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Inhibition of Human Immunodeficiency Virus Replication and Growth Advantage of CD4$^+$ T Cells from HIV-Infected Individuals That Express Intracellular Antibodies Against HIV-1 gp120 or Tat

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ABSTRACT

Current clinical gene therapy protocols for the treatment of human immunodeficiency virus type 1 (HIV-1) infection often involve the *ex vivo* transduction and expansion of CD4$^+$ T cells derived from HIV-positive patients at a late stage in their disease (CD4 count <400). These protocols involve the transduction of T cells by murine leukemia virus (MLV)-based vectors encoding antiviral constructs such as the rev m10 dominant negative mutant or a ribozyme directed against the CAP site of HIV-1 RNA. We examined the efficiency and stability of transduction of CD4$^+$ T cells derived from HIV-infected patients at different stages in the progression of their disease, from seroconversion to AIDS. CD4$^+$ T cells from HIV-positive patients and uninfected donors were transduced with MLV-based vectors encoding β-galactosidase and an intracellular antibody directed against gp120 (sFv 105) or Tat. (sFvtat1-C$_\kappa$). The expression of marker genes and the effects of the antiviral constructs were monitored *in vitro* in unselected transduced CD4$^+$ T cells. Efficiency and stability of transduction varied during the course of HIV infection; CD4$^+$ T cells derived from asymptomatic patients were transducible at higher efficiencies and stabilities than CD4$^+$ T cells from patients with acquired immunodeficiency syndrome (AIDS). Expression of the anti-tat intracellular antibody was more effective at stably inhibiting HIV-1 replication in transduced cells from HIV-infected individuals than was sFv 105. The results of this study have important implications for the development of a clinically relevant gene therapy for the treatment of HIV-1 infection.

OVERVIEW SUMMARY

This study examined the efficiency and stability of transduction of T cells from human immunodeficiency virus (HIV)-infected individuals with a murine leukemia virus based vector encoding an anti-HIV intracellular antibody to gp120 (sFv105), or to Tat (sFvtat1-Cκ). The paper demonstrates that T cells, derived from HIV-positive patients, were stably transduced with the transgene and that they were protected from HIV infection by the presence of the gene encoding the anti-HIV intracellular antibody. The results of this paper provide proof of principle that an anti-HIV intracellular antibody can be used as a putative gene therapeutic agent to protect CD4$^+$ T cells from HIV-infected individuals. Therefore, this data provides support for a proposed clinical phase 1 study of a gene therapy for HIV infection utilizing the transduction and *ex vivo* expansion of T cells with a construct encoding an anti-HIV intracellular antibody.

INTRODUCTION

IN THE FACE OF THE GROWING human immunodeficiency virus type 1 (HIV-1) pandemic and the limited success of current interventions to control both the transmission and the effects of

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HIV in the infected individual, novel strategies such as gene therapy have been developed to combat the retrovirus (Sarver *et al.*, 1990; Dropoulic and Jeang, 1994; Yu *et al.*, 1994). A putative gene therapy for HIV infection can involve the introduction of a gene encoding an antiviral construct into the principal target cell for HIV infection, the mature $CD4^+$ T cell (Baltimore, 1988; Anderson, 1994). A variety of antiviral constructs including decoy RNA, ribozymes, dominant negative mutant HIV proteins, and intracellular antibodies directed against HIV proteins have been developed (Winter and Milstein, 1991; Chen *et al.*, 1992; Malim *et al.*, 1992; Bevec *et al.*, 1994; Chang *et al.*, 1994; Marasco *et al.*, 1993). Many of these antiviral constructs, such as a ribozyme binding at the CAP site of HIV RNA, polymeric RNA decoys to the tat activation region (TAR) of the provirus, the dominant negative m10 mutant of the HIV-1 protein Rev, and intracellular antibodies directed against gp120, Tat and Rev have been shown to inhibit HIV replication in $CD4^+$ T cell lines that constitutively express the antiviral construct (Weerasinghe *et al.*, 1991; Winter and Milstein, 1991; Chen *et al.*, 1992; Malim *et al.*, 1992; Bevec *et al.*, 1994; Chang *et al.*, 1994; Yamada *et al.*, 1994; Duan *et al.*, 1994; Mhashlikar *et al.*, 1995). In addition, when the ribozyme to the CAP site of HIV RNA was stably transduced into normal T cells from an uninfected donor using a MuLV based vector, inhibition of HIV replication was demonstrated when these transduced cells were challenged with both primary and laboratory isolates of HIV, at low multiplicities of infection (moi) (Leavitt *et al.*, 1994; Vandendriessche *et al.*, 1995).

However, if gene therapy for HIV infection is to be considered a viable therapeutic option *in vivo*, then the antiviral constructs must be capable of inhibiting the replication of primary isolates of HIV in $CD4^+$ T cells from HIV-infected and uninfected individuals (Vandendriessche *et al.*, 1995; Woffendin *et al.*, 1996). Woffendin *et al.* and Vandendriessche *et al.* demonstrated that anti-sense TAR, antisense *tat* and *rev* sequences, and the Rev m10 transdominant mutant protein were capable of inhibiting replication of primary isolates of HIV in T cells from HIV-infected individuals (Vandendriessche *et al.*, 1995; Woffendin *et al.*, 1996). Furthermore, because the disease progresses clinically from primary infection, through an asymptomatic phase to AIDS, each stage being characterized by its own clinical, immunological, and virological features, the antiviral construct must be capable of inhibiting HIV replication in T cells derived from patients at these different stages in the disease (Nabel *et al.*, 1994; Vandendriessche *et al.*, 1995; Leavitt *et al.*, 1996; Woffendin *et al.*, 1996). Finally, $CD4^+$ T cells bearing an anti-HIV construct have recently been shown to have a selective advantage and to expand *in vitro* despite challenge with HIV-1 (Lisziewicz *et al.*, 1995; Leavitt *et al.*, 1996). This finding has allowed the ultimate goal of a gene therapy for HIV infection to be contemplated—to repopulate an individual with autologous $CD4^+$ T cells that are protected from HIV infection and therefore have a selective advantage for survival and expansion in the HIV-infected individual.

