Gene Therapy for Human Immunodeficiency Virus Infection in the Humanized SCID Mouse Model

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Abstract

Background: The humanized SCID mouse model is an attractive tool for testing gene therapy to combat human immunodeficiency virus infection *in vivo*.

Objectives: To devise a more specific gene therapy directed against HIV, replacing the formerly used interferon with either soluble CD4 molecule immunoadhesin (sCD4-IgG) and/or anti-gp41 monoclonal antibody (2F5), or HIV-negative transdominants genes (Tat, Rev).

Methods: Human monocytoid cell line (U937) was transfected with IFNα, β or γ genes. 3T3 murine fibroblastic cell line was transfected with sCD4-IgG or 2F5, or both genes, and a human T4 cell line (CEM) was grafted to SCID mice. Negative transdominant genes (Tat, Rev or both) were also transduced in CEM T cell line. Animals were then challenged with HIV-1. Viral load was followed.

Results: IFNα or β were potent anti-HIV, reducing viral load *in vivo* and inhibiting reverse transcriptase activity in human-removed cells from animals. sCD4-IgG immunoadhesin and gp41 monoclonal antibody resulted in a dramatic reduction of HIV-1 cellular and plasmatic viral load in humanized SCID mice. The simultaneous introduction of negative Tat and Rev genes resulted in a synergistic inhibition of HIV-1 replication *in vivo*.

Conclusions: Despite the marked reduction of HIV-1 propagation by IFN genes or by negative Tat and Rev transdominants, the gene therapy using soluble CD4 immunoadhesin or anti-gp41 was a more efficient preventive treatment against HIV infection.

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For Editorial see page 883

Gene therapy represents an attractive approach to the treatment of human immunodeficiency virus infection. To test the effects of several genes, we first developed an animal model for this infection in mice transplanted with human cells. The humanized SCID mouse can be infected *in vivo* with HIV since it lends itself to the evaluation of the putative therapeutic effect of gene transfers [1]. In a first series of experiments the IFN genes were investigated in this model [1] and the results are summarized below. We then studied the efficacy of a specific approach using the genes for soluble CD4 molecule immunoadhesin and for an anti-gp41 monoclonal antibody [2]. Potent inhibition of HIV infection was observed, as described in this article. Initial investigations in our laboratory demonstrated the feasibility and partial efficacy of gene therapy with negative transdominant Tat and Rev genes [3]. New data concerning both these mutant genes are reported here.

In humans, pilot studies have demonstrated the possibility of the anti-HIV gene therapy approach [4,5]. Further clinical studies are now required to determine whether the strategies of our or other groups represent a novel and efficient curative treatment of HIV/AIDS patients.

IFN = interferon
HIV = human immunodeficiency virus

Methods

Gene therapy with interferons

Human monocytoid U937 cells were transfected with IFNα, IFNβ, or IFNγ, involving the integration of Tat-inducible IFN genes into the cells as previously described [1]. Briefly, the expression of the IFN genes was regulated by the HIV-1 long terminal repeat sequence and the splice/polyadenylation signals from SV40 (pA). The IFN cassette was linked to the *Streptomyces alboniger* puromycin acetyltransferase selective marker expressed under the control of the SV40 promoter (Svpro) and polyadenylation signals (SvpA). To check the efficacy of gene transduction before cell grafting, the induction of IFN production was measured in cell supernatants after a few days of cell culture.

SCID mice were injected intraperitoneally with controlled or genetically modified (IFNα, β, or γ genes) U937 cells ($20x10^6$ cells/animal). On day 10 post-transplantation, the humanized SCID mice received an intraperitoneal inoculation of 1,000 $TCID_{50}$ of HIV-1 Lai. The viral load was checked by quantitative competitive-polymerase chain reaction, comparatively in spleen cells of mice with genetically modified U937 cells and in control mice. Reverse transcriptase activity was also measured in spleen cells at the time of mouse sacrifice, according to Schwartz et al. [6]. Briefly, 50 µl cell-free supernatants were mixed with 10 µl lysis buffer A containing 0.5 mol/L KCl, 50 mmol/L DTT and 0.5% Triton X-100; then 40 µl buffer B containing 1.25 mmol/L ethylene glycol-bis (-aminoethylether)-N,N,N',N'-tetraacetic acid, 125 mmol/L Tris-HCl pH 7.8, 12.5 mmol/L MgCl2, after which 3 µCi ^{3}H-deoxyribonucleoside triphosphate (Amersham International, UK) and 0.05 optical density poly rA-oligo-dT were added. The microtiter wells were covered and

incubated for 1 hour at 37°C. Stop solution (20 µl) containing 120 mmol/L $Na_4P_2O_7$ in 60% trichloracetic acid was added and plates were kept at 4°C for 15 min. Precipitates were filtered on glass filters using a cell harvester (Skatron Instruments, Suffolk, UK) and washed with 12 mmol/L $Na_2P_2O_7$ in 5% trichoracetic acid. The filters were dried and radioactivity was counted in a liquid scintillation β-counter.

