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GENE THERAPY APPROACHES TO HIV-INFECTION : IMMUNOLOGICAL STRATEGIES : USE OF T BODIES AND UNIVERSAL RECEPTORS TO REDIRECT CYTOLYTIC T-CELLS

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1. ABSTRACT

Combined regimens of classical antiviral treatments have not, until now, lead to the eradication of HIV-1. A specific anti-HIV immune response may have to be boosted or transferred to patients after suppression of viral replication, in order to eradicate residual infected cells from their sanctuaries. Cytotoxic T cells engineered to express recombinant chimeric receptors can be redirected against HIV-infected cells and could represent the basis of a new type of immunotherapy. Several HIV epitopes have been targeted successfully *in vitro*. Two types of binding domains (antibody fragments, CD4) fused with various signal transducing units (zeta chain of the CD3 complex, Fc epsilon RI gamma chain) have been tested for their ability to redirect effector cells to HIV infected lymphocytes. CD4-zeta-expressing myeloid and natural killer cells conferred SCID mice protection against challenge with tumor cells expressing HIV-env.

Finally, the safety of the adoptive transfer of syngeneic CD4- zeta -modified T cells in HIV-infected individuals is currently under evaluation.

2. INTRODUCTION

The elaboration of strategies of adoptive cellular immunotherapy is based on the current interpretation of the immune mechanisms that are, or should be, responsible for controlling HIV-1 replication during acute and chronic infection.

Several studies have provided evidence supporting that HIV specific CD8+ CTLs would play a crucial role in controlling virus replication and preventing disease progression. The expansion of HIV-1 specific CTLs following primary infection correlates with a sharp decrease of viral replication (1) (2), which progressively rises again with the decline of HIV-specific CTL activity (3). Conversely, lack of progression has been associated with high frequencies of CTL precursors endowed with broad specificities (4). It was recently shown that higher frequencies of HIV-1-env-specific CTLs correlate with lower levels of plasma HIV-1 RNA and PBMC-associated infectious virus (5). HIV-1 specific CTLs were found in individuals with repeated exposure to the virus, and that did not become infected (6) (7). *In vitro* studies have confirmed that purified CD8+ cells have the ability to inhibit the cellular cycle of replication of HIV (8). Finally, during primary or chronic SIV infection, viral replication is not controlled in monkeys depleted of CD8+ T cells (9). It is therefore accepted that through the development of a vigorous CTL response earlier after infection, HIV-1 specific CTLs could be able to eradicate HIV-1 infection in certain transiently infected patients and would at least be able to contain HIV-1 spread in most other cases. However, in most cases, the host response ultimately fails to control HIV replication and disease progression. The progression of HIV infection despite the development of specific host CD8+ CTL responses to HIV, implies that cellular-based immunotherapeutic strategies should have to overcome significant obstacles in order to be effective.

To induce CTLs, vaccination with live attenuated viruses has been proposed and indeed appears to be effective in animal models (10) but such an approach presents too many risks to be widely proposed to healthy humans. Synthetic vaccines would probably be safer but should consist in a mixture of many different T cell epitopes in order to prevent viral escape. An appropriate epitope mix could be optimized for a given individual but will not be effective in other, unrelated, individuals because of HLA restriction.

Although vaccines have failed to elicit neutralizing antibody responses against field isolates of HIV-1 (11), appreciation of the complementary nature of T cell and antibody-based immunotherapy stimulated interest in developing new approaches that would combine the advantages of both arms of the immune response and minimize limitations of each kind of therapy.

3. ANTI-HIV T BODIES: RATIONALE

The T body approach uses a chimeric receptor made of an antibody variable region (Fv) as the extracellular, recognition domain spliced to the TCR constant domain or any T cell signalling receptor subunit as transmembrane and intracellular domains. Such chimeric receptors, when expressed in T cells, confer on them antibody specificity and redirect T cells to any predefined target in a non-MHC restricted manner (12). This approach has been proven successful for tumor therapy in model systems (13).

The expected advantages of that type of strategy in the fight against HIV are numerous. Firstly, the T bodies would have a relatively high avidity for HIV, yet, T bodies bearing high affinity receptors migth not necessarily represent the neutralizing ones, *in vivo*. While HIV-specific antibodies are always found in the serum of HIV patients, their neutralizing potential appears to be limited. There seems to be a good correlation between efficient neutralization and high affinity of interaction between the antibody and the epitope expressed on the virus itself or on the surface of infected cells (14). On the other hand, the nature of the epitope that is targeted appears to be less important. In comparison, T cells make use of receptors endowed with particular fast off-rates of dissociation from their cognate ligands (15). It was therefore proposed (16) that several non-neutralizing HIV-specific antibodies could be "recycled" as effective T cell receptors. Indeed, we have shown that an antibody that could not neutralize efficiently a particular HIV variant, could still be used to construct chimeric T-bodies that specifically recognize that variant (16).