Intracellular antibodies have recently been designed that bind several different HIV proteins and have been shown to inhibit HIV replication in $CD4^+$ T cell lines in which the intracellular antibody is stably expressed (Marasco *et al.*, 1991; Duan *et al.*, 1994; Mhashilkar *et al.*, 1995; Shaheen *et al.*, 1996). In this study, the effect of the anti-HIV intracellular antibodies directed against Tat (sFvtat1-C_κ) or gp120 (sFv105) on HIV replication in $CD4^+$ T cells from HIV-infected individuals is examined and the implications for the design of phase 1 protocol for testing a gene therapy for HIV infection in HIV-infected patients are discussed.

MATERIALS AND METHODS

Retroviral vectors and packaging cell culture

LN-based murine leukemia virus (MLV) retroviral vectors were used in all experiments (see Fig. 1) (Miller, 1992). The vectors LGC.sFv105 and LGC.sFvtat1-C_κ encoded the intracellular antibodies sFv 105 and sFv tat1-C_κ, which bind gp120 in the endoplasmic reticulum (ER) and Tat in the cytoplasm, respectively (Marasco *et al.*, 1993; Mhashilkar *et al.*, 1995). The production of RNA encoding the intracellular antibody was driven by the cytomegalovirus (CMV) promoter. The LG, LGC.sFv105, and LGC.sFvtat1-C_κ vectors also encoded the enzyme β-galactosidase (β-Gal) containing an intranuclear localization signal and the expression of the marker gene was driven by the 5′ long terminal repeat (LTR) of the MLV-based vector. GP+env AM12 amphotropic MLV retroviral vector packaging cell lines were made and cultured by standard meth-

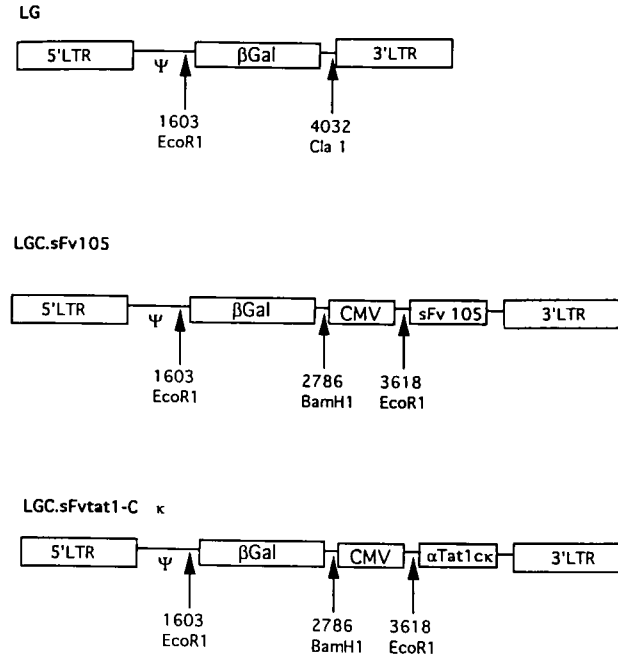

FIG. 1. LN-based vectors. The three LN-based vectors used in this study are shown in this figure. These include LGC.sFv105, LGC.sFvtat1-C_κ, and the LG vector. The intracellular antibody gene expression is driven by the CMV promoter in the construct. Each vector contains the β-Gal gene with an encoded intranuclear localization signal. Numbers against each construct related to cloning sites on the original LN vector from which all the vectors utilized in this study were derived (Miller, 1992).

ods (Kotani *et al.*, 1994; Yamada *et al.*, 1994). Vector-containing supernatants were filtered through 0.45 μm filters and titered by limiting dilution onto 3T3 cells. The titer of vectors generated from the AM12-based packaging cells varied between 5×10^5 infectious units/ml and 1×10^7 infectious units per milliliter. All vectors were applied to target primary $CD4^+$ T cells at a titer of 2.5×10^5/ml.

Patient selection

Peripheral venous blood was obtained from 3 patients immediately following a documented HIV seroconversion illness, 23 patients with asymptomatic HIV infection (who had not had an AIDS-defining illness), and 22 patients with a diagnosis of AIDS. A medical history, including the history of antiretroviral drug usage was taken at the time of venesection and peripheral blood was sent for hematological indices and CD4/CD8 count. Peripheral blood was also drawn from 26 age- and sex-matched HIV uninfected donors who were attending the genito-urinary medicine clinic for routine investigations. Hematological indices and CD4/CD8 T cell count were also performed on the HIV-negative samples at the time of venesection.

Tissue culture and transduction of T lymphocytes

Peripheral blood samples were layered onto Ficoll-Hypaque (Pharmacia, Uppsala) and the mononuclear cell fraction prepared by standard methods. Mononuclear cells were initially cultured for 24 hr in RPMI-1640, 10% fetal calf serum (FCS), and 10 IU/ml of phytohemagglutinin (PHA) (Difco, Detroit). Nonadherent lymphocytes were obtained from PHA-stimulated cultures and maintained at a density of 1×10^6 cells/ml in RPMI-1640 and 10% heat-inactivated FCS, 10 IU recombinant interleukin-2 (rIL-2) (Boehringer, Mannheim), and penicillin (100 U/ml) and streptomycin (100 μg/ml). The $CD4^+$ T lymphocyte subpopulation was prepared from nonadherent peripheral blood mononuclear cells (PBMC) using Dynabeads (Dynal) according to established techniques. The purity of $CD4^+$ T cell populations prepared in this way was determined by immunostaining for CD4 and CD8 (DAKO, UK) using a standard methodology (Cordell, 1984). Two days after culture in rIL-2, lymphocyte subpopulations were exposed to vector-containing supernatants over four sequential 24-hr time periods. On each occasion, T cell subpopulations were exposed to a 50:50 mix of vector containing supernatant and complete medium containing rIl-2 as above. Following each round of transduction, cells were washed and resuspended in a fresh mix of vector containing supernatant and complete medium. Following the four rounds of transduction, cells were cultured in RPMI-1640, 10% heat-inactivated FCS, and rIl-2-containing medium and cultured at a density of 1×10^6 cells/ml.