Gene therapy with anti-gp41 or CD4 immunoadhesin

Murine 3T3 fibroblastic cells were transduced with either the genes coding for an anti-HIV-1 gp41 monoclonal antibody (2F5) or the genes coding for the soluble CD4 molecule linked to a fragment of human IgG (sCD4-IgG) as previously described [2]. Briefly, retroviral vector RVTG6371 encoded the human monoclonal antibody (mAb) 2F5. It carried the long terminal repeat of Moloney murine sarcoma virus (5' LTR), the Moloney murine sarcoma virus/Moloney murine leukemia virus hybrid packaging sequences (psi), the mouse phosphoglycerate kinase-1 gene promoter sequences (pgk), the mAb 2F5 heavy and light chains cDNA, respectively (2F5 HC and 2F5 LC), the internal ribosome entry site of encephalomyocarditis virus, the long terminal repeat (3' LTR) of myeloproliferative sarcoma virus, the puromycin acetyltransferase (Pac) gene and the simian virus polyadenylation signal.

Retroviral vector RVTG8338 encoded the human soluble CD4-IgG immunoadhesin (sCD4-IgG). A comparable 2F5 construction was performed. based on the ligation of the leader variable (V1/V2) segment of human CD4, 2F5 hinge region (hinge CH2-CH3) and the 2F5 heavy chain (2F5 HC).

The cells were incorporated into "neo-organs" made of collagen and paratetrafluoroethylene. The neo-organs were placed into the peritoneal cavity of SCID mice. Three weeks later, the animals were injected i.p. with human CD4+ CEM cells ($20x10^6$ cells/animal), and a week later they were challenged with HIV-1 Lai. This methodology was described recently [2,3].

This *in vivo* production of 2F5 and sCD4-IgG was sequentially measured. Cell viral load was determined by QC-PCR and plasma viral load was measured using the NASBA kit (Roche, France). HIV-1 reverse transcriptase activity was monitored in supernatants of cell cultures from the spleen, the liver and the above-described tumor.

Gene therapy with negative Tat and Rev transdominants

CEM cells were transfected according to a technical laboratory manual [7], under the control HIV-1 LTR sequence, with the transdominant negative Tat form (LTR MSV-Tat TD-proSV40-puro-LTRMPSV), with the transdominant negative Rev form (LTR MSV-Rev TD-proSV40-puro LTRMPSV), and with both negative transdominant forms (LTR MSV-Tat TD-proSV40-puro-LTRMPSV + LTR MSV-Rev TD-proSV40-neo-LTRMPSV). Non-transfected CEM cells were used as negative and positive controls. After *in vitro* amplification in the presence and absence of selective media, $20x10^6$ CEM cells were injected i.p. in SCID mice. Ten days later 1,000 $TCID_{50}$ of HIV-1 (HIV Lai) were inoculated to the mice. Three weeks after HIV-1 inoculation the animals were sacrificed and their organs (spleen, liver, tumor) were aseptically removed and cultured. Culture supernatants were collected and frozen for RT activity test as described.

LTR = long terminal repeat

QC-PCR = quantitative competitive polymerase chain reaction

Results

Gene therapy with interferons

- *In vitro production of IFN*

Before cell transplantation, interferon production was checked *in vitro*. IFNα or γ were induced by HIV-1 in the culture of genetically modified U937 cells and measured for 14 days. The peaks of IFNα and γ were obtained respectively on day 4 (1,600 IU of IFN/ml) and day 14 (325 pg/ml of IFN/ml).

- *In vivo effect of gene therapy on HIV viral load*

Fifteen days after HIV-1 challenge of humanized SCID mice, the quantitative detection of HIV provirus was carried out in spleens removed from mice previously grafted with U937 cells transfected or not by IFN genes. Cellular viral load was found to be 6 logs lower in IFNα mice than in control mice. It was 5 logs lower in IFNβ mice and only 2 logs lower in IFNγ mice [Table 1].

