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Construction and Characterization of a Chimeric Virus (BIV/HIV-1) Carrying the Bovine Immunodeficiency Virus *gag-pol* Gene: Research Letters

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Construction and Characterization of a Chimeric Virus (BIV/HIV-1) Carrying the Bovine Immunodeficiency Virus *gag-pol* Gene [Research Letters]

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HIV-1HXB2 5′LTR region, most of BIVR29 *gag-pol* segment and HIV-1HXB2 *pol* IN-3′LTR region were respectively amplified. A chimeric clone, designated as $pHBIV_{3753}$, was constructed by cloning three fragments sequentially into pUC18. MT4 cells were transfected with $pHBIV_{3753}$. The replication and expressions of the chimeric virus ($HBIV_{3753}$) were monitored by RT activity and IFA. The results firstly demonstrated that it is possible to generate a new type of the BIV/HIV-1 chimeric virus containing BIV *gag-pol* gene.

The development of safe, effective HIV vaccines is considered to be one of the most important ways to control the incidence of HIV infection [1]. Among the various types of vaccines developed, attenuated vaccines have the advantage that they mimic natural virus infection in the host. Although an HIV vaccine for humans has not yet been produced, SIV or SHIV vaccines attenuated by gene modification have protected monkeys from homologous and heterologous challenge viruses [2]. However, their safety for human use is questionable because they can cause fatal immune system disease [3].

Bovine immunodeficiency virus (BIV), a non-primate lentivirus, more closely resembles human and non-human primate immunodeficiency viruses in structure, immunology and genetics, and is unable to infect humans or human cells [4]. There is, therefore, the possibility of constructing a new chimeric virus to be used as an attenuated vaccine against HIV. In this work, we sought to construct a series of chimeric viruses of BIV and HIV-1 to generate a chimeric virus that can be used as a candidate for attenuated vaccine. We successfully generated a himeric virus/H(BIV) that contains the *gag-pol* gene from BIV.

The complementary DNA sequence data of BIV_{R29} and $HIV\text{-}1_{HXB2}$ are from GenBank. The following gene segments were obtained by polymerase chain reaction (PCR), using pHXB2 or pBIV plasmids as templates. The first segment was the entire HIV-1 5′ long-term repeat (LTR) region (1 ~ 19 nt and 788 ~ 776 nt). The second segment contained most of the BIV_{R29}*gag-pol* segment (701 ~ 727 nt and 4444 ~ 4421 nt). The third was the $HIV\text{-}1_{HXB2}$ *pol* IN -3′LTR region (4377 ~ 4411 nt and 9719 ~ 9696 nt). Two ends of three pairs of primers included different enzyme sites, respectively. PCR products were then digested by respective enzymes and ligated sequentially into predigested pUC18 vector (Figure 1a). The recombinant chimeric clone was designated as $pHBIV_{3753}$. It was verified by *Xba* I, *Bam* HI, *Hin* dIII, *Bgl* I, and *Pst* I enzyme digestion.

