases studied 164 were HIV positive .The commonest STD was genital herpes(25.6 %),followed by condylomata acuminata(24.4%) ,syphilis(12%) and molluscum conatgiosum(10%) .Other STD seen were, gonorrhoea (8%), non-gonococcal urethritis (NGU) (5%), genital candida infection(4%), vulvovaginal candidiasis(3%) ,bacterial vaginosis(3%) , scabies (2%) and donovaniosis (1%).We nalysed the significance of association vis-avis CD 4 counts in thre groups (<200,200-500,>500) Only genital herpes and condylomata acuminata had a statistically significant association with advanced stage (P <0.5)

In 25 % of case two or more STD were present.The3 commonest cutaneous condition seen were, xerosis,oral thrush and herpes zoster .The most common systemic infection seen was tuberculosis .

All patient responded to standard therapy

Conclusion: In this study viral STD like herpes genitalis and condylomata acuminata were common and had a correlation withy low CD4 counts(<200)

Concomitant with the lowered immunity and the endemicity of tuberculosis most of our cases had systemic tuberculosis.

P-MO-8

Regulation of CD4 Expression via Recycling by HRES-1/RAB4 Controls Susceptibility to HIV Infection

Gyorgy Nagy^{1,2}, Jeffrey Ward¹, Dick D. Mosser¹, Agnes Koncz¹, Peter Gergely Jr.^{1,3}, Katalin Banki¹, Andras Perl¹

1: *Departments of Medicine, Microbiology and Immunology, and Pathology, State University of New York, College of Medicine, Syracuse, New York, USA*

2: *Hospitaller Brothers of St John of God, Department of Rheumatology, Budapest, Hungary*

3: *National Institute of Rheumatology and Physiotherapy, Budapest, Hungary*

A novel 2986-base transcript encoded by the antisense strand of the HRES-1 human endogenous retrovirus was isolated from peripheral blood lymphocytes. This transcript codes for a 218-amino acid protein, termed HRES-1/Rab4, based on homology to the Rab4 family of small GTPases. HRES-1 nucleotides 2151-1606, located upstream of HRES-1/Rab4 exon 1, have promoter activity when oriented in the direction of HRES-1/Rab4 transcription. The human immunodeficiency virus, type 1 (HIV-1), *tat* gene stimulates transcriptional activity of the HRES-1/Rab4 promoter via *trans*-activation of the HRES-1 long terminal repeat. Transfection of HIV-1 *tat* into HeLa cells or infection of H9 and Jurkat cells by HIV-1 increased HRES-1/Rab4 protein levels. Overexpression of HRES-1/Rab4 in Jurkat cells abrogated HIV infection, gag p24 production, and apoptosis, whereas dominant-negative HRES-1/Rab4^{S27N} had the opposite effects. HIV-1, uses two receptors for cellular attachment and viral entry. Initial viral attachment occurs through the binding of the envelope protein gp120 to the CD4 molecule expressed on the surface of T lymphocytes and macrophages. Viral binding to CD4 is necessary but insufficient to mediate viral entry. CD4 appears to play a role in HIV entry distinct from merely serving as the attachment protein for the virus. CD4 undergoes endocytosis following T cell activation via the activation of protein kinase C and subsequent phosphorylation of CD4. HIV binding was also reported to induce phosphorylation of CD4 via a protein kinase C-dependent pathway; however, internalization of HIV does not require endocytosis of CD4. HRES-1/Rab4 inhibited surface expression of CD4 and targeted it for lysosomal degradation. HRES-1/Rab4^{S27N} enhanced surface expression, recycling, and total cellular CD4 content. Infection by HIV elicited a coordinate down-regulation of CD4 and up-regulation of HRES-1/Rab4 in PBL. Moreover, overexpression of HRES-1/Rab4 reduced CD4 expression on peripheral blood CD4+ T cells. Stimulation by HIV-1 of HRES-1/Rab4 expression and its regulation of CD4 recycling reveal novel coordinate interactions between an infectious retrovirus and the human genome.



Analysis of Positional Interdependencies of Mutations in Reverse Transcriptase

Bazsó F., Borgulya G., Zalányi L., Nepusz T.

*Department of Biophysics, KFKI Research Institute for Particle and Nuclear Physics
of the Hungarian Academy of Sciences H-1525 Budapest, P.O. Box 49, Hungary*

Mutations in one position may depend on mutations in other positions, therefore it is important to quantify, i.e. measure mutational influence between various positions. For this purpose a new methodological tool is used, which is based on information- and graph theory. Data about the Reverse Transcriptase (RT) available from the EuResist project's (www.euresist.org) integrated database are represented as a weighted directed graph. Positions in the RT sequence are represented as nodes, while the weight of the edge connecting nodes i and j is calculated from the Thiel's entropy coefficient $k(i, j) = I_{ij} / S_i$, where S_i is the entropy of amino-acid probability density at position i , whilst I_{ij} is the mutual information of amino-acid occurrence between positions i and j . The weight measures how much the knowledge of the amino-acid at position j statistically determines the knowledge of the amino-acid at position i . Graph is clustered using the Szemerédi's regularity lemma. Because of graph's directedness, to each position two cluster indices were assigned. The outcome of the clustering procedure is a grouping of RT positions into groups which influence each other's mutations in the strongest possible way. Simulations showed that best clustering results (without vacuous categories) were obtained when edges with weights 0.9 were considered, and the number of clusters was fixed to 15. The method can be applied to the analysis of other enzymes and genetic data. The clustering results give new perspective on positions responsible for the development of antiviral drug resistance, and also suggest new targets suitable for future drug- and vaccine design.

Acknowledgement. The authors were supported with EU FP6 Programme grant nr. IST-4- 027173-STP.

Corresponding authors' e-mails: bazso@sunserv.kfki.hu, borgulya@rmki.kfki.hu

P-MO-10

Two-year Follow-up Study of Levels and Avidity Maturation of HIV Antibodies in Treated Children

Sylvie Faucher

*National HIV and Retrovirology Laboratories, Public Health Agency of Canada
Ottawa (Ontario), Canada*

Objective: Establish the antibody levels and avidity maturation over time among treated children with controlled (group I) and uncontrolled viral load (group II).

Methods: Antibody end point titers and avidity to 11 HIV-1 proteins, CMV and EBV lysates were determined from 12 treated HIV-1 infected children during a period of up to 30 months. Plasma samples were incubated with a mix of 13 protein-coupled fluorescent beads (Luminex) and detection was performed with anti-human IgG-biotin and Streptavidin-PE. Antibody avidity was determined from the residual titer measured when antibodies were incubated in presence of a chaotropic agent.