In addition to viral load measurements, RT activity was determined in culture supernatants from these mouse spleens. A low RT activity was detected in IFNα and IFNβ transfected cells from the spleens, but such a reduction was less significant in IFNγ producing mice [1].

Gene therapy with anti-gp41 or CD4 immunoadhesin

- *In vivo release of 2F5 or sCD4-IgG*

SCID mice implanted with 2F5 neo-organs were assayed for 2F5 monoclonal antibody in plasma samples. High levels of 2F5 were found from week 2 throughout the observation period (until week 6). Similarly, human sCD4 molecule could be detected in mouse plasma by a qualitative enzyme-linked immunoabsorbent assay during the same period.

The follow-up of 2F5 production showed an increased secretion during the first 5 weeks post-grafting. Neo-organ-transplanted SCID mice showed mean serum levels of 2F5, increasing from 167 to 1,000 ng/ml between weeks 1 and 5. HIV-1 was inoculated at week 4 after neo-organ implantation. At week 6, a mean of 450 ng/ml of 2F5 was found in the serum of mice (data not shown). We did not have standard sCD4-IgG purified molecules to quantify the production of

RT = reverse transcriptase

Table 1. HIV-1 proviral DNA detection by QC-PCR in spleen cells of control and IFNα, β and γ SCID mice infected with Lai virus

Competitor copies^0	10^6	10^5	10^4	10^3	10^2	10	1
Control + HIV-1	X						
SCID-IFNα + HIV-1							X
SCID-IFNβ + HIV-1						X	
SCID-IFNγ + HIV-1			X				

X = the equimolarity of the copy number of the HIV-1 *gag* (mean of three animals) fragment between the target DNA and the competitor DNA.

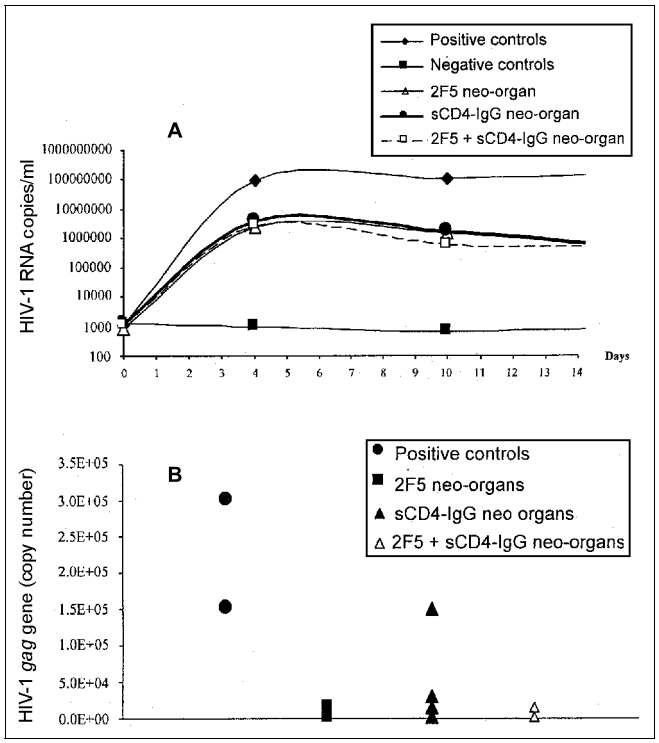

Figure 1. [A] Plasma HIV-1 viral load in SCID mice implanted with neo-organs (producing 2F5 Mab or sCD4 IgG or 2F5 + sCD4 IgG), transplanted with human T cells and challenged with HIV (Lai). **[B]** HIV-1 proviral detection, by QC-PCR, in spleen cells of control, 2F5 or sCD4 IgG or 2F5 + sCD4 IgG neo-organ grafted in SCID mice infected with HIV1 (Lai).

the immunoadhesin *in vitro* and *in vivo*. Nevertheless, we could detect the molecules in cell supernatants and in mouse sera by qualitative ELISA assay (data not shown).