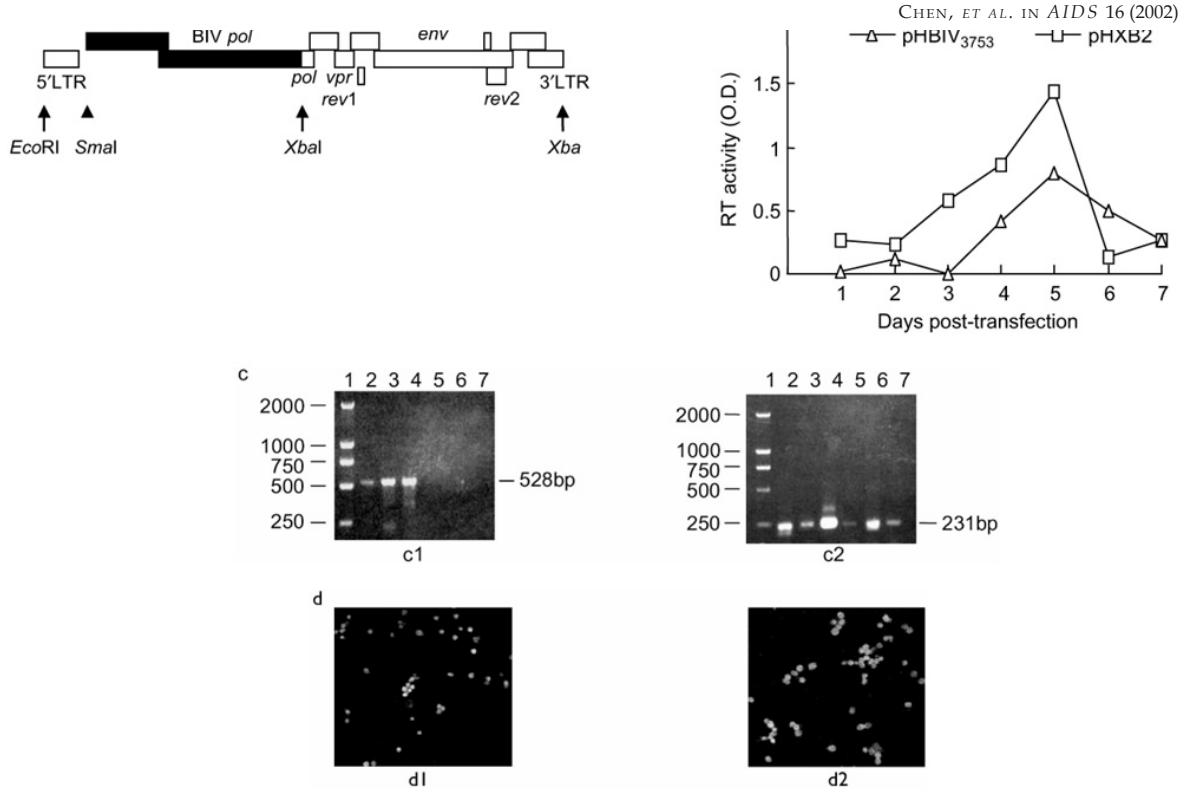

Fig. 1. Genetic structure and biological activity of $HBIV_{3753}$. (a) Genome organization of the chimeric clone. The arrowheads indicate the positions of enzyme sites used to make the recombinant. White boxes represent long-term repeats (LTR) and open reading frames from pHXB2. Black boxes represent *gag-pol*, not including the IN region of *pol*, from primate bovine immunodeficiency virus (pBIV). (b) Kinetics of $HBIV_{3753}$ replication in MT4 cells. Viral replication was monitored by reverse transcriptase (RT) activity of culture supernatants. (c) Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of BIV *gag* and HIV-1 *tat* reverse transcripts of $HBIV_{3753}$. Lanes 2-4: RT-PCR amplification of BIV *gag* fragments; lanes 5-7: PCR amplification of BIV *gag* fragments used as control; using the cellular RNA as templates at 2, 4, 7 days post-transfection, respectively (c1). Lanes 2-4: RT-PCR amplification of the full-length HIV-1 *tat* fragments, the cellular RNA from cells transfected with pHXB2 used as controls; lanes 5-7: RT-PCR amplification of $HBIV_{3753}$; collected 5, 6, 7 days post-transfection, respectively (c2). (d) BIV or HIV-1 antigen expressions in MT4 cells transfected with the chimeric clone. The smears were prepared at 48 hours post-transfection and analysed by indirect immunofluorescence assay with either mouse anti-BIV p26 monoclonal antibody (d1) or human anti-HIV-1 antibody (d2).

Equivalent quantities of $pHBIV_{3753}$ or pHXB2 plasmid were introduced into MT4 cells by electro-operation. The culture supernatant from transfected cells was collected from 1 to 7 days. To assess virus replication kinetics, the virion-associated reverse transcriptase (RT) activity was determined by a BrdUTP incorporation assay (Cavidi Tech AB, Sweden). The level of RT activity peaked at 5 days post-transfection (Figure 1b). BIV replicative capacity was lower than that of HIV-1. Although the promoters and regulatory protein of HIV-1 were used in $HBIV_{3753}$, the *gag-pol* gene came from BIV. Therefore, $HBIV_{3753}$ replicative ability was lower than that of HIV_{HXB2}.

At 2, 4, and 7 days post-transfection, the cells were harvested and the total RNA of transfected cells was extracted. BIV *gag* gene transcript of $HBIV_{3753}$ was amplified by RT-PCR (Figure 1c 1). For 5-7 days post-transfection, the HIV-1 *tat* gene transcript of $HBIV_{3753}$ was obtained by RT-PCR (Figure 1c 2). Our results showed that despite the HIV-1 *gag-pol* region being replaced by BIV *gag-pol*, $HBIV_{3753}$ could form full-length and splicing transcripts.

The antigenicity of $HBIV_{3753}$ was analysed by indirect immunofluorescent assay (IFA). Forty-eight hours post-transfection, the cells exposed to mouse anti-BIV p26 monoclonal antibody showed a fluorescence signal only in the cytoplasm. The cells also reacted with HIV-1-positive serum, and a fluorescence signal appeared both on the cell membrane and in the cytoplasm. These data indicate correct $HBIV_{3753}$ gene expression and also show correct antigenicity for two kinds of viral proteins (Figure 1d).

This is the first demonstration to show the use of a BIV gene to replace the structural gene of HIV-1, and thereby create a new type of $HBIV_{3753}$, which contains biological activity. The final goal of authors is to obtain a safe, effective, and attenuated chimeric virus. In our laboratory, a series of BIV/HIV-1 chimeric clones are being constructed and analysed.

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