Results: HIV-1 antibodies were detected with a predominant response towards p24, p55 and p66. Antibodies to the matrix protein p17 were detected in 9 patients with titers two log lower than other gag antibodies. Antibodies to gp41, gp120 and gp160 were detected in all patients with a predominant response toward gp160. Among the regulatory proteins, nef and tat antibodies were the most frequently detected and showed the most markedly decline in group I patients. Overall, 4/6 patients in group I, showed half a log titer decrease to at least 2 proteins whereas 1/6 patient in group II showed similar titer reduction. Antibodies to bystander viruses were found at low titers in both groups and remained unchanged over time. Antibody avidity to all HIV proteins was high (60-80%) for all but one patient (30% for tat) and remained unchanged over time.

Conclusions: The antibody titer profiles were sufficient to segregate patients into treatment responders/non-responders. Patients with controlled viral load showed higher reduction in antibody titers towards more proteins than patients with uncontrolled viral load. The antibody avidity was similar in both groups of patient suggesting that viral load did not affect maturation of antibody avidity.



Exposing and Measuring Suppressed HIV Specific Humoral Immunity

Carmen Soler, Jessica Jaiven, Tamar Jehuda-Cohen

Ort Braude College, Dept. Biotechnology, P.O. Box 78, 21982 Carmiel, Israel

Detection of HIV infection by serum antibodies is hindered by a delay in antibody production, post infection, as can be seen by the relatively long window period between infection and seroconversion. This delay could potentially mask the initial set of primed cells at the onset of the infection.

We used an in-vitro stimulation technology to induce the production of HIV specific antibodies at the seronegative state of the infection in order to detect those early infected individuals and analyze the profile of those early antibodies. Fresh blood samples from 200 very high risk individuals were tested for HIV antibodies with and without the pre-stimulation of the blood sample. Twenty one were seropositive without pre-stimulation, and 25 (21+additional 4) were positive after the pre-stimulation. All positives were confirmed by second ELISA assays. Using PCR, integrated HIV-1 was detected in 5/5 additional positives.

The antigenic profile of the antibodies in the serum of seropositive samples was compared to that of induced antibodies from the early, seronegative, stages of the infection. It was found that while most of the antigenic targets were the same, there were several peptides against which there were antibodies only after in-vitro stimulation. Thus the antibody repertoire in the seropositive individuals does not represent the whole “story” of the initial encounter with the HIV. Peptides from two strains of HIV were used and for both the epitops “seen” at the earliest stages of the infection were of peptides from the variable regions.

These findings could shed light not only on the early immune recognition of HIV but also on epitops against which the immune response has been “silenced” to be detected only by in-vitro stimulation that overcomes that suppression or peripheral tolerance. Thus it could have an impact both on HIV vaccine design and its clinical research.

P-MO-12

Reduced Repertoire Complexity of the IgG Derived Heavy Chain Complementarity Determining Region 3 of Long Term Non Progressors in Comparison to Healthy Donors

S. Deroo¹, S. Delhalle¹, N. Beaupain¹, J.M. Plesséria, J.C. Schmit^{1,2}

¹ *Laboratoire de Rétrovirologie, Centre de Recherche Public-Santé, 84, Val Fleuri, L-1526 Luxembourg, Luxembourg*

² *Service National des Maladies Infectieuses, Centre Hospitalier Luxembourg, 4, rue E. Barblé, L-1210 Luxembourg, Luxembourg*

Background: The heavy chain complementarity determining region 3 (HCDR3) is the most polymorphic region in both length and shape of the antibody and plays a key role in the determination of the antigen specificity. Length distribution analysis gives a clear perception of the repertoire variations between individuals and their immune responses during HIV infection. The objective of the present study was to compare the length distribution of the IgM and IgG derived HCDR3 loops of healthy donors and long term non progressors (LTNP).

Methods: Total RNA was extracted from PBMC isolated from 3 LTNP and 10 healthy donors. The human IgM and IgG CDR3 encoding regions were amplified separately starting from random primed cDNA. The fluorescent PCR fragments were separated by electrophoresis and their length was calculated by the use of a fluorescent size standard.

Results: The IgM derived HCDR3 length distribution pattern of the healthy donors and 2 LTNP was Gaussian-like. The HCDR3 repertoire of LTNP#2 displayed a skewed Gaussian-like profile. These distribution patterns were composed of 15 to 23 different CDR3 length classes. The smallest HCDR3 loops detected in the IgM repertoires were 2 amino acids. The longest HCDR3 loops composed of 24 residues were identified in the IgM repertoire of the healthy donors while the longest HCDR3 loops of the LTNP were 22 residues. The most common length of the IgM derived HCDR3 loops of healthy donors was 11 amino acids. In contrast, the most common HCDR3 length of LTNP varied between 8 and 12 residues. The length distribution of the IgG derived HCDR3 loops of healthy donors and LTNP displayed a skewed Gaussian-like profile composed of 15 to 18 different HCDR3 length classes. However, in the length distribution of LTNP and in particular, LTNP#2 different length classes were not represented. In comparison to the other LTNP, LTNP#2 had the lowest CD4 counts (CD4<500) and a detectable viral load (1500 copies/ml). The smallest fragment detected in the IgG HCDR3 repertoires of healthy donors and LTNP was 2 residues and the longest 21 residues.

Conclusions: Subtle differences between the IgM derived HCDR3 length distribution of healthy donors and LTNP were observed. More pronounced differences were observed between the IgG HCDR3 length distributions of healthy donors and in particular for LTNP#2. Missing length classes in the HCDR3 repertoires suggest a lower repertoire complexity and reveals the appearance of potential clonal expansions. Interestingly, for LTNP#2 these findings correlate with the lowest CD4 counts and a detectable viral load.

CD4 T Helper Responses to CMV Predict the Time “Off Therapy” in HIV-infected Patients under Guided Treatment Interruptions

M. Bofill¹, L. Darwich², C. Cabrera², J. Martinez-Picado², J.A. Esté², B. Clotet², L. Ruiz²

¹*Institució Catalana de Recerca i Estudis Avançats*, ²*Fundació IrsiCaixa, Badalona, Spain*

Background: Although the introduction of HAART has greatly reduced the clinical manifestation of cytomegalovirus (CMV) infection in HIV+ individuals, CMV viremia and related disease are not uncommon. CMV infection has been strongly associated to HIV disease progression which may in part be explained by the required control of CMV by the CD4 T helper response.

Methods: A cohort of 75 chronically infected HIV-1 individuals on HAART with CD4 levels above 500/mm³ and undetectable HIV-RNA levels participated in a guided treatment interruption clinical trial (TIBET). These patients interrupted therapy and were followed for a period of at least two years, or until their CD4+ counts reached values of less than 350 cells/mm³, or HIV-1 RNA levels increased above 100,000 copies/ml. The CD4 specific responses to CMV were measured by the capacity of these cells to produce IFN-gamma upon stimulation with CMV measured by ELISPOT assay. Kaplan-Meier survival curves and contingency tests were used to analyse the predictive value of the CD4 helper responses to CMV to stay “off” anti-HIV therapy.