- *In vivo effects of gene therapy with 2F5 or sCD4-IgG on HIV viral load*

At the time of sacrifice, 6 weeks following neo-organ implantation, the number of HIV-1 proviral DNA copies was low in spleen cells of each group of mice that received 2F5 neo-organs, sCD4-IgG neo-organs, or 2F5 + sCD4-IgG neo-organs, as compared to controls [Figure 1A]. In the last group characterized by the *in vivo* production of both 2F5 and sCD4-IgG, a more homogeneous and potent effect was observed, even though synergism could not be shown.

Similarly, the plasmatic viral load was significantly reduced by either 2F5 or sCD4-IgG in mice implanted with the corresponding neo-organ. The results were superimposable with 2F5 and sCD4-IgG, and already 1.5 log lower than in controls at day 4 (data not shown). The difference from controls was larger on days 10 and 15 [Figure 1B]. When both molecules were produced together in the same mouse, the reduction of viral load was comparable to that obtained with either molecule. At day 10, this plasmatic viral load tended to be lower in the group 2F5 + sCD4-IgG, but even then the reduction did not reach statistical significance when compared with either 2F5 or sCD5-IgG alone.

ELISA = enzyme-linked immunoabsorbent assay

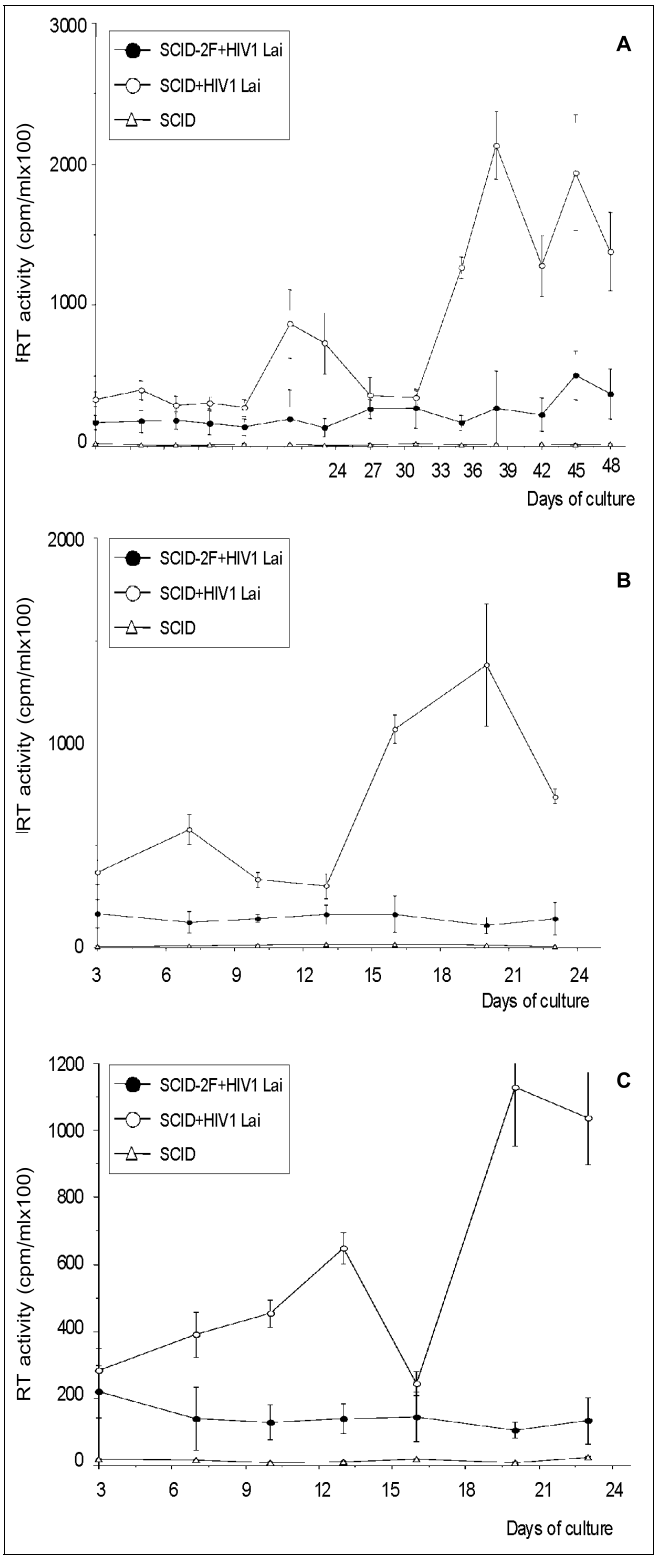

Figure 2. Reverse transcriptase activity in cultured cells from **[A]** spleens, **[B]** livers and **[C]** tumors in mice implanted with 2F5 neo-organs.

