cells. Most BLV-infected cattle remain asymptomatic, but about 30% develop persistent lymphocytosis, and perhaps 5% eventually acquire B-cell lymphoma. Mainly as a result of the latter condition, BLV is a significant economic problem for cattle producers worldwide. Moreover, because of the close genetic similarities between the two viruses, BLV may also be a useful model for investigating HTLV-associated diseases in humans. The prognosis of HTLV-I-associated disease is generally poor because of resistance to chemotherapy. Clinical trials using antiretroviral agents to treat HTLV-I-infected patients have been generally unsuccessful; there is clearly a need for additional therapies.

We are currently using an in vitro BLV infection assay as a model to screen various compounds for antiviral activity. Persistently BLV-infected fetal lamb kidney (FLK) cells are used as a source for infection of other cell types. For example, feline CC81 cells form multinucleated syncytia upon exposure to FLK-BLV cells. The formation of syncytia can be quantitated to reflect the levels of infection. We recently found that the BLV-induced syncytium formation can be inhibited by ribavirin or alpha interferon, but a combination of ribavirin and alpha interferon at doses similar to those used to treat human hepatitis C virus infection are much more effective. No cytotoxic effects were observed at the effective doses, and this inhibition is greater than that achievable with either drug separately. We are currently refining our infection assay as well as testing additional compounds. Furthermore, we are developing additional methods to quantitate the production of BLV antigen, as well as the expression of BLV-specific mRNA, to corroborate these results.

**Acknowledgements:** Supported by NIH grant number P20 RR0016469.

doi:10.1016/j.antiviral.2009.02.037

**33**

## Design of Artificial Polyepitope DNA Vaccine Constructs for Eliciting of HIV-Specific CD8+ CTL Responses

Svetlana Oreshkova*, Sergey Bazhan, Pavel Belavin, Tatyana Ilyicheva, Sergey Seregin, Alexander Ilyichev, Larisa Karpenko

*State Research Center of Virology and Biotechnology Vector, Koltsovo, Novosibirsk Region, Russia*

For designing of poly-CTL epitope immunogens, candidates for multi-CTL epitope-based HIV-1 DNA vaccine, we used 10 specially selected HLA-A2-restricted CTL epitopes from the main HIV-1 antigens Env, Gag, Pol, Nef and Vpr. To optimize the target multi-CTL-epitope immunogens for eliciting maximal immunogenicity, several variants of genetic constructs have been proposed. These constructs provided various coding and expression strategies for the target immunogens to ensure their effective proteasome degradation and liberation of determinants, their transport through the endoplasmic reticulum and binding to HLA class I molecules on the surface of antigen-presenting cells. Obtained constructs were cloned in vector plasmid pcDNA3.1 (Invitrogen, USA) and vaccinia virus (strain WR, NIAID, and NIH). Immunization of HLA-transgenic mice was used to induce a functional $T_{CD8+}$ response in humans. For that we used a "prime-boost" strategy, in which priming with recombinant DNA plasmids is followed by boosting with a recombinant vaccinia virus. The obtained results demonstrated that vaccine construct which was optimized on the three parameters is most promising. First, it contains ubiquitin for targeting polyepitope constructs for proteasom. Second, within this construct the epitopes are flanked by residues for proteasomal liberation of determinants. Third, all epitopes contain motives for TAP, which, as it was expected, should promote the transport determinants in the endoplasmic reticulum where they are involved in binding to molecules of MHC class I. As a result this construct provides maximal levels of peptide/MHC complexes *in vitro* and $T_{CD8+}$ responses to more peptides *in vivo* than other constructs.

**Acknowledgements**: This work was supported by BTEP#19/ISTC#2153 project.

doi:10.1016/j.antiviral.2009.02.038

**34**

## Feglymycin, a Unique 13 Amino-acid Peptide, With a Novel Mechanism of Anti-HIV-1 Activity

Dominique Schols[1,*], Geoffrey Férir[1], Bart Hoorelbeke[1], Anne Hänchen[2], Frank Dettner[2], Roderich D. Süssmuth[2]

[1] *Rega Institute for Medical Research, Leuven, Belgium;* [2] *Technische Universität Berlin, Berlin, Germany*

Feglymycin is a naturally occurring peptide isolated from *Streptomyces* sp. DSM 11171. The unusual primary structure of feglymycin consists of an alternating sequence of mostly aromatic (*S*)- and (*R*)-amino acids and, most remarkably, the X-ray model shows formation of a double stranded antiparallel β-helical dimer which is stabilized by a network of internal hydrogen bonds between phenolic OH-groups. Here, we report the first highly convergent total synthesis of the 13 amino-acid peptide of feglymycin by fragment condensation. The peptide strongly inhibits HIV-1 replication in several T cell lines (MT-4 and CEM) and in PBMCs ($IC_{50}$: 0.5–2.6 μM). In addition, the peptide is equally active against CCR5-using or CXCR4-using viruses and the antiviral activity appeared to be independent of the virus co-receptor use. Feglymycin remained equally active against an in vitro generated enfuvirtide-resistant HIV-1 NL 4.3 strain. The peptide does not interact with the main HIV receptors CD4, CXCR4 and CCR5 as examined with various mAbs binding assays and chemokine-specific $Ca^{2+}$-signaling assays. The peptide does not inhibit the binding of HIV-1 to $CD4^+$ T cells and also does not inhibit viral capture to Raji/DC-SIGN$^+$ cells, suggesting that the peptide interferes with post-binding events. However, the peptide inhibited syncytia formation between HIV-1-infected and uninfected $CD4^+$ T cell lines and this with comparable $IC_{50}$ values ($IC_{50}$: 2.5 μM) as in the HIV-1 replication assays. To study the potential interaction between feglymycin and gp120, we used in-house surface plasmon resonance (SPR) technology using a Biacore T100. Feglymycin clearly interacts with recombinant gp120 HIV-1 IIIB immobilized on a CM5 sensor chip resulting in a complex binding signal i.e. not a 1:1 Langmuir binding. Therefore it is only possible to determine the apparent equilibrium dissociation constant ($K_D$) resulting in 22 μM. The current study validates feglymicin as a potential lead peptide for anti-HIV-1 therapy and as a candidate topical microbicide to prevent heterosexual transmission of HIV-1.

doi:10.1016/j.antiviral.2009.02.039