Results: Patients were stratified into two groups, those with a number of CD4 helper responses below 500 spots/10⁶ cells (N=47) (weak response) or above 500 (N=28) (strong response). Kaplan-Meier survival curves showed that the subjects with a weak response to CMV had a higher chance to restart treatment than those patients with a strong response to (HR:0.39; 95%CI:0.15 to 0.61. p=0.006). During the 2 year follow-up, the number of patients with CD4 response <500 spots/10⁶ cells that required re-initiation of treatment was significantly (p=0.003) higher (28 out of 32) than those with >500 spots /10⁶ cells (23 out of 47 patients).

Conclusions: HIV-infected patients with weak CD4 helper responses to CMV were more likely to reinitiate HAART in guided treatment interruptions than patients with strong responses, suggesting that a weak capacity to control CMV could negatively influence disease progression. CD4 specific responses to CMV might be a predictive marker for the evolution of HIV infection.

P-MO-14

Baseline Expression of Some Surface Antigens on T-Cells Predicts Response to Antiretroviral Therapy

Yulia Sitdykova, Lidia V. Serebrovskaya, Alexey V. Kravchenko, Vadim V. Pokrovsky

Russian Federal AIDS Center, bd.2,15, 8 ul. Sokolinoy Gory, 105275 Moscow, Russia

Background: It is supposed that immunological status of HIV-infected patient prior to initiation of antiretroviral therapy may predict its efficacy.

Objective: To determine if the baseline levels of naïve/memory CD4 T-cells and expression of some surface antigens affect the response to the treatment.

Methods: We measured the level of naïve/memory CD4 T-cells and CD8 cells, expressing CD28 and CD57 in 45 HIV-infected treatment-naïve subjects, mean age 28±9,9. The analysis was performed using flow cytometry (Epics XL, Beckman Coulter). We determined the percent of CD4+CD45Ra+CD62L+ cells (naïve), CD4+CD45RO+ (memory), CD8+CD28+ and CD8+CD57+ cells. We calculated naïve/memory CD4 T-cells ratio prior to initiation of antiretroviral therapy. The patients were divided into two groups according to the value of the ratio – group 1 was consisted of the patients with lower values of the ratio, group 2 included the patients with higher values. CD4 T-cell increase was compared in both groups after 24 weeks of therapy.

Results: Patients with higher values of naïve/memory CD4 T-cells ratio demonstrated significantly higher CD4 T-cell increase after 24 week of treatment as it shown in the Table.

Group#	n	naïve/memory CD4 T-cells ratio	median %CD4 Week 0	median %CD4 Week 24	median CD4 cell/mm ³ Week 0	median CD4 cell/mm ³ Week 24	CD4 increase from baseline (%)
1	25	<0,6	19	21	281	291	3,6%
2	20	≥0,6	20	26	262	423	61,5%

The correlation between the value of the ratio and CD4 T-cell increase was found ($r = 0,3141$; $p=0,02$). We also noticed that CD4 T-cell increase was more prominent in patients with higher level of CD28 expression by CD8 cells and lower level of CD8+CD57+ cells, although statistically significant correlation was not found.

Conclusion: Patients who had higher level of naïve and lower level of memory CD4 T-cells, higher naïve/memory CD4 T-cells ratio, lower numbers of CD8+CD57+ cells and higher numbers of CD8+CD28+ cells prior to initiation of antiretroviral therapy demonstrated stronger immunological response to treatment.



Prevalence of HIV CCR5-Δ32 in the Slovak Population

Paulina Nogova, Monika Habekova, Danica Stanekova

NRC for HIV/AIDS prevention, Slovak Medical University, Bratislava, Slovakia

Aim of the study: To describe prevalence of 32 bp deletion in the gene for CCR5 coreceptor HIV /CCR5-Δ32/ in the Slovak population.

Patients and methods: CCR5-Δ32 was investigated in the group of 136 HIV-negative /95 men + 41 women/ and 72 HIV-positive / 70 men + 2 women/ Slovaks. For cell DNA isolation commercial kit Nucleospin Blood, (Machery Nagel) was used. CCR5-Δ32 was tested by the use of the kit EliGene CCR5 (polymorfizmus Δ) from Elisabeth Pharmacon. Provirus DNA was amplified by DNA-PCR. Amplification products were analyzed according to their molecular weights in agarose gel electrophoresis.

Results: CCR5-Δ32 was found in 0,73% HIV-negative individuals homozygous and in 16,9% HIV-negative individuals heterozygous for this mutation. In the group of HIV-positive persons no homozygosity of CCR5-Δ32 was found. Heterozygosity of CCR5-Δ32 in HIV-negative individuals was similar to that in HIV-positive patients /16.9% vs. 16,66%/.

Conclusion: Study of CCR5-Δ32 prevalence in the Slovak population revealed similar results compared to those described in other EU states. Significant difference between HIV-negative and HIV-positive group was not found. Results of the study do not indicate that relatively low prevalence of HIV-infection in SR could be due to the prevalence of CCR5-Δ32.

P-TU-1

The Novel Adipocytokine Visfatin/PBEF1 is One of Several Apoptosis- and Lipid Metabolism-Associated Factors Induced in Monocytes during *in vivo* HIV-1 Infection

Rafael Van den Bergh^{1,2}, Geert Raes^{1,2}, Marc Vekemans³, Huyen Thanh Thi Tran^{1,2}, Tom Boonefaes⁴, Johan Grooten⁴, Guido Vanham⁵, Patrick De Baetselier^{1,2}

¹*Department of Molecular and Cellular Interactions, VIB, Pleinlaan 2, B-1050 Brussels, Belgium*

²*Department of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium*

³*HIV Clinic, Institute of Tropical Medicine, Antwerp, Belgium*

⁴*Laboratory of Molecular Biology, Department for Molecular Biomedical Research, Universiteit Gent, Ghent, Belgium*

⁵*HIV Virology Research Unit, Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium*

