

Design and testing of coxsackievirus based vaccine vectors to express HIV-1 Nef and Gag

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Abstract

Background: Enteroviruses elicit protective mucosal immune responses that might be useful as part of a strategy to prevent sexual transmission of HIV-1. Poliovirus vectors have been used successfully in preclinical studies to alter the course of SIV infection, but plans to eliminate poliovirus circulation are potential impediments to this strategy. As an alternative, we have designed and tested vectors based on another enterovirus, coxsackievirus B3 (CVB3).

Methods: HIV-1 Nef and Gag gene sequences were PCR amplified and inserted into a molecular clone of CVB3, permitting HIV protein expression as a fusion with the amino terminus of the CVB3 polyprotein. Virus was produced by transfection, and serially passaged in vitro. HIV-1 protein expression was monitored by immunoblot, and the genetic stability of insertions into the vector was monitored by PCR-based assays and sequence analysis. Chromium release assays were performed with patient derived CTL clones, using CVB3 infected CaCo cells as targets.

Results: Vectors containing the HIV-1 Nef and matrix coding sequences (CVB-Nef and CVB-MA) expressed Nef and MA over multiple passages. In contrast, vectors encoding longer segments of Gag proved to be genetically unstable due to sequences encoding the 24 kD capsid protein, and HIV protein expression was lost in 3 to 5 passages. Cells infected with CVB-MA vector were susceptible to lysis by a CD8 T cell clone specific for the HIV-1 MA SL9 epitope often targeted during chronic HIV infection.

Conclusions: Coxsackievirus vectors were constructed that stably expressed HIV proteins over multiple passages, permitting recognition and cell lysis by cytotoxic T cells. These studies help elucidate the design of CVB3 vectors and suggest their potential as vaccine candidates.

Background

The search continues for safe, effective, and practical vaccine agents and strategies that could prevent or modify the course of infection with HIV. It is currently hypothesized that a successful prophylactic vaccine strategy would require both effective humoral and cellular immune responses. (16, 20) Responses localized to the genital and gastrointestinal mucosa may also be needed since the majority of HIV transmission occurs at these sites. Understandably, there has been interest in the potential use of poliovirus and other enteroviruses as potential vaccine vectors for HIV. Live poliovirus vectors have drawn interest since they elicit long-lasting immunity, are associated with the development of herd immunity, and produce potent mucosal immune responses following replication in the gastrointestinal tract. (5) In addition, recombinant live poliovirus vaccine vectors that express SIV epitopes have been shown to elicit neutralizing mucosal IgA antibodies, SIV specific cytotoxic T cell responses, and appear to prevent and attenuate SIV infection following viral challenge. (6-7) These findings mesh with recent studies that replication of HIV and SIV and depletion of CD4 T cells in the gastrointestinal tract associated lymphoid tissues are key early events in the pathogenesis of simian and human AIDS. (11)

Unfortunately, vaccine associated paralytic poliomyelitis can occur as a complication of poliovirus vaccines. This is rare, and usually involves infection of immunodeficient hosts and reversion to virulence during protracted in vivo passage. Despite its rarity, the potential for reversion to neurovirulence and plans to totally eliminate worldwide circulation of poliovirus (13) have led to reservations about using poliovirus as a vaccine vector for other diseases. Other enteroviruses, including the related coxsackieviruses, have therefore been considered as candidates. Halim et al. inserted HIV sequences into recombinant coxsackieviruses and demonstrated that they could induce HIV humoral and cellular immune responses in mice. However, these recombinants included only small portions of the capsid domain of HIV. (12) To further explore the potential utility of enteroviruses as HIV vaccine vectors, we constructed recombinant vectors based on coxsackievirus B3 that encode varying amounts of the Gag protein of HIV-1. We found that these vectors readily expressed the entire Gag protein and truncated forms of it. Vectors containing HIV capsid (CA) sequences were genetically unstable, but expression of the HIV matrix protein (MA) persisted through extensive in vitro passage, and targeted cells for lysis by MA-specific human CD8 T cell clones.