Secondly, specific cytolytic activity is not MHC restricted, therefore, a wider range of HIV epitopes can be targeted on the surface of the infected cells. Furthermore, the recognition via the chimeric receptor will not be affected by HIV-1 induced down-regulation of HLA class I antigens (17).

Thirdly, cytotoxic cells engineered in that way could be more effective than the soluble antibody at controlling cell to cell spread of the virus, which probably represents the main mode of transmission in solid tissues such as the lymphoid organs. Lymphoid tissues have been demonstrated to contain large amounts of trapped virus particles and serve as a chief site for ongoing HIV replication in CD4+ T cells and macrophages (18). Soluble antibodies have a limited capacity of diffusion into solid tissues, and even when rendered bispecific, they remain bound to target and effector cells for only 10-80 hours (when they are proteolytically degraded) (19). In contrast chimeric receptors are expressed by transduced CTLs for the life time of host cells (20) and T bodies would be able to migrate inside the virus sanctuaries that are represented by solid organs. Moreover, they could be induced to secrete soluble antiviral cytokines at sites of viral replication.

Lastly, CTLs can be redirected against a variety of B cell epitopes (and, not necessarily neutralizing epitopes) in order to limit the possibilities of viral escape.

4. *IN VITRO* EXPERIMENTS

Antibody based targeting and cell mediated cytolysis have been combined by grafting effector cells with a chimeric receptor composed of an antigen-binding domain joined to transmembrane and signal transducing domains that initiate cellular activation after receptor cross-linking by antigen. The first chimeric antibody/receptor design consisted in the replacement the TCR V alpha and V beta regions by V_H and V_L antibody domains (21-25). Subsequently, a simplified design used the single chain Fv (scFv) of an antibody as the extracellular recognition unit of the chimeric receptor (26). The basis for this design results from the observation that fusion proteins utilizing signaling chains carrying a motif for tyrosine kinase activation mediate T-cell receptor signal transduction (27) (28) (29) (30) (31). Both the Fc epsilon RI gamma chain (26) (32) and CD3 zeta chains (26) (33) (32) (20) have been successfully used as signal transducing units.

The only HIV products expressed at the surface of infected cells are MHC-bound peptides and the envelope protein of HIV-1 (gp120-gp41 complex) (34-36). Although antibodies that bind particular MHC-peptides complexes have been described (37), there are no antibodies available that would interact with complexes of MHC and HIV-derived peptides. Hence, T-bodies can only be redirected against the native envelope protein of HIV-1, no other native HIV proteins are displayed on intact cells. It should be noted that only productively infected cells express the HIV envelope, while latently infected cells do not express detectable amounts of it. Furthermore, HIV gp120-gp41 complex is only expressed during late stages of the HIV replication cycle (38, 39), in cells that start to actively produce virus. Strategies that would nevertheless enable the targeting of latently infected cells are discussed below.

Various HIV-binding domains have been used to construct chimeric T cell receptors. In an attempt to circumvent escape mechanisms based on the numerous mutations occurring during viral replication, CD4 (the high affinity co-receptor for gp120) was first chosen as extracellular domain for the design of a first generation of anti-HIV cTCR called 'universal receptor' (UR). Such chimeric receptors would interact with gp120 expressed on infected cells irrespectively of the viral strain. Romeo and Seed described chimeras composed of CD4 extracellular domains (aa 1-369) fused to the transmembrane and intracellular domains of TCR/CD3 (zeta) or IgGFc receptor-associated gamma chain signal transducing elements. They showed that those chimeras are capable of directing CTLs to recognize and kill cells expressing gp120 (30).

Roberts and al. have reported the construction of two types of such universal receptors with extracellular domains interacting with the envelope glycoprotein (env) of HIV-1. The env-specific moiety of those chimeric receptors was represented by, as previously, the extracellulars domains of CD4 or by antibody fragments (derived from the anti-gp41 98.6 MoAb (40) (41) or from the gp120-specific human MoAb 447-D (42)). Single chain variable domains of antibodies (ScFv) are generated by joining the V_H and V_L regions of a monoclonal Ab (mAb) via a flexible linker (43). Both types of cTCR are able to

initiate an efficient effector T-cell response against HIV-infected cells, including cytokine secretion, proliferation and cytolytic activity upon interaction with target cells expressing surface viral antigen (44). It was also demonstrated that such UR bearing CD8+ T cells can inhibit HIV-1 replication *in vitro* (45).