Monocytes, and macrophages as their more differentiated counterparts, play a fundamental role during HIV infection, since they act as both antigen-presenting cells and effector cells of cellular immunity. Additionally, they are important target cells of HIV infection. In contrast with T lymphocytes, however, they do not die as result of infection but instead develop severe dysfunctions. These dysfunctions can manifest at the level of the monocyte/macrophage itself (e.g. delayed apoptosis, resulting in the formation of viral reservoirs) or at the level of interactions between monocytes/macrophages and other (immune) cells (e.g. killing of uninfected lymphocytes and concomitant protection against apoptosis of infected lymphocytes). To date this aberrant monocyte phenotype, and in particular the genetic basis thereof, remains poorly characterised. Therefore, we performed a transcriptome analysis of monocytes isolated from HIV-infected patients, using both a commercially available genome-wide microarray platform and a custom designed, focussed 'Macrophage Activation State' cDNA array. In this fashion we identified several functional clusters of genes which appear to be differentially expressed in monocytes of HIV patients *versus* those of healthy controls. Overrepresentational analysis of these clusters revealed significant perturbation of cellular processes associated with apoptosis and lipid metabolism/insulin signaling. The novel adipocytokine *pre-B-cell colony enhancing factor 1* (PBEF1 or visfatin), which has steadily been gaining clinical interest in the past few years, is one of the significantly upregulated genes in monocytes of HIV patients. Induction of PBEF1 mRNA and protein (both intracellular and secreted) was therefore characterised in monocytes of therapy-naïve and HAART-treated HIV-patients using real-time quantitative PCR, ECL-Western Blot and ELISA: a positive correlation between PBEF1 expression and viral load was found. Interestingly, this factor (primarily associated with visceral fat metabolism) has properties pertaining to both (protection against) apoptosis and insulin signaling and could consequently play a major role in one or more of the described monocyte dysfunctions. A thorough functional analysis of the role of PBEF1 in HIV-infection, and in particular in HAART-associated disorders such as lipodystrophy/metabolic syndrome, is therefore warranted.

The Role of the gp120 V3 Loop Sequon for Transmissibility of HIV-1

Clevestig P, Pramanik L, Lindgren S, Ehrnst A.

*Department of Microbiology, Tumor, and Cell biology, and the Department of Clinical Science,
Karolinska University Hospital Huddinge, Karolinska Institutet, Stockholm, Sweden*

Background: It is becoming increasingly clear that the R5 phenotype is necessary for HIV-1 transmission to occur. Therefore protective vaccine candidates must be targeting protection against R5 transmission. We have shown that a small element, the gp120 V3 loop N-linked glycosylation motif, or sequon, is closely linked to the R5 phenotype in an analysis of all submitted sequences to the Los Alamos Database (Clevestig et al., 2005). We wanted to analyse its role in mother-to-child transmission in a group of mothers carrying both the R5 and the X4 phenotype, adding a detailed analysis of HIV-1 clones from each individual.

Materials and methods: We chose to study mothers (n=7), carrying subtype D, a subtype which we have identified as being more commonly associated with the X4 phenotype. From 11 pregnancies 10 children were born. One became HIV-1 infected. Samples were collected during 33 time-points, from the first trimester up to 7 months post partum. Co-receptor use was determined for 29 viral isolates, using U87.CD4 cells, expressing either CCR5 or CXCR4, (n=17 from PBMC; n=12 from plasma). Nested PCR was used with primers, specific for the V3 region of the gp120 gene. The sequence data was assembled into an alignment and a phylogenetic tree was constructed using the neighbour-joining method for each mother. The presence of the V3 loop sequon was determined as well as the V3 loop charge. HIV RNA and CD4 cell counts were recorded.

Results: The co-receptor use was determined as CCR5 by R5 virus, (n=8), CXCR4 by X4 virus, (n=20), or dual-tropic, using both CCR5 and CXCR4 (n=1). We also produced 240 clones, representing one viral DNA molecule from peripheral blood mononuclear cells (PBMC), an average of 14 per sample and 58 per case. Although four of five mothers carried both phenotypes, there was a great individual difference in their proportions when grouping the clones around their respective phenotype in the phylogenetic tree. Despite this, there was a highly significant association between the presence of the N-linked glycosylation site of the V3 loop and the R5 phenotype on the one hand and between positive charge and the X4 phenotype on the other. Presence of this NNT motif seemed indifferent for CXCR4 use. Apart from the mother with an infected child, the majority of the X4-associated clones lacked the V3 loop sequon.

The mother with an infected child lacked prominent risk markers. Her CD4 cell counts were between 540 and 720 and her HIV RNA copies between 6.000 and 26.000 copies/ml. All her viral isolates and clones contained the V3 loop sequon. One mother of two uninfected children with low CD4 cell counts and high RNA levels between 51.000 to 690.000 copies/ml had a vast majority of clones, lacking the V3 loop sequon. In the other mothers of uninfected children, three had low RNA levels, few clones were achieved, and they all had the V3 loop sequon.

Conclusion and discussion: We showed in a viral population study that there was a close relation between the presence of the NNT-motif in the V3 loop and the R5 phenotype. The absence of this sequon may render HIV less transmissible. Therefore, an increased presence of an X4 phenotype, lacking the sequon, may be less likely to be transmitted perinatally.

P-TU-3

Improving the Immunogenicity of HIV-1 Envelope Glycoproteins

N.C. Sheppard*, C.L. Robson, M. Seaman, L. Kong, P. Kwong, S.A. Jeffs, N. Almond,
I.M. Jones, Q.J. Sattentau

**University of Oxford, Department of Pathology, South Parks Road, Oxford, OX13RE, UK*

The weak immunogenicity of HIV-1 envelope glycoproteins (Env) is one factor that limits the efficacy of Env-based immunization strategies. We sought to improve the antibody response following vaccination with gp140 and gp120 candidates from clades B and C by altering the glycosylation profile and by addition of novel exogenous adjuvants. A complete model of gp120 glycan was constructed and this showed that surface access was dominated by glycan. It is known that Env binds to mannose receptors and mannose-binding lectin via the mannose residues in its high-mannose and hybrid type glycans. These interactions are reduced by the presence of sialic acid in the complex and hybrid-type glycans. We show that of the glycan modifications, sialic acid has a dominant role in modulating the immunogenicity and antigenicity of gp120, with greater antibody titres obtained in mice immunized with gp120 lacking sialic acid with or without other complex glycan residues. Differences in antigenicity were ligand- and strain-dependent and the glycan model showed that these could not be explained simply by the bulk of sialic acid. As the receptors for CpG-ODN and poly(I:C) (TLR9 and 3 respectively) are located in endosomal compartments we sought to improve their activity as exogenous adjuvants using transfection reagents to deliver them to the correct compartment and protect them from nucleases. Poly(ethylenimine) (PEI) is a transfection reagent previously shown to activate genes with a Th1/Th2 profile in the context of gene therapy. PEI substantially improved the antibody responses to gp140 when used alone in mice and rabbits and its efficacy was equivalent to a much larger molar dose of alum. At an optimal ratio, combinations of PEI and CpG-ODN or poly(I:C) induced significantly greater overall and/or Th1-biased antibody responses to gp140.

Enhanced Inhibition of Drug Susceptible and Nucleoside Resistant Strains of HIV-1 by Combining IM²⁸ with Zidovudine, Lamivudine or Indinavir: Is IM²⁸ a Vector for Gene Therapy?