Estimations of transduction efficiency and cell viability were performed on the cultures at 2, 8, 20, 30, and 40 days post transduction. A total of 10^5 cells were removed from the culture and stained for intranuclear β-Gal activity and trypan blue exclusion by established methods. Transduction efficiencies were also determined in $CD4^+$ T cells that were derived from PBMC activated by PHA and rIL-2 and exposed to azidothymidine (AZT) (GLAXO-Wellcome, UK) at a final concentration of 1 μM, 2 days after activation and 2 days prior to transduction.

Monitoring HIV replication and HIV challenge

Three assay systems were utilised for monitoring the inhibitory action of the transduced antiviral constructs in $CD4^+$ T cells. Peripheral blood from asymptomatic HIV-infected individuals was used exclusively for these experiments because individuals with AIDS, and consequently lower $CD4^+$ T cell counts, did not tolerate the repeated venesection of larger quantities of peripheral blood necessary for the performance of these experiments.

The first assay system involved the culture of $CD4^+$ T cells in the presence of rIL-2 and the subsequent removal of samples of culture supernatant at 3, 6, 9, 14, and 28 days post transduction for HIV-1 p24 antigen estimation by enzyme-linked immunosorbent assay (ELISA) (Coulter, Miami). Second, HIV-1_{MN} strain, which was adapted to grow on PBMC, was added to cultures of transduced lymphocytes at an moi of 1.0 (Prakash *et al.*, 1991). The titer of the challenge HIV-1_{MN} was determined on peripheral blood lymphocytes from an uninfected donor by established means. HIV-1_{MN} challenged transduced cells were then washed three times in RPMI-1640 and cultured at a density of 1×10^6 cells/ml in medium containing rIL-2. Supernatant from these cultures was removed at regular intervals for HIV-1 p24 estimation for up to 28 days. Third, 1×10^6 PBMC from an uninfected donor were added to cultures of transduced lymphocytes from asymptomatic HIV-infected and HIV uninfected donors and then co-cultures of cells were maintained in rIL-2 for up to 28 days. Supernatant was removed from these co-cultures for HIV-1 p24 antigen estimation at regular intervals up to 28 days following the establishment of the co-culture. All experiments were performed in triplicate for transduced $CD4^+$ T cells from each patient with asymptomatic HIV infection.

RESULTS

Patient details

Peripheral blood lymphocytes were obtained from uninfected donors ($n = 26$), HIV-infected individuals experiencing a seroconversion illness ($n = 3$), patients with asymptomatic HIV infection who had not progressed to AIDS ($n = 23$), and patients with AIDS ($n = 22$). The median value and range of $CD4^+$ T cell counts for each group of patients were: asymptomatic HIV-positive patients, 470×10^6/liter, 380–620; HIV-positive patients with AIDS, 130×10^6/liter, 40–260; age- and sex-matched uninfected controls, (780×10^6/liter, 680–980). Seven of the asymptomatic HIV-positive patients and 4 of the patients with AIDS were receiving a combination of antiretroviral drugs at the time of venesection.

Characterization of transduced peripheral blood lymphocytes

Peripheral blood $CD4^+$ T lymphocytes, prepared from each donor were transduced with the vectors LG, LGC.sFv105, or LGC.sFvtat1-C_κ in parallel. $CD8^+$ T cell contamination of $CD4^+$ T cell preparations from asymptomatic HIV^+ individuals and uninfected controls was determined at a level consistently less than 1% (range 0.2–0.8%; median 0.41%) by im-

munostaining for CD8. Transduced lymphocytes were not grown in selective medium but maintained in rIL-2 containing medium for up to 40 days as described above. The proportion of cells expressing β-Gal at 5 days following transduction is shown in Fig. 2A; uninfected donors (median = 20.1%; range = 10–28%), seroconvertors (median = 12.5; range 9–15%), asymptomatic HIV infection (median = 19%; range = 11–29%), and patients with AIDS (median = 6%; range = 0–12). Transduction efficiencies were not found to be significantly altered by the addition of AZT to medium 2 days prior to the transduction (Fig. 2B).

The proportion of transduced cells in cultures was also monitored over time in these expanding cultures. The proportion of transduced cells did not vary significantly over time in $CD4^+$ lymphocyte cultures derived from uninfected individuals (Fig. 3A). However, the proportion of transduced T cells from asymptomatic HIV-positive individuals increased in those cultures transduced with the LGC.sFvtat1-C_κ or the LGC.sFv105 vector, when the proportion of transduced cells was normalized for the number of viable cells in the cultures (Fig. 3B). The proportion of transduced cells from individuals with asymptomatic HIV disease at day 2, was 10% + 2.6% (mean%, SEM), 11.2% + 1.8% and 9.1% + 1.7% for cells transduced with the LG, the LGC.sFv105, and LGC.sFvtat1-C_κ vectors, respectively. The proportion of transduced T cells from asymptomatic HIV-infected individuals by day 40 rose significantly to 15.1% + 1.9% and 15.8% + 2.2% for cells transduced with the vectors LGC.sFv105 ($p = 0.043$, day 7 and day 40 compared by the Student's t-test) and LGC.sFvtatI-C_κ ($p = 0.039$, day 7 and day 40 compared by the Student's t-test). The proportion of T cells transduced with the LG vector that did not encode an anti-HIV intracellular antibody did not alter significantly over the similar period of time in culture ($p = 0.15$, Student t-test). In contrast, the proportion of transduced $CD4^+$ T cells in cultures from patients with AIDS failed to expand significantly and declined regardless of which of the vectors they had been transduced with (Fig. 3C).

