

Developing Multi-HIV Antigen Specific T Cells as a Component of a Cure Strategy

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Although antiretroviral therapy (ART) can suppress HIV in patients, it cannot cure infection, and patients require lifelong therapy. However, ART is unable to target persistent latent reservoirs, which are a major obstacle for an HIV cure. Some promising strategies involve the use of epigenetic modifying drugs and other latency reversing agents (LRAs) which reactivate HIV out of latency, but *in vivo* depletion has not been seen because of inefficient clearance of exposed target cells by impaired endogenous immunity (1).

One strategy to restore T cell function to facilitate killing involves the *ex vivo* expansion of antigen specific T cells from immune suppressed patients with subsequent adoptive T cell transfer. Similar successful strategies have been employed in the setting of Epstein Barr viral infection, for example (2). This approach has so far been limited in HIV because previous studies have generated T cells targeting only single CD8 restricted epitopes (3). Here we describe a novel strategy to expand polyclonal T cells recognizing multiple HIV antigens using overlapping peptide libraries spanning gag, pol, and nef. This strategy will be potentially beneficial for an HIV cure strategy combining agents that induce reactivation of latency with adoptive T cell transfer using HIV-specific T cells.

Introduction

Antiretroviral therapy (ART) has been effective at suppressing HIV replication, but its inability to target the latent reservoirs means that the infection cannot be cured using this approach. Cessation of ART immediately leads to viral rebound as a consequence of viral reactivation. Therefore, the persistent viral reservoir requires lifelong therapy with the ART drug cocktail which is associated with appreciable side effects and expense (4,5). A cure strategy that would enable targeting of this latent reservoir would greatly improve the lives of millions of people living with HIV/AIDS.

The latent reservoir is comprised of resting CD4+ T cells (and most probably other cells). The absence of viral replication means that these infected cells do not express HIV antigens and are therefore not killed by immune effectors.

Recent efforts to "coax" HIV out of their latent state have focused on reactivating the resting cells, the hypothesis being that upon reactivation, viral cytopathic effects or immune clearance would then clear the reservoir (6). To this end, latency reversing agents such as epigenetic modifying drugs have been developed. While they successfully re-induce HIV expression (7,8), subsequent clinical trials have so far failed to show clinical efficacy (9).

Studies suggest that, following treatment with latency reversing agents, viral cytopathic effects are insufficient to kill off reactivated cells (9,10), and the generalized immune suppression in HIV patients limit immune clearance (9,11).

To overcome dysfunctional endogenous immunity, T cells can be expanded and stimulated *ex vivo* and subsequently infused into HIV patients following reactivation. Preclinical experiments suggest this is a viable approach (12). However, durable control still seems lacking in the absence of ART (3).

The lack of efficacy may stem from the variable nature of the virus. Therefore, using single epitope specific T cells may lead to immune escape (3). This contrasts with the use of polyclonal virus specific CTLs used in trials against infections (or disease) caused by EBV, CMV, and adenovirus (2,13-16) in immunocompromised settings. Hence, we proposed that developing an HIV specific T cell product with broader antigen recognition would increase the ability of the T-cells to target HIV-infected cells without immune escape.

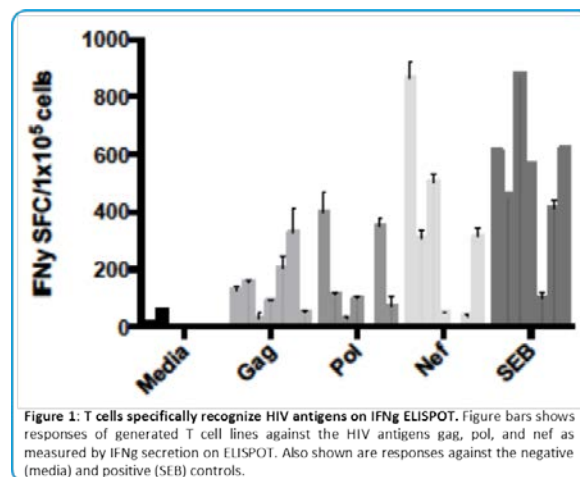
Materials & Methods

T-cells and antigen presenting cells were isolated from HIV patients on ART who were treated at the University of North