D. Mavoungou**, H. Loemba*, K. Diallo*, M. Olivier*, M. A. Wainberg*

**McGill AIDS Centre, Jewish General Hospital, 3755 Côte Ste-Catherine Road, Montreal, Quebec,
Canada H3T 1E2.*

***Centre de Recherche sur les Pathologies Hormonales (CRPH), BP 12134, Libreville, Gabon*

Background: Recently it has been hypothesized that gene therapy strategies which down regulate CCR5 expression should prevent viral entry and disease progression.

Aims of the study: To investigate the ability of IM²⁸ to suppress the replication of HIV-1 in the context of two-drug combinations with antiretroviral agents in tissue culture assays. To verify the capacity of IM²⁸ to enhance inhibition of drug susceptible and multinucleoside resistant strains of HIV-1 by the combination of IM²⁸ with Zidovudine, Lamivudine or Indinavir.

Methodology: Incubation of cells infected with laboratory adapted IIB strain of HIV-1 in the presence 3TC of IM²⁸ and Zidovudine, Lamivudine and Indinavir.

Results: The drug combinations resulted in a synergistic suppression of HIV-induced syncytia formation and RT activity. The combination of 3TC and IM²⁸ also substantially inhibited a clade C drug susceptible clinical isolate of HIV-1. The enhancement of viral suppression was especially evident at drug concentrations closed to IC₅₀ values when a wild type subtype B clinical isolate of HIV-1 was challenged by the combination of IM₂₈ with either AZT, 3TC or Indinavir. In addition a synergistic inhibition of an AZT/3TC/DDC/nevirapine resistant clinical isolate of HIV-1 was observed when IM²⁸ was combined with AZT, 3TC or Indinavir at doses corresponding to each drug IC₅₀ down to lower drug concentrations around half of the IC₅₀ value. No significant additive drug cytotoxic effects were observed when IM²⁸ was combined with each antiretroviral agent.

Conclusion: Combinations of IM²⁸ with RT and protease inhibitors may be beneficial for therapeutic usage and should be investigated in salvage regimen against drug resistant strains of HIV-1. Because molecule which block viral entry targeted CCR5 or CXCR4, IM²⁸ a steroid hormone known by its capacity to induce gene transcription as all steroids should be considered as vector for gene therapy as a lipid carrier.

P-TU-5

Circulating CD4+ and CD8+ Lymphocytes in Immunosuppressed Individuals

Norah O Akinola; Lateef Salawu; *Gregory Erhabor; Muheez A Durosinmi

*Department of Haematology and Immunology and *Department of Medicine,
Obafemi Awolowo University, Ile-Ife, Nigeria*

Human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis* (Tb) infections cause immunosuppression. There is limited data on the subsets of circulating lymphocytes in Nigerians with these infections.

Consenting adults, attending the Communicable Disease Clinic at the Obafemi Awolowo University Teaching Hospitals, Ile-Ife, for the management of HIV and pulmonary Tb (PTB) at diagnosis, were studied together with some age- and sex-matched individuals without infection (controls), after ethical approval was obtained. Demographic data, haematological indices (full blood count – FBC and white cell differentials), CD4 and CD8 cell counts and ratio and body mass index (BMI) were obtained from all volunteers. Data was analysed using descriptive and inferential statistics.

Results from 50 PTB patients, 43 HIV patients and 20 controls were analysed. There was no significant difference in the mean age of the three groups, although HIV patients had more males (M:F = 4:3). The total white cell count (WCC) was within normal limits for all groups, but PTB patients had a significantly higher mean value than the controls ($p < 0.01$). The mean total lymphocyte count (TLC) was lower in HIV patients ($1879 \pm 1538/\text{cmm}$) when compared with the controls ($2660 \pm 1071 /\text{cmm}$; $p < 0.05$). The mean CD4 count was significantly lower in HIV patients ($145 \pm 116/\text{cmm}$) than PTB patients ($502 \pm 175/\text{cmm}$; $p < 0.01$). Controls had significantly higher counts than both patient-groups ($637 \pm 136/\text{cmm}$; $p < 0.0001$). The mean CD8 count however did not show a significant difference in any of the groups, but the mean CD4/CD8 ratio was lower in HIV patients (0.67 ± 0.68) than PTB (2.21 ± 1.22) and controls (2.62 ± 1.04 ; $p < 0.01$). There was no gender difference in the CD4+ and CD8+ lymphocyte counts in any of the groups. HIV patients had a greater tendency to be anaemic than PTB patients with a mean packed cell volume of $32 \pm 7\%$ and $35 \pm 7\%$ respectively ($p < 0.05$). The mean BMI was similar in HIV ($19.5 \pm 6.3 \text{ kg/m}^2$) and PTB ($17.8 \pm 2.6 \text{ kg/m}^2$) patients, but significantly lower than that of the controls ($25.1 \pm 5.3 \text{ kg/m}^2$; $p < 0.001$). There was a positive correlation between CD4 and CD8 count in PTB patients ($r = 0.34$; $p < 0.01$) and controls ($r = 0.5$; $p < 0.01$), but this was not observed in HIV patients in whom there was a strong correlation between CD4 count and CD4/CD8 ratio ($r = 0.74$; $p < 0.001$) instead. The relationship between CD4 count and TLC ($r = 0.46$) and WCC ($r = 0.42$) was observed in HIV patients only.

This study shows that prior to treatment, circulating CD4+ lymphocytes are fewer in HIV infection than in PTB. The compensatory effect of CD8+ lymphocytes is not significant in either HIV infection or PTB. CD4/CD8 ratio however is a good correlate of CD4+ cell count in HIV-positive treatment-naïve patients, but not in untreated PTB patients.

Delayed-Type Hypersensitivity (DTH) Responses May Predict Long-Term Clinical Benefit of Peptide-Based Therapeutic Immunization

Kran A.M.B., Sørensen B., Sommerfelt M.A., Baksaas I, Kvale D.

Ullevål University Hospital, Department of Microbiology, Oslo, Norway

Background: Therapeutic immunization aims to attenuate disease progression by modulating HIV specific immune responses, but correlates of effective immunity remain to be defined. We here suggest that vaccine-specific in vivo-responses in terms of delayed-type hypersensitivity (DTH) skin reaction may correlate to effective immunity in therapeutic immunization.

Methods: In a phase II dose-finding clinical trial, we have recently immunised 38 patients with a mixture of four p24-like conserved peptides (Vacc-4x) targeting skin dendritic cells. Vacc-4x-specific cellular responses were evaluated in vivo by delayed type hypersensitivity (DTH) skin test measured as infiltrate areas 48h after intradermal injection of Vacc-4x peptides, and in vitro by T cell proliferation using a standard 6d CFSE-assay. After 10 immunizations over 26 weeks on antiretroviral treatment (ART), ART was intermittently stopped for four weeks and again at week 38 for 14 weeks according to protocol. Patients were followed post-study every third month, and ART was resumed according to current guidelines. Clinical follow-up was performed 2 and 4 years after enrolment. Kaplan-Meier product-limit estimates were used to analyse time until ART was resumed.