Viability of transduced $CD4^+$ T cells from HIV-1-infected and uninfected donors was also monitored (Fig. 4). The rate of growth of lymphocytes from uninfected donors was similar for each of the vectors used (LG, LGC.sFvtat1-C_κ, or LGC.sFv105). These cultures expanded from the viable cell densities of 1×10^6/ml cells to approximately 8.6×10^6/ml after 40 days in medium containing rIL-2 (Fig. 4A). The density of viable cells increased in cultures of T cells from asymptomatic HIV-positive patients that had been transduced with the LGC.sFvtat1-C_κ or the LGC.sFv105 vector, whereas the density of viable cells from cultures of T cells transduced with the LG vector plateaued or declined after 30 days (Fig. 4B). The density of viable T cells from individuals with asymptomatic HIV disease at day 2, was 1.0×10^6 cells/ml + 0.1×10^6 cells/ml (mean + SEM) for cells transduced with the LG, the LGC.sFv105, or LGC.sFvtat1-C_κ vectors. The proportion of transduced T cells from asymptomatic HIV-infected individuals had risen significantly by day 40 post transduction to 7.1×10^6 cells/ml + 1.8×10^6 cells/ml for the vector LGC.sFv105 ($p = 0.0023$, day 7 and day 40 compared by the Student's t-test) and to 8.1×10^6 cells/ml + 2.5×10^6 for the vector LGC.sFvtat1-C_κ ($p = 0.0010$, Student's t-test). In contrast, the viability of $CD4^+$ T cells from HIV-1-positive individuals with AIDS and transduced with one of the three vectors

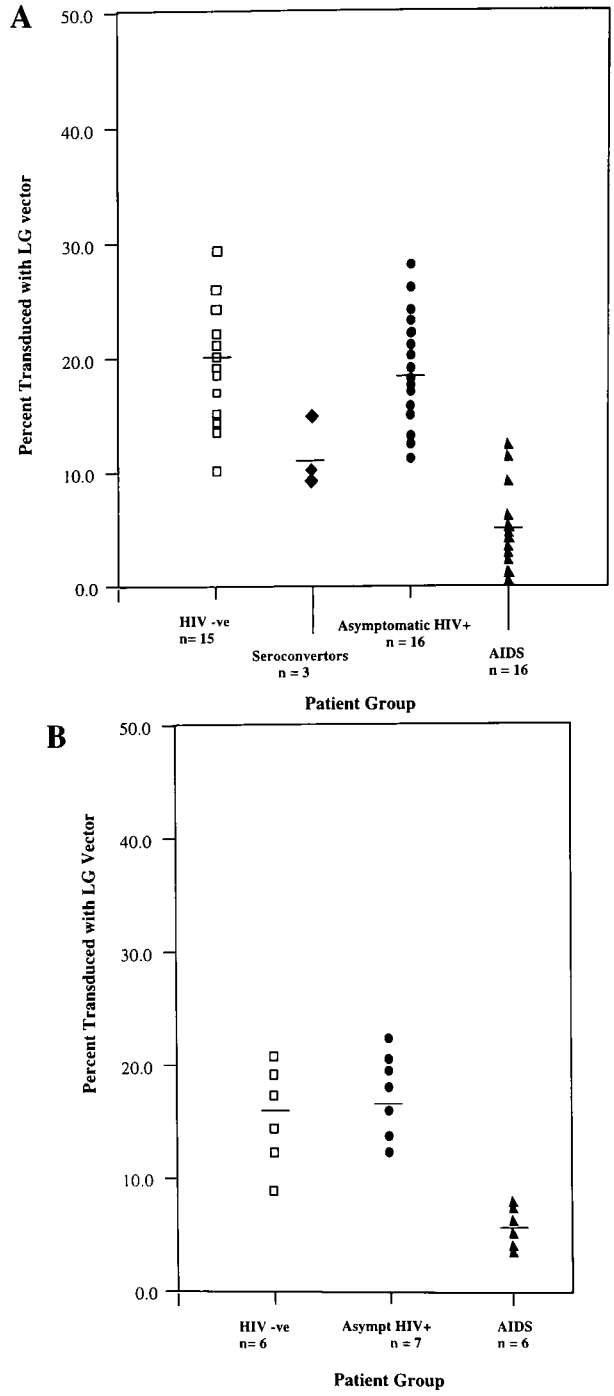

FIG. 2. Transduction efficiencies measured in $CD4^+$ T cells 5 days post transduction in the absence (A) and presence (B) of 1 μM AZT. Transduction efficiencies were determined by the measurement of the proportion of cells transduced with the LG vector that expressed intranuclear β-Gal activity. Transduction efficiencies were determined for $CD4^+$ T cells from HIV negative controls (□), HIV-positive individuals undergoing a seroconversion illness (◆), asymptomatic HIV-positive patients (●), and patients with AIDS (▲) (A). Median transduction efficiencies for $CD4^+$ T cells from HIV-negative donors, asymptomatic HIV-positive individuals, and patients with AIDS were not significantly altered by the presence of AZT (1 μM) added to culture medium 48 hr after activation of PBMC with PHA and rIL-2 (see B).