One concern is that CD4-zeta receptors might possibly put the UR-expressing cells at risk of being infected themselves. Indeed, T cells engineered to express high surface levels of CD4 could potentially represent *in vivo* a new cellular reservoir of virus.

Our group therefore focused on ScFvs to construct HIV-specific receptors. Very few human monoclonal antibodies endowed with broad neutralizing properties have been so far described (46) (47) (48). We have chosen to target gp120 because it is expressed on the surface of HIV infected cells. In addition, the epitope recognized by b12 mAb (46) presumably projects more from the surface of the infected cell than the gp41 epitopes, and could therefore be better presented to effector T cells (16).

5. ANIMAL MODELS

Hege *et al.* have described the generation of T cell-independent systemic immunity in SCID mice reconstituted with CD4-zeta -expressing myeloid and NK cells following bone marrow transplantation (50). It is the first study demonstrating the efficacy of such an hematopoietic stem cells-based immunotherapy approach *in vivo*. Gene modification of hematopoietic stem cells (HSC) may be preferable to modification of terminally differentiated effector cells, such as T or NK cells. Indeed, multiple effector cells can be simultaneously redirected using a stem cell approach (by passing the requirement to isolate and express mature effector cells, which may negatively impact on their *in vivo* trafficking or function. Such an approach may also provide long lasting memory cells and, thereby, could serve as a renewable source of gene modified effector cells, allowing a prolonged antigen-specific immune surveillance.

6. CLINICAL USE

The safety and efficacy of adoptive T-cell therapy in humans have been established in the prophylaxis against viral diseases caused by CMV (52) and EBV (53) as well as in the treatment of hematological malignancies and melanoma (54).

Extrapolation of the data obtained from animal models in which tumor or viral infections can be eradicated by adoptive transfer of antigen specific T-cells, together with the findings from clinical studies using adoptive transfer into human subjects, suggest that patients may have to receive cell dosages on excess of 10^9 antigen-specific lymphocytes to obtain a therapeutic anti-viral effect. Cells grafted with a chimeric receptor are particularly suited to evaluation in a clinical trial, and can be used to obtain large populations of antigen specific T cell within a few weeks, in contrast to the long time required for selection, characterization and expansion of CTLs with native specificity for target antigen.

The *in vivo* life span of gene modified T cells was studied in HIV-discordant syngeneic twin pairs in which peripheral blood lymphocytes from the healthy twin were retrovirally transduced with the *neo* gene (55). This marker gene was detected by PCR in both blood and lymph nodes for at least 25 weeks post infusion demonstrating the possibility of adoptive T-cell therapy in this disease .

A study of the adoptive transfer of syngeneic gene modified $CD8^+$ lymphocytes in HIV-infected identical twins was started in 1995 (56). The goal of this protocol is to redirect cytotoxic T cells against HIV infected target cells, using the CD4-zeta chimeras described above (table 1). This study was designed to determine the safety and activity of healthy UR expressing T cells after transfer to HIV-1-infected individuals. In an initial phase I/II study, PBMC obtained from HIV-1-seronegative donor twins were enriched for CD8+ expression, activated with IL-2 and anti-CD3, and transduced with a murine retrovirus containing the CD4-zeta gene. Following dose escalation, 30 HIV-1-infected twins received up to 6 infusions over 1 year of either 10^{10} CD4- zeta transduced or control CD8+ T cells (57). In a second study, designed to test the effects of providing HIV-1-specific CD4+ T cells help, 17 twins subsequently received 3 additional infusions of 10^{10} CD4-zeta-modified CD4+ and CD8+ T cells, at 2 weeks intervals (58). In this latter study, preactivated modified T cells were detected in 21/21 recipients of gene-modified $CD8^+$ T cells alone, with peak levels of 10^4 copies/10^6 PBMC in 16 patients. Nevertheless, rapid clearance of modified cells was seen in 9 recipients. In contrast, all 17 recipients of gene-modified CD4+ and CD8+ T cells showed prolonged, high level persistence of gene-marked cells. Fractions of circulating gene-marked CD4+ and CD8+ T cells ranged from 10^3 to $>10^4$ copies/10^6 PBMC, increased with time in some patients, and persisted for 100 days. No treatment-limiting side effects related to the cells were observed. Therefore, adoptive transfer of genetically engineered, HIV-1-specific T cells appears to be safe. Compared to gene-modified CD8+ T cells alone, CD4+ and CD8+ T cells given together resulted in increased cell survival in the circulation, and provided preliminary evidence of *in vivo* proliferation of the engineered cells. Moreover this study demonstrated tissue trafficking by finding CD4-zeta T cells in rectal-mucosa-associated lymphoid tissue in 2 patients.