Results: 90% developed specific T cell responses both in vitro and in vivo after all immunizations were completed. Four years after the last immunization, 81% still had positive DTH-responses, and 61% had specific proliferative T cell-responses. The magnitude of DTH responses at week 38 was related with improved control of viral replication and stable CD4 counts at the end of study, 14 weeks after ART was stopped.

The patients were stratified according to the magnitude of their Vacc-4x-specific DTH responses at study week 38. At follow-up both 2 and 4 years after enrolment in the study, Kaplan-Meier analysis of time-dependent resumption of ART showed that DTH-high-responders having DTH induration area above median before treatment interruption, remained without ART for a longer period of time compared with DTH low-responders ($p=0.01$ and $p=0.07$, respectively, Cox-F-test).

Conclusion: These data show an association between the magnitude of post-immunization vaccine-specific in vivo-responses and prolonged time without the need for resuming ART almost 4 years after treatment interruption following therapeutic immunization. Although this needs to be confirmed in controlled clinical trials, these data indicate that monitoring specific cellular immune responses in vivo by DTH skin tests might help to predict efficacy in clinical trials with therapeutic immunizations.

P-TU-7

Immunogenicity of Synthetic Peptide Constructs Based on Conserved and Variable Regions of HIV-1 Envelope Proteins

Debra Meyer*, Christina Philippeos, Raymond Hewer

Biochemistry Department, University of Johannesburg, P.O. Box 524, Auckland Park, 2006, Gauteng Province, South Africa

**corresponding author debram@uj.ac.za*

Correlates of protective immunity against HIV are not yet conclusively defined but there is a general belief that both humoral and cellular immune responses will have to be induced by an effective HIV vaccine. Immunogenic, broadly reactive epitopes of the HIV-1 envelope glycoproteins could serve as important targets of the adaptive humoral immune response in natural infection and potentially as components of an AIDS vaccine. We therefore design, synthesize and evaluate synthetic peptides based on the surface regions of HIV-1 envelope proteins for induction of neutralizing antibodies. Constructs based on the epitopes recognized by the neutralizing monoclonal antibodies 4E10 and 2F5 were designed for the membrane proximal external region (MPER) of gp41 of HIV-1 subtype C. These synthetic constructs were highly antigenic when tested against naturally infected HIV-1 positive sera. As immunogens these constructs induced strong humoral immune responses in mice and rabbits. Synthetic peptide constructs based on the HIV-1 subtype C, third variable loop (V3) of gp120 induced polyclonal antibodies able to neutralize a neutralization sensitive subtype B isolate (SF 162) as well as a difficult to neutralize Du 151 subtype C primary isolate. These polyclonal antibodies were also able to identify the V3-consensus sequence peptide produced in a *Bacillus flagellin* expression system, in western blot analysis.

In Silico Criterion for Assessment of LPL Mutations as a Risk Factor for Cardiovascular Disease in HIV Infection

Sanja Glisic, Vladimir Perovic, Jelena Prljic, Nevena Veljkovic

Centre for Multidisciplinary Research, Institute of Nuclear Sciences VINCA, Belgrade 11001, Serbia

With the introduction of highly active antiretroviral therapy (HAART) the course of HIV disease has been significantly modified, with longer survival and improved quality of life of HIV-infected subjects. On the other hand, increased concerns are raised about the long-term effects of HAART treatment, including protease inhibitors (PIs)-related dyslipidemia, which is associated with an increased risk of cardiovascular disease (CVD). There is strong evidence that PIs decrease the enzymatic activity of lipoprotein lipase (LPL), which is a key enzyme in lipid metabolism. Decrease of the LPL enzymatic activity leads to elevated triglycerides (TG) and reduced high-density lipoprotein (HDL-C levels), both risk factors for CVD. Accordingly, LPL mutations, which decrease the LPL activity, may confer susceptibility to CVD. Therefore, HIV-infected patients under HAART therapy who are carriers of pathogenic LPL mutations have increased risk of CVD.

Here, the informational spectrum method (ISM), a virtual spectroscopy method for structure/function analysis of nucleotide and protein sequences, is applied for identification of evolutionary highly conserved information encoded by the primary structure of LPL. It was demonstrated that mutations, which alter the LPL enzymatic activity also alter this information. Based on this finding, an efficient and simple bioinformatics criterion for assessment of the pathogenic effect of LPL non-synonymous single nucleotide substitution as a risk factor of CVD has been proposed.

Identification of pathogenic LPL mutations in HIV patients before HAART therapy will be beneficial because appropriate lifestyle and therapeutic intervention will decrease risk of CVD.

hiv-1, informational spectrum method, LPL, mutation, CVD

P-TU-9

Mechanism of Immunomodulation by Flavonoids in HIV-1 Infection

Sanja Glisic, Vladimir Perovic and Nevena Veljkovic

Centre for Multidisciplinary Research, Institute of Nuclear Sciences VINCA, Belgrade 11001, Serbia

HIV-1 gene expression and transcription is an essential step in the viral life cycle, which is considered to be a possible target for inhibition of HIV-1. NF- κ B, termed as the central mediator of immune responses, plays a key role in the regulation of HIV-1 gene expression. NF- κ B induces the expression of numerous cytokines, chemokines, and immunoregulatory genes, many of which promote HIV replication. Therefore NF- κ B is potential target for inhibition of HIV-1.

Here, the informational spectrum method (ISM), a virtual spectroscopy method for structure/function analysis of nucleotide and protein sequences, is applied for identification of NF- κ B pathway inhibitor candidates. ISM analysis of all human proteins from UniProt database was performed. The results of the analysis indicate that flavonoids represent inhibitors of NF- κ B pathway through prevention of I κ B alpha phosphorylation.

We proposed a novel mechanism of immunomodulation in HIV infection by flavonoids, pointing out this nontoxic and safe chemical class as a promising source of antiretrovirals for AIDS therapy.

A Novel Anti-HIV Vaccine Strategy Based on the Adjuvant Effect of HBV Surface and Core Proteins on Cellular Immunity and Crossreactive Antibody Responses

Enrique Iglesias, Julio C. Aguilar, Rafael Thomson, Yamilka Carrazana, Yadira Lobaina, Daymir García, Jorge Sánchez, José García, Otto Cruz, Emma Brown, Alejandro Martín, Verena Muzio Gerardo Guillén

*Center for Genetic Engineering and Biotechnology, Havana City, Cuba
Laboratory for AIDS Research (LISIDA), San Jose, Havana Province, Cuba*

We have previously reported the cross-adjuvant effect on the resulting immune response obtained with the aggregated recombinant Hepatitis B Virus surface (HBsAg) and core (HBcAg) antigens. A therapeutic vaccine candidate for Hepatitis B was developed and it is under clinical trials. Potent and multi-specific cellular immune responses correlate with a better prognosis and viral clearance during the course of chronic diseases, additionally, widely cross-reactive humoral responses could be a potential solution to abrogate viral hiper-variability.