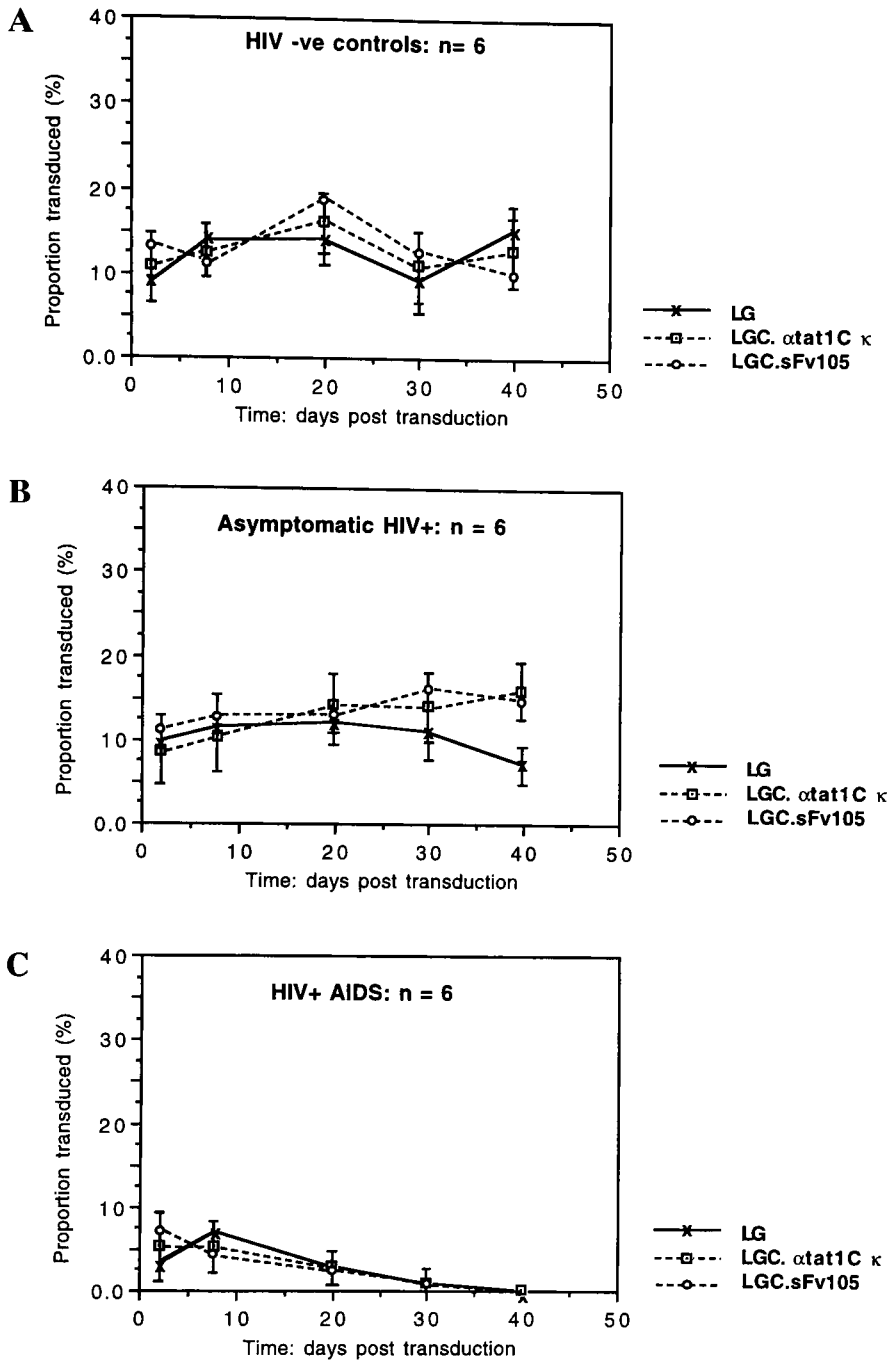

FIG. 3. Stability of transduced CD4$^+$ T cells: The proportion of cells staining positive for the transduced β-Gal gene was assessed at intervals up to 40 days for CD4$^+$ T cells transduced with each of the three constructs, LG, (—×—), LGC.sFv105 (—○—), and LGC.sFvtat1-C$_\kappa$ vectors (—□—) in uninfected controls (A), HIV-infected individuals without an AIDS diagnosis (B), and HIV-infected individuals with AIDS (C). Standard errors of the mean values are shown for the T cells transduced with the LG and LGC.sFvtat1-C$_\kappa$ vectors. All 6 patients in each of the three categories were transduced with each of the three vectors. The transduction efficiencies in time-course experiments at day 2 and 7 post-transduction, illustrated in A, B, and C, not statistically significantly less than in the separate set of single time point transduction experiments represented in Fig. 2A, which were determined at day 5 post transduction.

plateaued and ultimately declined during the 40-day culture process (Fig. 4C).

Protection of lymphocytes from HIV infection

HIV replication in expanding transduced but unselected lymphocytes was monitored in three assay systems. First, endogenous HIV replication in cultures was monitored in cultures of mock-transduced or transduced $CD4^+$ T cells. HIV p24 antigen production was barely detectable in lymphocytes from all groups of HIV-infected individuals (data not shown). Cultures of $CD4^+$ T cells from patients with asymptomatic HIV infection intermittently produced p24 at the lowest levels detectable with the assay system, *i.e.*, up to 80 pg/ml of HIV-1 p24 antigen.

