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HIV and tropism : evaluation of HIV fitness using recombinant viral constructs

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HIV entry into host cells is mediated by the interaction of viral envelope glycoproteins with the cellular CD4 receptor and either of the coreceptors, CCR5 or CXCR4. The choice of one of the coreceptors over the other, the so-called coreceptor tropism, determines which type of cells will be infected by a particular virus. Viruses which prefer CCR5 as a coreceptor are referred to as R5 viruses, while those using CXCR4 are termed X4. A third group of viruses, the R5X4 viruses, utilize both CCR5 and CXCR4 as coreceptors. In general, HIV-1 infection and pathogenesis is initiated by R5 viruses. However, in HIV-1 subtype B infections a shift in coreceptor utilization, the "coreceptor switch", will occur in about half of all chronically infected patients. This switch is usually associated with rapid CD4+ T-cell depletion and fast progression to AIDS. Many hypotheses were made to define the mechanism and to identify factors involved in this switch.
Despite these continuous efforts, the molecular bases of the coreceptor switch are not fully understood due to lack of "fast and easy to perform" research tools. Therefore, the objective of this study was to generate fluorescently labeled chimeric viruses that can be used to study possible mechanisms of coreceptor switch, viral fitness and the presumed role of different compounds involved in the switch.
An In-Fusion cloning strategy was used to clone selected envelope sequences from two biological clones, VI 943-3 (R5) and VI 943-1 (X4) and two reference strains, BaL (R5) and HxB2 (X4) into a fluorescently labeled pBRNL43 IRES eGFP/DsRedExpress viral vector to generate chimeric viruses. Of the generated 25 recombinant plasmids, 11 were found to be infective both in peripheral blood mononuclear cells (PBMC) and in various cell lines. In addition to the chimeric viruses, we have also generated a Δenv pBRNL43 eGFP/DsRedExpress backbone which can be used to insert different envelope sequences depending on the study objective.
Fitness of the chimeric viruses was evaluated by competing R5 viruses with their X4 counterparts. In these experiments R5 chimeric viruses from both biological clones and reference strains were more fit than the X4 groups. Relative fitness of chimeric viruses was also assessed in the presence of visfatin, an adipocytokine presumed to inhibit R5 viruses. A decrease in the relative fitness of R5 chimeric viruses derived from biological clones was observed in the presence of visfatin.

The study had proved that fluorescently labeled chimeric viruses containing envelope sequences of our choice can be generated and can be used to study inherent viral tropism. The study has also shown that viral fitness can be influenced by host derived factors, but further investigation is needed to evaluate the infectivity, especially in primary cells, before reaching any conclusion. Additionally these viruses can be used to study other models involving the study of viral fitness under different conditions.

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Evaluation of viral parameters involved in the outcome of the HIV Pseudovirus neutralization assay

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HIV pseudovirus assays play a crucial role in investigating the breadth of neutralization of mAbs against viral clones and this has great value in HIV vaccine development. With the enormous genetic diversity of HIV, high throughput approaches are required to keep pace with rate of mutation in the virus. The pseudovirus assay is ideal for such investigations. This study tested the hypotheses that differing HIV-1 envelope to backbone ratios during cotransfection of 293T/17 cells in generating HIV-1 *env*-pseudoviruses has an influence on viral titers and also that harvesting HIV-1 *env*-pseudoviruses at different time points, 48 hours and 72 hours, after cotransfection of 293T/17 cells affects viral titers and infectivity. Our findings suggest that using different envelope to backbone ratios has a strain dependent effect. For BaL26cl1, increasing backbone DNA while keeping envelop DNA concentration constant leads an increase in pseudovirus titers. For 92Br025 and SF162 there is an optimal amount of backbone DNA that can be added beyond which pseudovirus titers decrease. Secondly, our data show that HIV *env*-pseudoviruses harvested 48 hours after cotransfection of 293T/17 cells are more infectious, requiring lower titers to achieve the same level of infectivity as compared to those harvested after 72 hours. We also show that neutralization profiles of four mAbs 4E10, 2G12, 2F5, and b12, and TriMab (a combination of 2G12, 2F5 and b12) against three HIV-1 env-pseudovirus batches (BaL26cl1, 92Br025 and SF162) are neither affected by the change in *env*-backbone ratio nor the difference in harvesting time points i.e. 48 and 72 hours after cotransfection of 293T/17 cell line. TriMab had the best neutralization as compared to the four mAbs when tested individually. Our work thus shows that it is important to perform preliminary studies of each new pseudovirus to determine the optimal *env*:backbone ratio for economical reasons. However, it is reassuring that, when properly tittered on target cells, the Env/Backbone nor the time of harvesting the pseudoviruses will influence the result of neutralization studies. Thus our work has contributed to standardizing HIV neutralization assays, which are a cornerstone parameter in HIV clinical and preclinical vaccine studies.