7. PROBLEMS IN SOLUTION

The therapeutic efficiency of UR-T cells might benefit from the elucidation of certain mechanisms of HIV-1 immunopathogenesis. Mechanisms that can result in a decrease of natural HIV-1 specific CTL activity such as loss of virus specific help, viral escape, or clonal

Table 1. Summary of engineering of anti-HIV effector cells

Extracellular domain	Expression vector	Effector cells	Target	Read out	Ref.
4 extracellular Ig-like domains of human CD4 (1-369)	recombinant vaccinia virus	Cytotoxic human T cell line WH3	Hela cells expressing HIV-env	Specific lysis	(29)
4 extracellular Ig-like domains of human CD4 (1-372)	retroviral vector (kat-system)	Human CD8+ CTLs Human NK cells	Human 293 or CEM cell lines expressing env (IIIB) HIV-IIIB Infected CD4+ T cells JR-CSF infected monocytes HIV-1 IIIB infected CEM, Raji-env	Specific lysis Inhibition of HIV replication Specific lysis	(44) (49) (32)
ScFv from the gp41-specific human MoAb 98.6. (40) (41)	retroviral vector (kat-system)	Human PBMC	HIV-1 IIIB infected CEM Env-expressing human cell line 293.	Specific lysis	(44)
4 Ig-like domains of the CD4 receptor (1-372)	retroviral vector (kat-system)	Murine Bone Marrow progenitor cells	Raji-cells expressing HIV-env (HXB2)	Protection of SCID mice from Raji-env challenge	(50)
ScFv from the gp120-specific human 447-D (42)	retroviral vector (kat-system)	Murine Bone Marrow progenitor cells	Murine Bone Marrow progenitor cells	Lack of activity due to low expression level.	(50)
ScFv from the gp120 specific human antibody IgG1b12(46)	pRSV-neo plasmid	Cytotoxic murine hybridoma MD45 (51)	BHK cells expressing primary strains-derived env.	Gp120 induced IL-2 secretion	(16)

exhaustion, may also limit the *in vivo* use of engineered T cells.

The CD4+ T helper deficiency itself could represent an important limiting factor in these hosts (59). A correlation between the decline of CD4 T cells and loss of HIV-specific CD8 T cell responses during the progression of AIDS has been reported (60). The maintenance of a CD4+ T helper-cell response is concomitant with vigorous CTL response in long term non progressors (60). Strategies aimed at providind help to the transferred CD8+ CTLs were established, like concomitant infusion of IL-2 or T helper cells, genetically modified to resist to HIV (61), genetic modification of the CD8+ CTL with chimeric receptors containing cytoplasmic domains of the interleukin 2 receptor (62) to be able to function in a CD4 deficient environment.

Indeed, signaling through the chimeric receptor, without appropriate signals of coactivation, could induce an *in vivo* state of anergy of the transduced cells. Therefore, these cells would have to be preactivated or costimulated in order to fully respond to chimeric receptor engagement. It was recently proposed by Finney and al. to bypass that requirement for a cosignal by improving the design of chimeric receptors in such way that they could deliver both primary and costimulatory signals. They showed that the intracellular costimulatory signaling domain of CD28 can be fused with the zeta chain from the TCR/CD3 complex (63). In the same way the generation of double transfectants simultaneously expressing scFv-CD28 and scFv-zeta chimeras demonstrates that antigen-specific co-stimulatory signals can also synergize with signals mediated through chimeric zeta chains to secrete maximal levels of interleukin-2 (64).

In vitro selection of transduced T cells requires coexpression of a resistance gene that could elicit an immune response *in vivo*. Although transfer of autologous CD8+ HIV-specific T cell clones modified to express the hygromycin phosphotransferase gene (HyTK) is limited by the induction of a potent HyTK specific CTL response (65). It should be noted that similar responses were not reported after the infusion of specific CTLs expressing the *neo* gene (66-72). It can also be argued that transduced T cells are likely to change their *in vivo* homing properties following the *in vitro* activation required in order to get the cells cycling before retroviral transduction, or during propagation in tissue culture. Nevertheless, it could be shown that under such circumstances, at least a fraction of the transferred HIV-specific cells could migrate to lymph nodes (73).

