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Functionally Active HIV-Specific T Cells that Target Gag and Nef Can Be Expanded from Virus-Naïve Donors and Target a Range of Viral Epitopes: Implications for a Cure Strategy after Allogeneic Hematopoietic Stem Cell Transplantation

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Allogeneic hematopoietic stem cell transplantation (HSCT) can potentially cure human immunodeficiency virus (HIV) by eliminating infected recipient cells, particularly in the context of technologies that may confer HIV resistance to these stem cells. But, to date, the Berlin patient remains the only case of HIV cure despite multiple attempts to eradicate infection with HSCT. One approach to improve this is to administer virus-specific T cells, a strategy that has proven success in preventing other infections after transplantation. Although we have reported that broadly HIV–specific T cells can be expanded from HIV+ patients, allogeneic transplantations only contain virus-naïve T cells. Modifying this approach for the allogeneic setting requires a robust, reproducible platform that can expand HIV-specific cells from the naïve pool. Hence, we hypothesized that HIV-specific T cells could be primed ex vivo from seronegative individuals to effectively target HIV. Here, we show that ex vivo–primed and expanded HIV-specific T cells released IFNγ in response to HIV antigens and that these cells have enhanced ability to suppress replication in vitro. This is the first demonstration of ex vivo priming and expansion of functional, multi-HIV antigen–specific T cells from HIV-negative donors, which has implications for use of allogeneic HSCT as a functional HIV cure.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only approach that has resulted in a functional cure and long-term independence from antiretroviral (ARV) drugs in a human immunodeficiency virus (HIV)–positive patient, reported in the 2009 case of the Berlin patient [1]. The exact mechanisms contributing to this cure are not completely understood, but it is believed that the homozygous polymorphism conferring HIV resistance in donor cells, the preparative regimen, and the graft-versus-host response may have played important roles [2]. Replicating successful use of allogeneic HSCT as a functional cure has so far proven difficult. Homozygotes for the polymorphism are seen most frequently only in Europe, with a frequency of approximately 1% [3]; identifying such donors among those who are HLA matched to a particular patient will, therefore, be a daunting task. The chemotherapy regimen, believed to kill latently infected cells, cannot account for elimination of the reservoir, as no differences in HIV DNA and RNA were observed in HIV+ patients with lymphoma before and after chemotherapy [4]. The graft-versus-host effect in allogeneic HSCT can theoretically eliminate HIV reservoirs similar to graft-versus-leukemia [2], but concomitant graft-versus-host disease (GVHD) and the viral rebound that results upon ARV treatment interruption [1,5] are major barriers. In at least some instances, this rebound was quite symptomatic and patients became critically ill.

HIV rebound after transplantation may be analogous to the reactivation of cytomegalovirus (CMV) and Epstein-Barr

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virus (EBV) after allogeneic transplantation with a seronegative donor; therefore, success in these settings may also apply to HIV. Ex vivo–expanded, donor-derived, multivirus-specific T cells have repeatedly shown efficacy in preventing or treating infection after transplantation [6-8], and such cells may provide the best source of graft-versus-virus responses that can eradicate the HIV reservoir [2]. Challenges involved with the manufacture of HIV-specific T cells from a naïve donor, however, remained a critical limitation. To date, no HIV-specific T cells have been generated from non–HIV-infected individuals. Our previous success generating CMV-specific T cells from virus-naïve cord blood [9] and adult donors [10] demonstrated that priming can be effectively performed ex vivo and can be used as source of cells for immunotherapeutic applications. Our objective was to develop a strategy to expand HIV-specific T cells ex vivo from eligible seronegative HSCT donors (dHXTCs) to prevent viral relapse after transplantation. We hypothesized that multi-HIV antigen–specific T cells could be derived from HIV-negative donors using a good manufacturing practice–compliant methodology and would recognize multiple viral epitopes and effectively suppress viral replication in vitro.

METHODS

Overview

T cells and antigen-presenting cells were isolated from HIV-seronegative adult donors. Pepmixes spanning HIV-gag and HIV-nef were used as antigen to stimulate T cells, which were expanded in culture for approximately 23 to 26 days. Expansion was calculated from cell counts of viable cells, phenotype was determined using flow cytometry, specificity was demonstrated by IFNγ enzyme-linked immunospot (ELISPOT), and function was evaluated in viral inhibition experiments.