Figure 1

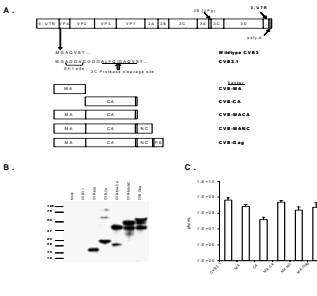


Figure 1: Construction of CVB3 vectors. (A) Schematic diagram of CVB3 genome and constructs used to express heterologous HIV sequences. In pCVB3.1 a SfiI cloning site and a synthetic viral 3C protease cleavage site are inserted downstream of the initiation codon of the coxsackie polyprotein. HIV sequences were PCR amplified with primers containing a SfiI restriction site and ligated into CVB3.1. (B) Expression of HIV sequences as detected by western blot. Membrane blotted with pooled serum from HIV infected patients. (C) Determination of the titer of viruses produced by transfected cells. Titer determined by plaque assay on HeLa (RW) cells. Values shown are in PFU/ml, (mean \pm 2 standard deviation).

Figure 2

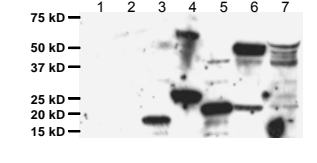


Figure 2: HIV-1 specific immunoblot of first passage of recombinant CVB3 vectors. Membrane probed with pooled serum. AS49 cells were infected with lysates from cells transfected with each of the CVB3 constructs. Lane 1: Mock infection. Lane 2: CVB3.1. Lane 3: CVB-MA. Lane 4: CVB-CA. Lane 5: CVB-MACA. Lane 6: CVB-NC. Lane 7: CVB-Gag.

Figure 3

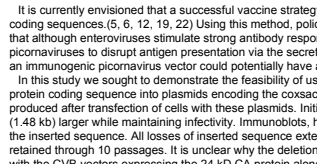


Figure 3: Expression of HIV proteins during serial passage. Lysates from serial passages of the CVB3 vectors were examined for stability of HIV-1 protein expression. Diagrams show P1 region of coxsackievirus and, if applicable, where any deletions were found during several passages. Filled in arrowheads signify expected size of proteins. If applicable, empty arrowheads indicate the majority deletion product.

Figure 4

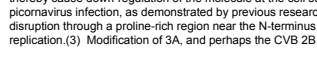


Figure 4: Chromium release assay of cells infected with CVB3 vectors. CaCo-2 cells were infected with CVB-MA or CVB-EGFP vectors at an MOI of 3. 10h later, 1 cell clone specific to the SL9 epitope of HIV-1 were added. Graph shows percent specific lysis of cells, cells infected with CVB-MA virus, cells loaded with SL9 peptide, and cells infected with CVB expressing GFP.

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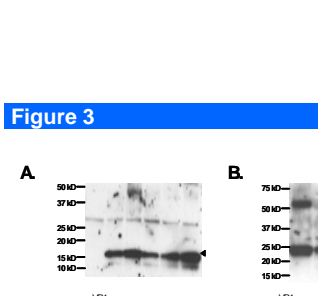


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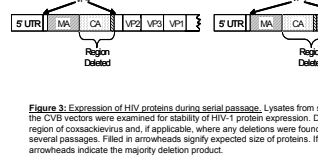


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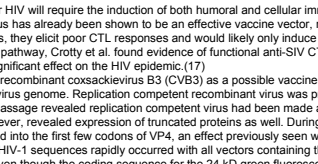


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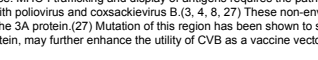


Figure 6: Expression of HIV-1 Nef protein during serial passage. (Top) Schematic diagram of HIV-1 Nef protein sequence inserted, as described previously, into the SfiI cloning site of pCVB3.1. (Bottom) 293T cells were used to transfect CVB-Nef, while AS49 cells were used for infection and passage. Lysates from Transfection to 10⁶ passage were examined for Nef protein expression as detected by western blot. Membrane was probed with Nef-specific antibody.