In line with previous results, the Center for Genetic Engineering and Biotechnology has developed during the last seven years a wide project to explore and exploit the adjuvant activity of HBsAg and HBcAg using as a target a number of antigens from several microorganisms. Among them, and the most important, a recombinant protein and peptide-based antigens from the Human Immunodeficiency Virus, designed for cellular and humoral immunity, respectively.

In order to study the effect of recombinant HBV antigens on the immune response to the protein CR3; several formulations containing the recombinant HBV antigens and CR3 -composed by CTL and Th epitopes from HIV1- were evaluated after subcutaneous and nasal administration to Balb/c mice. The formulations were tested for anti-HBV/HIV responses. Humoral and also CD4+/CD8+ proliferative and IFN γ secreting responses were measured by CFSE staining and ELISA/ELISPOT techniques.

A second line of experiments were developed in order to potentiate the immunogenicity and crossreactivity of the humoral immune response developed by multiple antigenic peptides (MAPs) synthesized based on previously demonstrated cross-reactive sequences from the V3 loop of HIV.

The results obtained evidenced that the antigen formulation comprising the mixture of CR3, HBsAg and HBcAg induced superior Th1-responses to the CR3 protein compared to the rest of the formulations assayed. Anti HBV responses remained strong.

By the other hand, it was possible to induce an improvement in the crossreactivity of the V3 sequences superior to the 90% based on the analysis of a panel of 50 unrelated V3 sequences from different clinical isolates.

In conclusion, it is possible to induce a Th1 immune response against HBV and HIV antigens after mucosal and parenteral administration of formulations containing HBsAg, HBcAg and the HIV-derived protein CR3 as well as highly cross-reactive anti V3 immune responses. In our opinion these results has implications for the development of new preventive or therapeutic vaccines for HIV and also for combined vaccines against both HIV and HBV considering the similitude in transmission routes, geographical distributions and co-infection rates.

P-TU-11

Nodular Tuberculide- A Rare Skin Manifestation of IRIS Caused by M Tuberculosis

Kabir Sardana

Maulana Azad Medical College, Delhi, India

Tuberculids (papulonecrotic tuberculid, erythema induratum, and lichen scrofulosorum) are cutaneous hypersensitivity reactions to *Mycobacterium tuberculosis*. We report the first case case of a recently described tuberculid, nodular tuberculide in a HIV positive patient form India

Tuberculids includes papulonecrotic tuberculid , erythema induratum of Bazin , and lichen scrofulosorum. A new distinct type of tuberculid described in 4 patients from South Africa by was named nodular tuberculid (NT). The distinguishing feature of NT was that the granulomatous vasculitis occurred neither in the papillary dermis as in PNT nor in the subcutaneous fat as in EIB but at the junction of the dermis and subcutaneous fat.

26-year-old HIV positive woman presented with 1-month history of lower extremity skin lesions. The lesions were initially tender and red, later becoming asymptomatic and dark brown. On physical examination she had 1.5 cm- to 2.0-cm, nontender, moveable, firm subcutaneous nodules with overlying brown hyperpigmentation or faint erythema on the bilateral hands and feet

(Fig 1a,1b) .She had previously been treated for Pulmonary Kochs and her chest X ray revealed bilateral diffuse infiltration. Skin biopsy specimen of a leg nodule demonstrated multiple caseating granuloma with langhans giant cells (Fig 1d).Laboratory tests showed *M tuberculosis* in multiple sputum samples by culture. A purified protein derivative (tuberculin) (PPD) test was negative. Her her CD4 count was 25 at the onset of HAART and on re testing was found to be 205.The case was diagnosed as a case of IRIS and the HAART therapy was continued .Within a month all the lesions healed leaving behind atrophic scar (Fig 1 c)

This is arguably the first case of IRIS manifesting as cutaneous nodular tuberculide caused by M tuberculosis
We would like to discuss the interaction of HIV and TB in association with IRIS and deliberate on the immunological synchrony therein.

Malignant Transformation of Donovanosis in a HIV-Positive Patient

Kabir Sardana

Maulana Azad Medical College, Delhi, India

Donovanosis is caused by *Calymmatobacterium granulomatis*. It is endemic in new Guinea, the Caribbean, S.Africa, S.E.Asia, Australia, Brazil and southern India. Although donovanosis has been reported to coexist with HIV infection, to our knowledge there have been no reports of co-existent SCC.

We present a 21 year old unmarried male, student of Uttar Pradesh with multiple asymptomatic penile ulcerations of 1 year duration. A tissue smear from the ulcer margin stained by rapid Giemsa showed Donovan bodies within mononuclear cells. Lesional FNAC showed multiple a typical squamous cells and occasional granulomas and inflammatory cells in the background. Biopsy from the ulcer edge showed nests of squamous cells with high degree of mitosis. FNAC from lymph node showed Reactive lymphoid hyperplasia. Serologic testing for HIV type I was positive, CD₄⁺ lymphocyte count was 345 cells/mm³. The patient was treated with HAART, Tb. Doxycycline (100 mg BD for 3 weeks) and Tb. Septran DS (BD for 21 days). In view of the histology report Total Penectomy with B/L modified inguinal lymph node dissection is planed.

Genital ulceration leads to 4.7 times higher risk of HIV transmission after sexual exposure to an HIV infected person. Coinfection with HIV is likely to persist for a longer duration, and thus require more intensive and prolonged antibiotic therapy compared to HIV negative patients with donovanosis Carcinoma is the most serious complication of donovanosis but is relatively rare-0.25% in Rajam and Ranjiah's series. This is the first case report of HIV positive patient with donovanosis undergoing malignant transformation which adds to the dismal therapeutic outcome in our case.

We will also deliberate on the link between Immunological dysfunction,CD 4 count and STD /Genital ulcer presentation in HIV/AIDS and the effect of HAART on the therapeutic outcome



fig 1 a



fig 1b

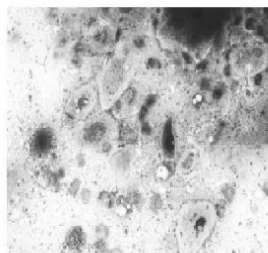


fig 1c

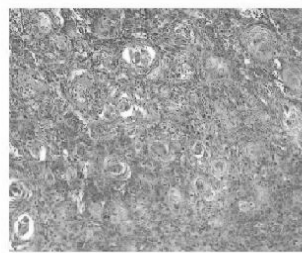


fig 1 d

P-TU-13

Motor Neuron Disorder in Patients with HIV-1 Infection

Ashok Verma, Joseph R. Berger

*Kessenich Family MDA-ALS Center, University of Miami Miller School of Medicine, Miami, Florida and
University of Kentucky College of Medicine, Lexington, Kentucky, USA*

Background: The etiological relationship of HIV infection to motor neuron disease is uncertain.