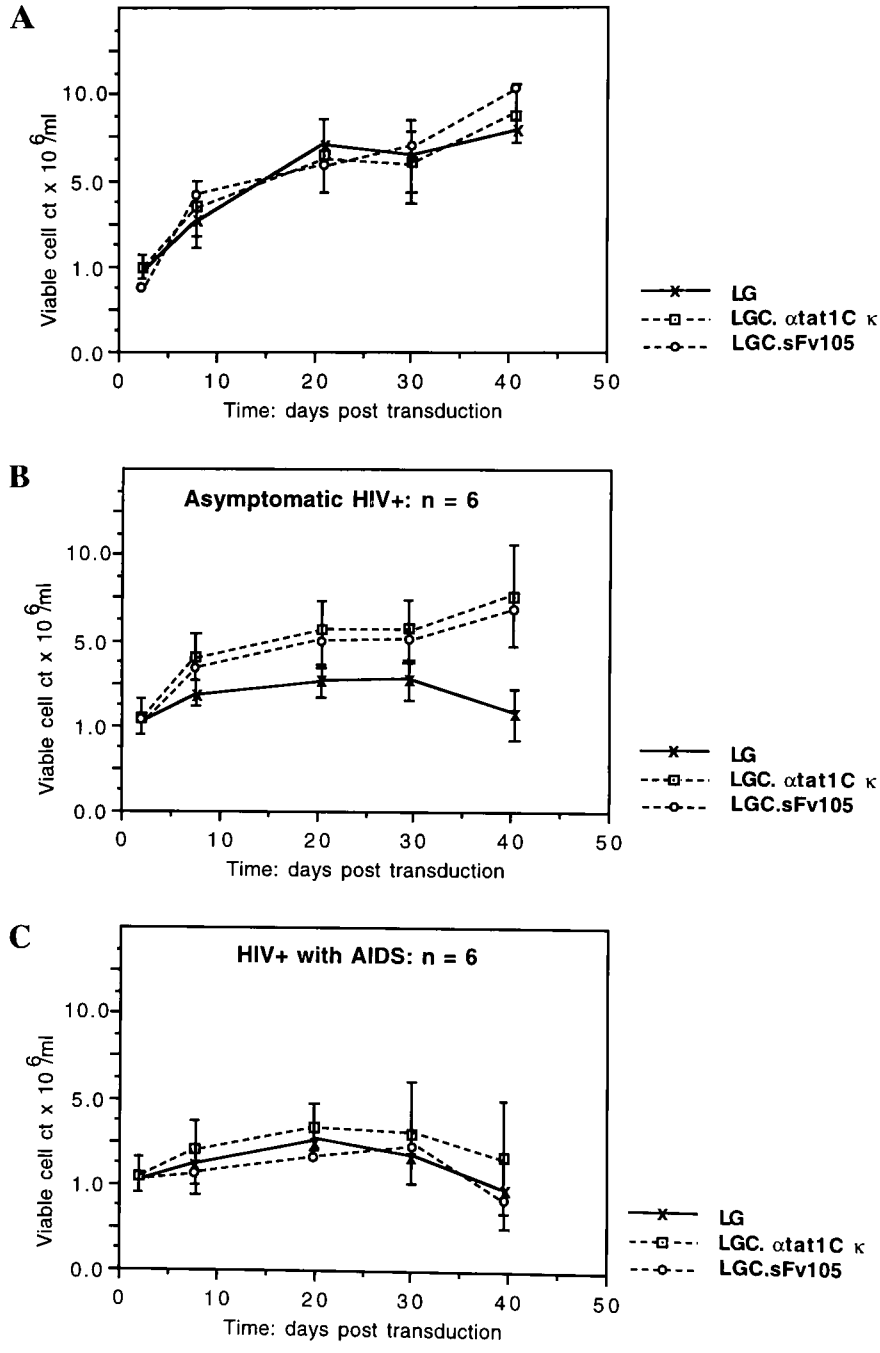

FIG. 4. The viability of transduced $CD4^+$ T cells. The proportion of viable cells in expanding T cell cultures was assessed at intervals up to 40 days for $CD4^+$ T cells transduced with each of the three constructs, LG (—×—), LGC.sFv105 (—○—), and LGC.sFvtat1-C_κ vectors (—⊡) in uninfected controls (A), HIV-infected individuals without an AIDS diagnosis (B), and HIV-infected individuals with AIDS (C). Standard errors of the mean are shown for the T cells transduced with the LG and LGC.sFvtat1-C_κ vectors.