- *RT activity in cultured cells from spleens, livers and tumors*

Cell cultures were performed using human T cells (CEM cells) recovered from various organs (spleen, liver and, in certain cases,

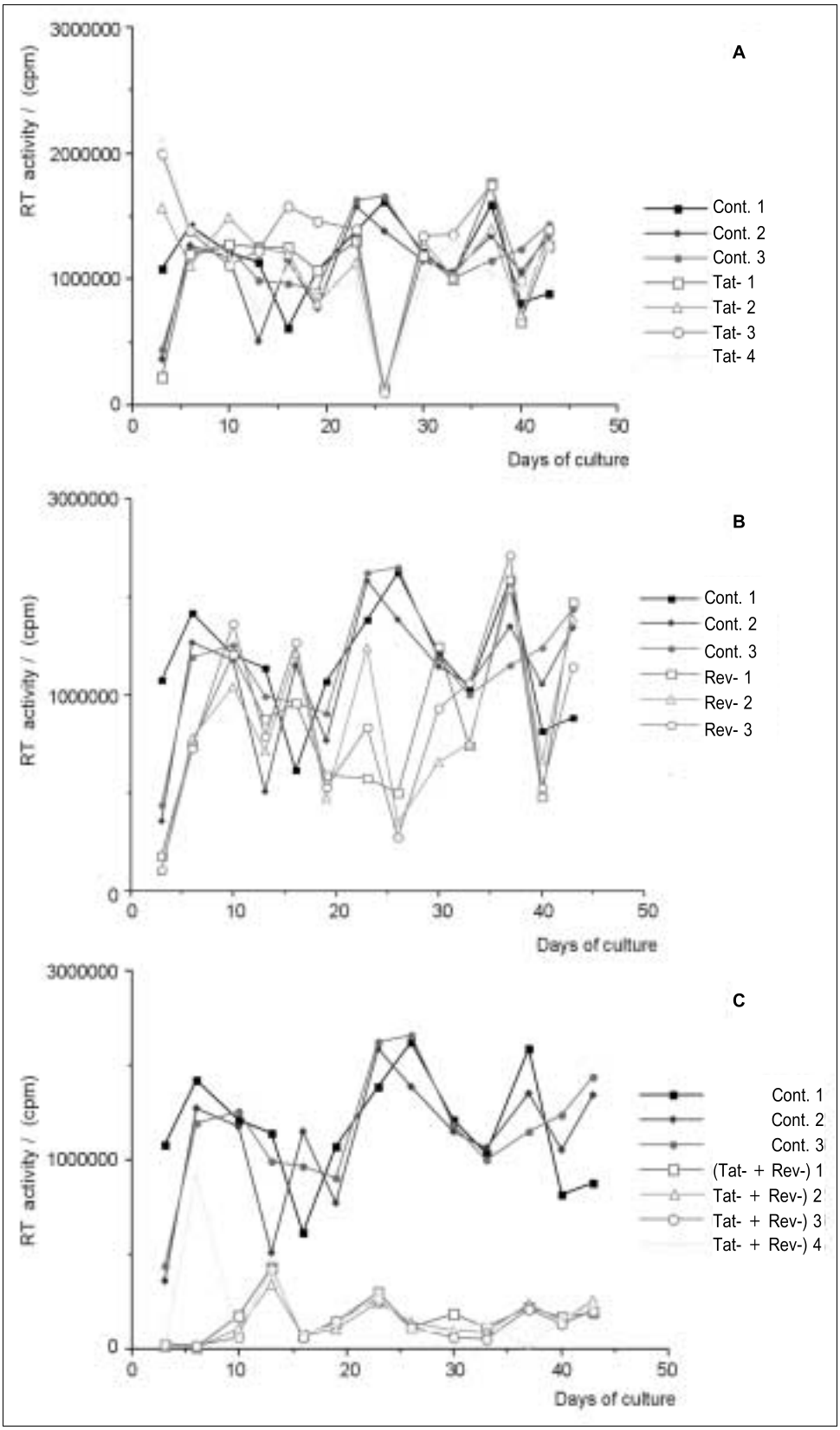

Figure 3. Reverse transcriptase activity in cultures of genetically modified CEM cells removed from spleens of SCID mice implanted with **[A]** Tat-negative transdominant (four Tat^- mice), **[B]** Rev-negative transdominant (three Rev^- mice) and **[C]** Tat + Rev-negative transdominant (four Tat^- + Rev^- mice) genes. Control (three control mice) and gene-treated animals were inoculated with HIV-1 (Lai)

abdominal tumor induced by CEM cells) of grafted SCID mice. When these CEM cells were recovered from mice implanted with 2F5 neo-organs, consistently low RT activity was observed, as compared to cultures obtained from control SCID mice also infected with HIV1 [Figure 2A, B and C].