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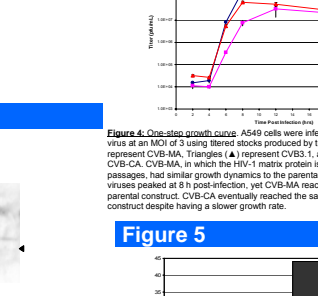


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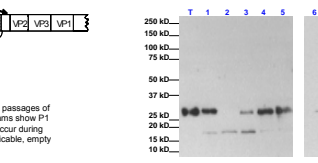


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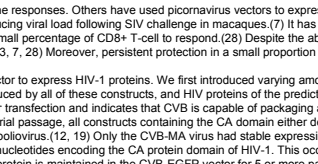


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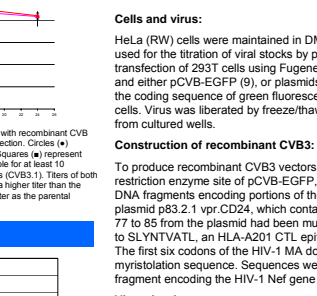


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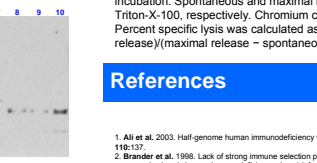


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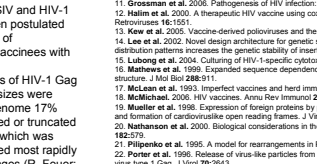


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Materials & Methods

Cells and virus: HeLa (RW) cells were maintained in DMEM + 10% FBS (complete DMEM). Cells were used for the titration of viral stocks by plaque assays. Virus stocks were produced by co-transfection of 293T cells using Fugene6 reagent with a plasmid expressing T7 polymerase and either pCVB-EGFP (9), or plasmids into which HIV sequences were inserted in place of the coding sequence of green fluorescent protein. Cell passages were performed using AS49 cells. Virus was liberated by freeze/thaw cycles, using dry ice, on cells scraped and pelleted from cultured wells.

Construction of recombinant CVB3: To produce recombinant CVB3 vectors, HIV sequences were inserted into the unique SfiI restriction enzyme site of pCVB-EGFP, an infectious CVB3 viral clone that expresses EGFP. DNA fragments encoding portions of the HIV-1 Gag gene were amplified by PCR from plasmid p83.2.1 vpr-CD24, which contains a portion of the HIV1MA-3 genome. Gag residues 77 to 85 from the plasmid had been mutated to change the predicted amino acid sequence to SLYNTVATL, an HLA-A201 CTL epitope found in many adults with chronic HIV infection. The first six codons of the HIV-1 MA domain were bypassed to preserve the CVB myristoylation sequence. Sequences were verified using primers at the insertion site. A DNA fragment encoding the HIV-1 Nef gene was amplified using a similar method.

Virus titration: Plates were seeded with HeLa (RW) cells and infected with dilutions of virus and overlaid with methylcellulose media. After 72 h, media was removed and plaques were counted.

One-step growth curve: AS49 cells were simultaneously infected at an MOI of 3 with each virus. At the indicated time points virus was harvested and titered as described above.

Immunoblot analysis: When significant cytopathic effect was seen following transfection or infection, cells were resuspended in loading buffer and proteins separated by gel electrophoresis. The gel was then transferred to PVDF-F membranes and probed with pooled antiserum. Bound antibody was detected using a chemiluminescence kit horseradish peroxidase conjugated sheep anti-human antiserum.

Chromium release assays: CaCo-2 cells infected with CVB vectors served as targets for the CTL clones as previously described. (15) Briefly, the target cells were labeled with ⁵¹Cr with or without the appropriate peptide followed by washing and plating. The CTL clones were then added at 5:1 for a 4 h incubation. Spontaneous and maximal lysis were measured without CTLs or by addition of Triton-X-100, respectively. Chromium counts were determined by microscintillation counting. Percent specific lysis was calculated as: 100 x (experimental release - spontaneous release)/(maximal release - spontaneous release).

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