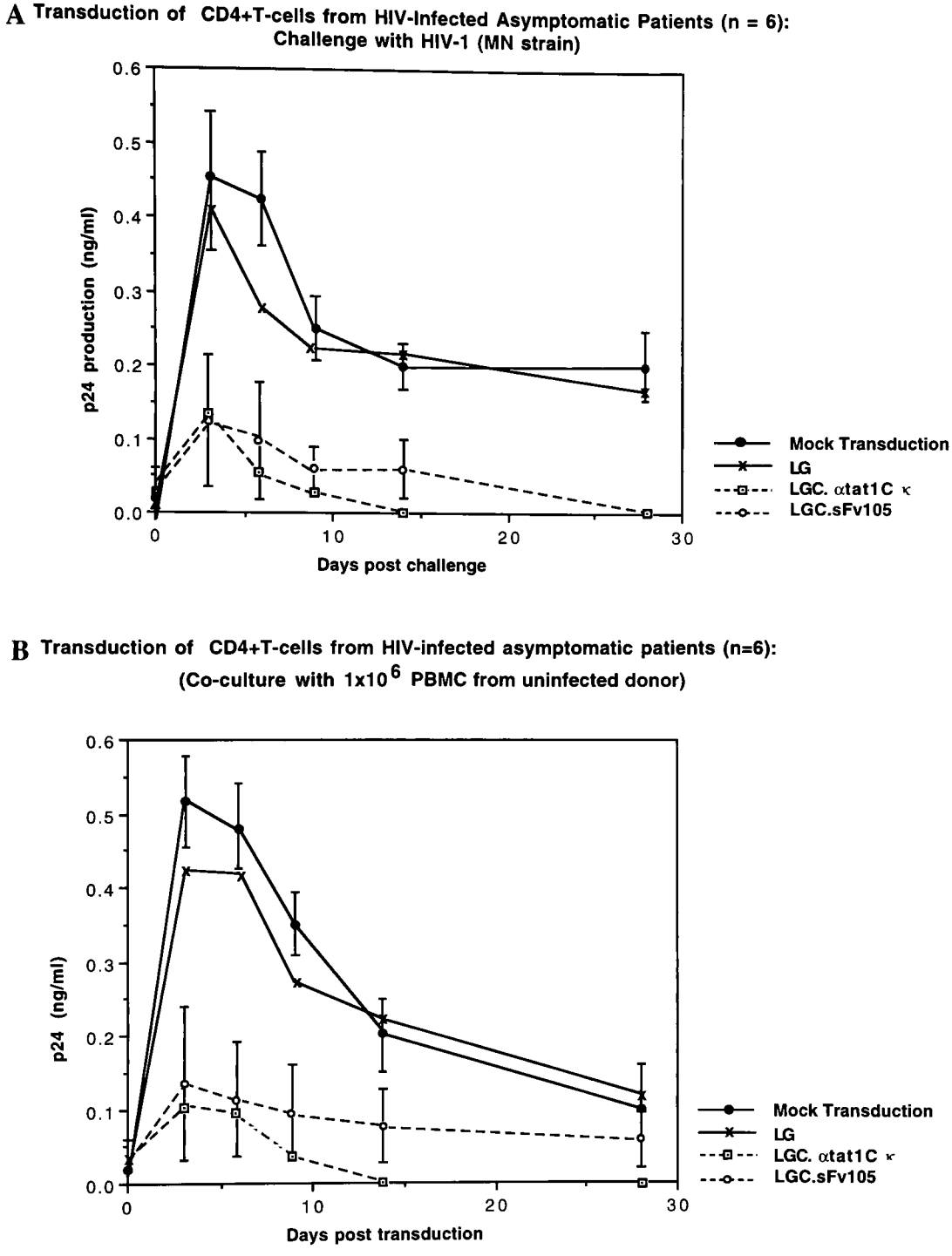

FIG. 5. HIV-1 replication in $CD4^+$ T cells transduced with genes encoding intracellular antibodies against gp120 and tat. $CD4^+$ T cells from patients with asymptomatic HIV disease were transduced with LG (—×—), LGC.sFv105 (—○—), and LGC.sFv-tat1-C_κ (—⊡—) vectors or underwent a mock transduction process involving the exposure of T cells to supernatants from GP+envAM12 cells (—●—). These cells were then either challenged with HIV_{MN} (PBMC adapted) at a moi of 1 (A) or co-cultured with heterologous activated PBMC from an uninfected donor (B). HIV-1 replication was then monitored in these cultures at regular intervals (day 3, 6, 9, 14, and 28 post transduction), using an assay for HIV-1 p24 antigen and measured in nanograms per milliliter of medium. Confidence limits of 95% are shown for the T cells transduced with the LGC.sFvtat1-C_κ vector or undergoing a mock transduction with exposure to supernatant from the AM12-based packaging cell line alone. Data presented results from experiments performed in triplicate for transduced $CD4^+$ T cells from each patient with asymptomatic HIV infection. Standard errors of the mean are shown for the T cells undergoing mock transduction and transduced with the LG and LGC.sFv105 vector.

Second, transduced lymphocytes from patients with asymptomatic HIV infection ($n = 6$) were challenged with HIV-1_{MN}, as described above. HIV replication in $CD4^+$ T cells of these patients was significantly reduced if cells were transduced with the LGC.sFv105 and LGC.sFvtat1-C_κ vectors (Fig. 5A). Inhibition of HIV replication was greater for LGC.sFvtat1-C_κ- than for LGC.sFv105-transduced cells. No inhibition of HIV replication was seen in $CD4^+$ T cells from any of the patient groups that had undergone a mock transduction with medium derived from GP+envAM12 cell cultures (containing no packaged vector) or following transduction with supernatants containing the packaged LG vector, encoding the marker gene for β-Gal, alone. HIV-1 p24 antigen production peaked in $CD4^+$ T cells transduced with the LG vector or under conditions of mock transduction at 0.41 ng/ml + 0.07 ng/ml (mean + SEM) and 0.45 ng/ml + 0.09 ng/ml, respectively, whereas cultures of T cells transduced with the vectors LGC.sFv105 and LGC.sFvtat1-C_κ gave significantly lower p24 peaks of 0.13 + 0.14 ng/ml, respectively ($p = 0.018$ and $p = 0.02$; Student's t-test comparing peak p24 production of cells transduced with the LG vector versus peak HIV-1 p24 production by cells transduced with the LGC.sFv105 and LGC.sFvtat1-C_κ vectors). HIV replication following challenge with HIV-1_{MN} in mock-transduced $CD4^+$ T cells from HIV-negative individuals and from asymptomatic HIV positive individuals did not differ significantly from each other (data not shown).

Finally, HIV replication in co-cultures of transduced $CD4^+$ T cells from HIV-infected patients with activated PBMC from uninfected donors was examined. HIV replication was significantly inhibited in co-cultures of $CD4^+$ T cells transduced with the LGC.sFv105 and LGC.sFvtat1-C_κ vectors with activated heterologous PBMC (Fig. 4B). No inhibition of HIV replication was seen in co-cultures of $CD4^+$ T cells and heterologous activated PBMC that had undergone a mock transduction or in co-cultures containing $CD4^+$ T cells from HIV-infected individuals that had been transduced with the LG vector. HIV-1 p24 antigen production peaked in $CD4^+$ T cells transduced with the LG vector or under conditions of mock transduction at 0.42 ng/ml + 0.09 ng/ml (mean + SEM) and 0.45 ng/ml + 0.1 ng/ml, respectively, whereas cultures of T cells transduced with the vectors LGC.sFv105 and LGC.sFvtat1-C_κ gave significantly lower p24 peaks of 0.14+ and 0.11 ng/ml, respectively ($p = 0.018$ and $p = 0.021$; Student's t-test comparing peak p24 production of cells transduced with the LG vector versus peak HIV-1 p24 production by cells transduced with the LGC.sFv105 and LGC.sFvtat1-C_κ vectors).

DISCUSSION

This study demonstrates that $CD4^+$ T cells from HIV-infected individuals are transducible using MLV-based retroviral vectors at efficiencies of up to 28% using an optimized transduction methodology. These levels of transduction efficiency were similar to both the transduction of uninfected controls and those previously reported in primary $CD4^+$ T cells from uninfected donors (Bunnell *et al.*, 1995; Leavitt *et al.*, 1996; Rudoll *et al.*, 1996). Transduced T cells from infected individuals were maintained in expanding cultures up to 40 days following transduction, without selection in medium containing rIL-2. A smaller proportion of T cells from patients with AIDS were transducible by the MLV-based vectors than those from patients with asymptomatic HIV infection. The transduction process and the expression of the encoded genes did not affect the overall viability of the cultures. HIV replication as a result of the expansion of an endogenous viral population or following challenge with exogenous virus was significantly reduced in those cultures of unselected T cells that contained cells transduced with the construct encoding the sFv105 and sFvtat1-C_κ intracellular antibodies.