Gene therapy with mutant Tat and Rev

As shown in Figure 3, no significant RT activity was observed, except between 23 and 27 days of culture for mutant Tat transfected cells [Figure 3A] and between 17 and 33 days of culture for mutant Rev transfected cells [Figure 3B]. It seems that native forms of Tat and Rev genes were more active on HIV-1 replication than mutant forms of both genes used separately. When both genes were introduced simultaneously in CEM transfected cells, *in vivo* HIV-1 challenge of SCID mice resulted in a synergistic effect with a dramatic inhibition of viral replication, as shown in Figure 3C. The HIV-1 replication was always more significantly inhibited, as compared to mutant Tat, mutant Rev or positive control cells.

Discussion

These results demonstrate that a carefully designed genetic modification of either human cells or murine cells can confer a strong anti-HIV activity in humanized SCID mice inoculated with HIV-1. When transfected with IFNα or IFNβ expression vectors, human cells maintained in various organs of an SCID mouse were protected against *in vivo* HIV-1 infection. The inhibition of viral propagation was concomitant to the expression of IFNα or β in the transfected cells. These data confirmed the antiviral activity of interferon in HIV infection [8,9].

The development of interferon gene therapy for human AIDS faces

two potential problems: a) the lack of specificity of interferon; b) based on pre-industrial manufacturing (Transgene Company, personal communication), the production of very large quantities of IFN genetically modified cells proved to be difficult due to the antiproliferative effect of interferon, which results in self-limitation of the activity.

We then investigated the potentials of two anti-HIV genes: an anti-gp41 antibody gene and the sCD4-IgG gene. The genetically modified fibroblasts were inserted into neo-organs that were placed intraperitoneally into humanized SCID mice. Each gene, as well as the addition of both genes, led to the expression of the therapeutic molecules, resulting in a very significant experimental reduction of HIV load *in vivo*. Because of their high affinity for highly conserved epitopes of the gp120 and gp41 glycoproteins of the HIV-1 envelope, the sCD4-IgG and 2F5 molecules display a broad spectrum of action [8]. Both were shown to recognize either laboratory or primary isolates [10,11]. Their potential antiviral efficiency was directly related to their ability to interact with the HIV-1 envelope. When the two molecules were produced together, an additive effect occurred in several mice. It is known that the CD4 molecule induces conformational changes in the gp120, permitting access to gp41 [12].

Gene therapy with sCD4 alone was shown to have a limited effect [12] due to the very short plasma half-life of CD4 [13]. Immunoadhesins involving a complex of sCD4 and human IgG have been significantly more efficient, with a longer half-life. Alternatively, a multimeric CD4 fusion protein can be used for this gene therapy approach [14].

Mutant forms of Tat transdominants were originally designed to compete with cognate Tat for the binding to the viral TAR sequence. The data presented here show the inability of TAR blocker to significantly affect HIV expression when mutant Tat gene was used alone. Concomitantly used Tat and Rev mutants were comparatively much more efficient.

The above-described gene therapies in humanized SCID mice demonstrate the validity and efficacy of this therapeutic field in HIV infection. Several experiments are still required to define the safest and optimal conditions for use in humans, but it is now reasonable to envision the future application in patients in whom HIV developed resistance to the presently available multitherapies. Ranga et al, in Nabel's group [15], first carried out a small clinical trial using Rev M10, a mutant HIV gene that inhibits viral replication, as a complementary treatment to antiretroviral compounds. Recently, a phase II randomized study of HIV-specific T cell gene therapy in subjects with undetectable plasma viremia, in a combination antiretroviral therapy, was initiated using CD4+ and CD8+ T cells bearing a chimeric T cell receptor against HIV [5]. Although it is too early to evaluate precisely the effects of this clinical trial, we are confident that further improvement in these gene therapies will result in a useful therapy for the growing number of AIDS patients.

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