If, as suggested above, HIV-specific CTLs are able to persist and remain functional *in vivo*, they could possibly mediate deleterious effects (74). In adult mice infected with LCMV (75) (76), LCMV-induced neurologic disease is directly mediated by virus-specific CTL (77). It might therefore be necessary to have an ability to control the fate, and/or function, of the cytotoxic $CD8^+$ T cells, once transferred *in vivo*. Different strategies have been developed in order to regulate transgene expression in eukaryotic cells (78). Tetracycline (Tet)-regulatable system

(TRS) seems to be particularly well suited due to the relatively low concentrations of tetracycline necessary to regulate transgene expression (79, 80). Tet-suppressible expression of a cTCR in T cells has been already demonstrated *in vitro* (81).

T bodies could also become anergized *in vivo*. The chimeric receptor colud be blocked through direct interaction with soluble gp160. As previously described, cTCR expressing T cells can be inhibited in several models by soluble antigen (24) and it is known that soluble gp160 can be found in the serum of HIV infected patients (82) (83). Roberts et al. have shown that serum from HIV

infected donors does not contain sufficient levels of antibodies or free antigen to inhibit cytolytic activity of chimeric receptor expressing T cells (44). This issue was also directly addressed in our laboratory where T cells with b12 mAb specificity were tested in the presence of soluble gp160. We found that 50 microgram/ml are required to efficiently prevent the activation of gp160-specific T bodies, while seric concentration of gp160 never exceeds 90 ng/ml in HIV patients (83). In patients with low levels of viremia, the concentration of soluble gp160 is well below this level, and it is therefore unlikely that soluble antigen could effectively modulate the *in vivo* activity of anti-HIV speciific T bodies.

Finally, the ability of the transduced T cells to differentiate into memory cells remains to be shown.

8. FUTURE STRATEGIES

The effector function of the genetically engineered T bodies should not exclusively rely on their cytotoxic activity. The chimeric receptor expressing cells can be induced to secrete endogenous cytokines at the target site or serve as a platform to carry and release such transgenic cytokines at the desired site. Rosenberg and al. modified tumor infiltrating lymphocytes (TIL) with the gene coding for tumor necrosis factor (TNF) in an attempt to deliver high concentrations of this tumor suppressive cytokine to the tumor site without dose limiting systemic toxicity (66). A T body approach has also been used to target lymphocytes to tumors and deliver a toxin locally (84). A similar strategy could be used to deliver anti-HIV cytokines. CTL expressing chimeric receptors would migrate through circulation to infected tissues and secrete interleukins upon activation. In terms of soluble factors, a possible candidate should be represented by beta interferon that has been shown to mediate high potent viral effect in animal models (85, 86).

New combination drug regimens seem to be increasingly effective (87) but no viral eradication has been so far obtained (88). The tight control of HIV replication that is observed in successfully treated patients is nevertheless correlated with a drastic reduction of the frequency of anti-HIV CTLs (89). Specific anti-HIV immune response may be therefore have to be boosted, or passively transferred in order to prevent the relapse of the disease after interruption of the antiviral treatment. T bodies could also be used as vehicles in order to deliver inhibitory soluble factors (88). It was recently proposed (90) that latently infected cells could be compelled to express HIV antigens by injecting patients with stimulators like anti-CD3 antibodies (for T cells) or GM-CSF (for macrophages). Upon activation and proliferation, such cells would become unmasked and cleared from the system by effector-killer cells.

Transduced T cells may be already used in a syngeneic setting, but their non-MHC restricted specificity may authorize their use in unrelated individuals, provided that the allogeneic response of the host would be inhibited. Such conditions could be met by immunocompromised patients.

Future application of this strategy may also involve the use of " Universal Donors " providing T cells with altered immunogenic properties leading to increased tolerance in MHC-mismatched recipients (91). A modified T cell that would combine the features of MHC-unrestricted specificity with the ability to be transplanted across MHC barriers, may provide a novel approach to the treatment of both viral and malignant diseases in genetically different individuals.

Finally, the strategy of gene transfer into hematopoietic stem cells or mature effector cells should be improved as it probably represents the main factor limiting the possibilities of success of gene therapy. Very efficient lentiviral systems of gene tranduction have been recently described (92-95). The potential problem for a wide application of HIV vectors to human studies is obviously safety, yet, there might be less restrictions in their use for gene therapy of HIV infected individuals.

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Abbreviations: b12,Human Monoclonal Antibody IgG1b12, cTCR, Chimeric T cell receptor, env, Envelope, ScFv, Single chain Fragment variable

Key words: HIV, Recombinant TCR, Single chain fragment variable

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