Objective: To describe four patients with HIV infection and motor neuron diseases (MND) or ALS (amyotrophic lateral sclerosis) mimic syndrome and to review the previously reported cases of HIV-associated MND.

Patients/design: We studied HIV-infected four patients who also had MND or ALS-like syndrome. We employed the *PubMed* for literature research using the following terms: motor neuron disease, amyotrophic lateral sclerosis, progressive muscular atrophy, primary lateral sclerosis, and Lou Gehrig disease. During the last 20 years, at least 23 cases of MND or ALS have been reported in HIV seropositive individuals. These were reviewed for comparison and contrast with the characteristics of sporadic ALS.

Results: Our two patients fulfilled the *El Escorial* criteria for a clinically definite ALS and are reported elsewhere (*J Neurol Sci* 2006;240:59-64). The other two patients had isolated pyramidal tract degeneration and their disease process stabilized following highly effective antiretroviral therapy (HAART). A review of the 23 previously described patients with ALS and HIV infection revealed that they could be categorized into clinically definite ALS (6 cases) or clinically probable or possible ALS cases (17 cases). Motor symptoms commenced at different stages of the HIV disease. Sixteen of 18 patients with HIV-associated ALS syndrome receiving HAART demonstrated at least partial recovery of their motor deficit.

Conclusions: Motor neuron disease similar to sporadic ALS may occur in association with HIV infection. Patients with HIV-associated MND may improve following antiretroviral therapy. An aggressive HAART regimen to reduce viral load should be pursued in all such cases.

HIV-leprosy Co-infected Cases Attending the Outpatient's Clinic at Agra, India: Case Report of 14 Patients

Tahziba Hussain*, Assistant Director and Kiran Katoch#, Deputy Director (Senior Grade),

Address for correspondence :

*Tahziba Hussain**

HIV/AIDS UNIT & Clinical Division#, National JALMA Institute for Leprosy and Other
Mycobacterial Diseases (Indian Council of Medical Research), Tajganj, Agra - 282001. INDIA.*

Phone No. + 91 - 0562 - 2331751 - 4, ext. 287

FAX - +91 - 0562 - 2331755

E-mail - tahziba_hussain@hotmail.com

Several authors have studied the potential effects of HIV infection on leprosy infections. While some studies reported that HIV infection is an important risk factor for leprosy, others found no association between the two diseases. Studies of the interactions of these two diseases assume importance in regions of geographical overlap of HIV epidemic with areas of leprosy endemicity. Surveillance studies at our Institute showed that the trend of HIV infection among Leprosy patients, over a decade, is low. There has been a slight rise in HIV-positivity from 0.124% (5/4025) during 1989-1993 to 0.376% (8/2125) during 1999-2004. Follow-up of these patients at an interval of 6 months, revealed that the incidence of downgradation in clinical spectrum into a severe form of leprosy as well as reversal/ENL reactions and neuritis (chronic/acute) was not observed among the leprosy patients. HIV-positive leprosy cases did not develop either ARC or AIDS. The present study reports the clinical profile of HIV-leprosy co-infected patients attending the Outpatient's clinic at Agra, India. This is the first report of a decade of HIV screening of leprosy patients in this region of the country and the longest follow - up of HIV - leprosy co-infected cases. This report comprises of the details of the Age, Gender, Type of Leprosy, Bacterial Index (B.I.) : Initial Smear, number and type of Patches and Lesions, Nerve involvement, Duration of Anti-Leprosy treatment, deformities, Whether admitted to Ward, Reactions, if any, Bacterial Index (B.I.) : after treatment, HIV status, CD4/CD8 ratio, number of follow-up visits, complications, etc.

Keywords: Leprosy, HIV, co-infection, case-report, follow-up, Outpatient's Clinic, Agra, India.

P-TU-15

The New Set of Viral Peptides for a Quality Control in HIV Vaccine Evaluation

Pokrath Hansasuta, Michitra Boonchan, Opas Choksapmanee, Supranee Buranapraditkul,
Kiat Ruxrungtham

Faculty of Medicine, Chulalongkorn University, Rama 4 road, Bangkok 10330, Thailand

Background: 'CEF' peptide set has recently been optimized as a quality control for the analysis of T cells in vaccine trials. However, the peptide set may not be optimal for vaccine trial in Asian countries including Thailand where HLA frequencies are not matched with the previously-described CEF peptide set. We have optimized a new set of peptides (CDEF) in order to use for a quality control in evaluation of HIV vaccine trials in Thailand.

Method: Twenty HIV-infected patients were enrolled from Immune Clinic, Chulalongkorn Medical School, Bangkok, and nine healthy blood donors were enrolled. Antigen-specific T cell responses were analyzed by Enzyme-linked Immunospot (ELISpot) assay. A total of 49 epitope peptides (derived from Cytomegalovirus, Dengue virus, Epstein-Barr virus and Influenza virus or CDEF) used in the assay were selected from database based on HLA frequencies in Thai population. A 'CEF' peptide set from US NIH was used as a control.

Result: The ELISpot using our CDEF peptide set seemed to be more sensitive than CEF peptide set both in HIV-infected and healthy donors. A total of 17/20 HIV-infected donors (85%) were positive for CDEF peptides, whilst 16/20 donors (80%) were positive for CEF peptides. On the other hand, a total of 4/9 healthy donors were positive for CDEF peptides, and 3/9 healthy donors were positive for CEF peptides. The higher sensitivity was also confirmed when individual peptide response was analyzed. Indeed, seventeen peptides in CDEF set but only seven peptides in CEF set were positive in either HIV-infected or healthy donors. Surprisingly, no response was detected upon stimulation with either influenza virus or dengue virus peptides. The most immunodominant peptide in this study was CMV-specific HLA-A*0201-restricted epitope (NLVPMVATV). The response to this epitope was detected in all HLA-A2+ CMV seropositive donors. The magnitude of response ranged from 780 to 4,002 sfu/10⁶ PBMC, with a mean of 2,091 sfu/10⁶ PBMC.

Conclusion: Our CDEF peptide set is more optimal to use as the quality control peptides in analysis of T cells in vaccine trials in Thailand. However, additional viral peptides may be needed to enhance the sensitivity of CDEF set.