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Development and evaluation of a real-time pcr based method for the quantification of the T-cell receptor of HIV infected patients receiving HAART

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Highly active antiretroviral therapy (HAART) does not completely restore the immune system of HIV-infected persons. The T cell receptor (TCR) repertoire recovery is skewed early after HAART. TCR Variable Beta (BV) diversity can be measured by flow cytometry and polymerase chain reaction (PCR) based methods at the protein and mRNA levels respectively. Our study focused on developing and evaluating a real-time PCR (Q-PCR) method for the absolute quantification of the TCR BV genes in HIV patients receiving HAART. We first optimized the primers for the amplification of TCR BC and BV families' mRNA from human peripheral blood mononuclear cells (PBMCs) using Q-PCR. Secondly, oligonucleotide standards for the TCR BC and BV families were designed and evaluated in order to calculate the relative expression of the BV families. Thirdly, titration of BV family expression from samples "spiked" with well characterized T cell lines was done using Q-PCR in comparison to flow cytometry (gold standard). The Q-PCR method was then used to determine the TCR BV repertoire of HIV negative individuals, HIV+ (not treated) and HIV+ (treated) patients. Finally the relative expression of the BV families was compared with T cell functionality. The Q-PCR method for the absolute quantification of the TCR BV families in HIV patients receiving HAART was developed and evaluated. The relative expression of the BV families in all groups (HIV negative individuals, HIV+ (not treated) and HIV+ (treated) patients) using comparative and absolute methods was comparable. The relative expression of all the BV families was highest in the HIV+ high CD4 nadir (>300(cells/µL)) group. BV 10, 16, 19 and 30 were significantly under expressed and BV 30 was negatively correlated with IFN-γ response to Staphylococcal enterotoxin B (SEB). Although the Q-PCR method developed in this study still needs optimization, it is cheap, automated, and accurate. Furthermore, this method promises to be an essential tool for identifying clonal expansions in TCR BV families in human PBMCs of HIV patients receiving HAART. The relative expression of the TCR BV families can be used together with the absolute CD4 T cell counts and viral load to monitor immune reconstitution after HAART in HIV patients.

Keywords: Relative TCR Variable Beta expression, real-time PCR (Q-PCR), HIV-1, HAART.

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Molecular epidemiology of Chlamydophila psittaci infections in birds and humans

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Chlamydophila psittaci is an obligate intracellular gram negative bacterium. It is the causative agent of psittacosis in humans and avian chlamydiosis in birds. *Cp. psittaci* has been found in 465 bird species (Kaleta & Taday, 2003).

The first part of the current thesis was to demonstrate the presence of *Cp. psittaci* in different avian samples in Belgium and in the United States as well as human personnel in turkey and chicken slaughterhouses in Belgium. To this purpose, Nested PCR and isolation in Buffalo Green Monkey cell culture were performed. Nested PCR revealed that 14.29% of wild birds, 40.63% of racing pigeons, 30.77% samples from the veterinary clinic, 84.62% of chickens, 57.14% of turkeys, 7.81% of human personnel in chicken slaughterhouse and 58.49% of human personnel in turkey slaughterhouse were *Cp. psittaci* positive. None of the turkey samples from the United States were positive in nested PCR. The positive nested PCR samples were confirmed by isolation in BGM cell culture. Isolation of bacteria presented 100% of wild birds, 69.23% of young racing pigeons, 50% samples from veterinary clinic, 64.55% of chickens, 70% of turkeys, 60% of human personnel in chicken slaughterhouse and 90.32% of humans in turkey slaughterhouse positive from the corresponding positive nested PCR. Interestingly, 7 out of 55 (12.73%) turkey samples in the Unites States were positive in isolation, although negative in Nested PCR. Seven cultured positive samples were further investigated by genotyping with real-time PCR, microarray and DNA sequencing. DNA sequencing revealed two samples (two parakeets) to be *ompA* genotype A and two samples (two pigeons) to be *ompA* genotype B.

In the second part, we evaluated the efficacy of two vaccination methods to protect SPF turkeys from a *Cp. psittaci* infection. The combination of a recombinant MOMP vaccine combined with immunostimulating complexes (or ISCOMs) as an adjuvant showed great results when administered to SPF turkeys, followed by a challenge with *Cp. psittaci* strain 92/1293 (*ompA* genotype D). Administration of the vaccine with a combination of ISCOMs and the natural antimicrobial protein ovotransferrin slightly worsened the

outcome of the experiment compared to the vaccine with ISCOMs alone, albeit not significantly.