The fact that HIV replication in both instances was not completely inhibited in these cultures may reflect the fact that only a minority of cells contained the gene encoding the intracellular antibody. Conversely, it was demonstrated that this minority, approximately 20%, of protected lymphocytes appeared to be capable of suppressing HIV replication in $CD4^+$ T cell cultures transduced with the LGC.sFv105 and LGC.sFvtat1-C_κ vectors. This was not thought to be due to the fact that the transduction efficiency determined by β-Gal staining underestimated the number of transduced cells actually in the culture. Quantitative DNA PCR for retroviral vector sequences was performed on cultures of transduced $CD4^+$ T cells (unpublished data) and it was found that up to 20% of transduced T cells contained integrated retroviral sequence. It is possible that the subgroup of cells that are permissive for MLV vector transduction may also represent a significant proportion of those $CD4^+$ T cells that are readily infectable by HIV. Therefore, $CD4^+$ T cells that are permissive for productive HIV infection are preferentially transduced by the vector encoding the protective anti-HIV intracellular antibody.

HIV replication in cultures containing cells transduced with sFvtat1-C_κ was more effectively inhibited than in cultures expressing sFv105. This may reflect both the pivotal function of tat in provirus activation as well as the variability of the gp120 amongst the viral isolates generated as a result of endogenous virus proliferation in $CD4^+$ T cells from an HIV-infected individual (Jeang *et al.*, 1991; Marasco *et al.*, 1993; Lisziewicz, 1995; Mhashilkar *et al.*, 1995).

Intracellular antibodies to both tat and gp120 have now been shown to be effective in inhibiting HIV replication in various *in vitro* systems and have been shown to be active against both laboratory and primary isolates of HIV (Marasco *et al.*, 1993; Chen *et al.*, 1994; Mhashilkar *et al.*, 1995). This study demonstrates the efficacy of intracellular antibodies in inhibiting HIV replication in T cells from HIV infected patients in a clinically relevant *in vitro* system. Furthermore, this study demonstrates that transduced cells expanded *in vitro* in the absence of selection continue to be capable of inhibiting HIV replication for up to 30 days. This relates to the *in vivo* setting in which transduced cells, expanded *in vitro*, would be reinfused and therefore mixed with untransduced cells (Yu *et al.*, 1994; Nabel *et al.*, 1994; Lisziewicz *et al.*, 1995; Woffendin *et al.*, 1996). It would be expected that transduced $CD4^+$ T cells would have a selective advantage, in that they would not be susceptible to HIV-induced cell killing. Data presented in this paper support this view in that $CD4^+$ T cells from patients with asymptomatic HIV infection that are transduced by vectors encoding the anti-HIV intracellular antibodies appear to have a selective advantage *in vitro* (see Fig. 3B and 4B). Endogenous HIV replication in these cultures of transduced cells was detectable at very

low levels (<80 pg/ml) but this was presumably adequate to provide the selective advantage for transduced CD4$^+$ T cells protected by the anti-HIV intracellular antibody.

The study may also point the way toward simplified gene therapy protocols in which T cells are prepared from the peripheral blood of HIV-infected patients, activated, and transduced *ex vivo* over 4–5 days and then reintroduced into the autologous donor. This would remove the complex *ex vivo* expansion step incorporated into all phase 1 studies of antiviral constructs to date, which require the use of antiviral drugs to be added into cultures to suppress HIV replication secondary to T cell activation and proliferation in the presence of IL-2 (Nabel *et al.*, 1994; Yu *et al.*, 1994; Lisziewicz *et al.*, 1995; Woffendin *et al.*, 1996). Endogenous HIV replication over this short period of time was barely recordable in T cells from HIV-infected patients in our culture system.

The presence of a subpopulation of transduced CD4$^+$ T cells bearing the intracellular antibody to gp120 and tat also inhibited the amplification of HIV replication in mixed cultures with heterologous activated PBMC. This co-culture system forms the basis for the technique used to measure cellular viremia and the technique was adopted for this study to assess whether the presence of transduced T cells would inhibit the spread of HIV from a cell population containing infected cells to activated uninfected PBMC. The fact that the presence of transduced T cells encoding sFv105 and sFvtat1-C_κ in the co-culture inhibited viral replication indicates that the transduction of a minority of cells can suppress viral replication in the co-culture as a whole.

The study has also demonstrated that T cells from patients with AIDS were less transducible, and that transduced cells from these patients survived for shorter periods of time *in vitro* than CD4$^+$ T cells from HIV-infected individuals who had not progressed to AIDS. This does not appear to relate to levels of viral replication following transduction because transduction efficiencies of CD4$^+$ T cells were not increased by the presence of AZT in the medium. This observation may result from the fact that the viral load at transduction in these patients with AIDS is higher than in HIV-positive patients who have not progressed to AIDS and that the consequent low transduction rates and reduced viability of cultures of these T cells relates to a higher levels of HIV-induced cell death in these cultures and which the expression of the anti-HIV intracellular antibody is unable to overcome (Ratner, 1989; Novak and McLean, 1990; Embretson *et al.*, 1993; Ho *et al.*, 1995). These data imply that the most successful strategy for an HIV gene therapy would target asymptomatic patients with HIV infection prior to an AIDS diagnosis, with CD4 counts in the range of 400–600. Like Lisziewicz *et al.*, we postulate that although treatment with a putative gene therapy would be best applied early in disease, an anti-HIV genetic construct may continue to have a lesser but still measurable effect on HIV replication in T cells from patients with late-stage AIDS (Lisziewicz *et al.*, 1995).

The aim of a gene therapy for HIV infection is to reconstitute the infected individual with a long-lasting population of CD4$^+$ T cells, protected from HIV infection and/or HIV replication. This is thought to be theoretically possible by introducing the antiviral construct into the CD34$^+$ progenitor cell that gives rise to CD4$^+$ T cells (Yu *et al.*, 1994). This study demonstrates that the introduction of a gene encoding an anti-HIV intracellular antibody into CD4$^+$ T cells from HIV-infected individuals gives them a selective growth advantage and can significantly reduce HIV replication in these transduced but unselected CD4$^+$ T cells *in vitro*.

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