Carolina-Chapel Hill under IRB approved protocols and with informed consent. **PepMix™ ULTRA Peptide Pools** spanning HIV-gag, HIV-pol and HIV-nef (PepMix™ ULTRA Peptide Pools are composed of peptides selected by a proprietary algorithm to provide broad coverage across all HIV clades; JPT, Berlin) were used as antigen to stimulate T cells. These peptide mixes were presented by autologous monocyte derived dendritic cells isolated by plastic adherence as previously described (16). T cells were initially stimulated by dendritic cells in the presence of IL7, IL12, and IL15, and subsequently stimulated with autologous peptide-pulsed PHA blasts in the presence of IL15 or IL2, and then with PHA blasts and irradiated K562 cells modified to express CD80, CD83, CD86, and 41BBL as described (17). After expansion in culture for approximately 23-26 days, the *ex vivo* expanded HIV-specific T cells (HXTCs) were phenotyped via flow cytometry and their specificity determined via IFN γ ELISPOT.

Results

We have previously published these results in Molecular Therapy (18), and have used this preclinical data to support an ongoing phase I clinical trial where we are evaluating the safety and efficacy of *ex vivo* expanded HIV1-antigen specific T cells in patients with viral suppression on ART (NCT02208167).



To evaluate whether we can expand T cells from HIV+ patients on ART to the numbers required clinically, we stimulated peripheral blood mononuclear cells with the overlapping peptide mix spanning the gag, pol, and nef proteins in the presence of anti-retrovirals (to prevent outgrowth of HIV infected cells) and observed a mean expansion of 145.6 fold [range 37.2-287.0] starting from 1 million T cells on day 0.

To determine the composition of the HXTC products, we phenotyped expanded HXTCs using flow cytometry and showed that while they were predominantly CD3+CD8+ T cells (mean 84.2%, range 65.97-97.14%), an appreciable proportion of antigen specific CD4+ T cells (mean=16.9% [2.9-34.0x10⁶]) were present. While the majority of the expanded T cells had an effector memory phenotype (CD3+CD45RA-CD62L-) (mean=74.0% [48.8-93.3x10⁶]). T cells with an effector memory phenotype showed functional effects in ART-independent viral control (19). Experience with T cell immunotherapeutics has demonstrated the beneficial effects of developing a product that has a mixed population of cytolytic antigen-specific CD4+ and CD8+ T cells in the culture as well as T cells with a central memory phenotype (18).

To confirm the specificity of the ex vivo expanded HIV-specific T cells (HXTCs), the T cells were tested for IFN γ secretion in ELISPOT assays after culturing with gag, pol, and nef peptides. HXTCs secreted IFN γ in response to HIV antigens gag (median=123 SFC/1x10⁵ cells [range 32-327.5]), pol (median=97.5 SFC [range 0.5-347.5]), and nef (median=49.5 SFC [range 0-863.5]) but did not respond to negative control PepMixes™ (median=3.5 SFC [0-12.5]) (Figure 1). Additionally, we observed that expanded products stained for HLA tetramers – showing epitope specificity of the HXTC product.

Discussion & Conclusions

The generated multi-HIV antigen T cell products (HXTCs) were obtained from HIV+ patients on ART that were not pre-selected based on HLA type or disease burden. Therefore, regardless of the patient characteristics, we were able to expand T cells from HIV+ individuals, suggesting that the protocol described is highly robust and would be broadly applicable for the clinical setting. HXTCs could therefore be expanded to the numbers required for clinical use from any HIV+ subject irrespective of their viral status or HLA type. Hence, HXTCs could be used as a component of a treatment strategy to achieve a functional cure by providing long term cure following eradication strategies that focus on reactivating the latent reservoir.

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