Isolation of Peripheral Blood Mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated from discarded lymphocyte filters (Children's National Medical Center Blood Bank) and buffy coats (National Institutes of Health Department of Transfusion Medicine). Use of coded blood samples was approved by the institutional review boards at Baylor College of Medicine and Children's National Medical Center.

Dendritic cells (DCs) isolated from plastic adherence of PBMCs were matured with IL-4 (1000 U/mL), granulocyte macrophage–colony stimulating factor (GM-CSF) (800 U/mL), IL-6 (100 ng/mL), TNF-α (10 ng/mL), IL-1β (10 ng/mL; all R&D), and Prostaglandin E2 (1 μg/mL; Sigma-Aldrich) [9,11] and were harvested after 24 to 48 hours of maturation. To generate phytohemagglutinin (PHA) blasts, PBMC were stimulated with PHA-P (5 μg/mL; Sigma-Aldrich) in the presence of IL-2.

Generation of Naïve-derived HIV-specific Cytotoxic T Cell Lines

Matured DCs were pulsed with gag and nef pepmixes (.2 μg/mL) (JPT, Berlin). Peptide compositions of pepmixes were selected by a proprietary algorithm to provide broad coverage across all HIV clades. For the initial stimulation/priming, IL-7 (10 ng/mL), IL-12 (10 ng/mL), and IL-15 (5 ng/mL) (all R&D Systems) were added. T cells were restimulated after 10 days with pepmix-pulsed autologous irradiated (30 Gy) PHA blasts at a stimulator-to-responder ratio of 1:4 and maintained with IL-15 (5 ng/mL) or IL-2 (50 U/mL) and then, 7 days later, restimulated with irradiated pepmix-pulsed PHA blasts and costimulatory K562 cells at a ratio of 1:1:4 (T cells:PHA blasts:K562) and maintained with IL-2. Each HIV-seronegative donor-derived T cell line depicted in this paper was labeled from 1 to 8.

Generation of Antigen-presenting Cells

DCs isolated after plastic adherence of PBMCs were matured with IL-4 (1000 U/mL), GM-CSF (800 U/mL), IL-6 (100 ng/mL), TNF-α (10 ng/mL), IL-1β (10 ng/mL; all R&D), and PGE2 (1 μg/mL) [9,11] and were harvested 24 to 48 hours later. To generate PHA blasts, autologous PBMC were stimulated with PHA-P (5 μg/mL; Sigma-Aldrich) in the presence of IL-2.

Phenotyping

T cells were phenotyped with anti-CD3, CD4, CD8, CD45RA, CD62L, CD56, and CD16. Control samples were labeled with appropriate isotype-matched antibodies. Data were analyzed using FCS Express Flow Cytometry Software (De Novo Software, Glendale, CA).

Epitope Mapping: IFNγ ELISpot

Antigen specificity was determined 7 days after third stimulation using IFN-γ ELISPOT assay. No peptides or irrelevant peptides (Oct4 or actin) were used as negative control, and Staphylococcus enterotoxin B or PHA was used as positive control. T cells were plated at 1×10^5/well. Positive responses are defined as having greater than double the spot forming cells (SFC) obtained in the negative control but at least 50 SFC/1×10^5 cells. For epitope mapping, the stimulants were 15-mer peptides overlapping by 11 amino acids spanning the consensus region of the gag and nef antigens pooled according to the matrices shown in Supplementary Figure 1 A,B. Using the matrices, samples that had enough T cells were tested against flanking peptides to determine the epitope.

HLA Epitope Specificity: IFNγ ELISPOT

Based on epitope mapping ELISPOTs, ex vivo–expanded HIV-specific T cells were tested for HLA specificity to gag, nef, or individual peptides. For HLA blocking, 1×10^5 cells/well were treated with monoclonal mouse antihuman HLA class I or HLA class II antibody in a 96-well round bottom plate for 1 hour at 37°C. Treated cells were transferred to ELISPOT plate, stimulated with peptide, and developed, as previously described.

Polyfunctionality

Polyfunctionality of ex vivo–expanded HIV-specific T cells was determined using Bio-plex Pro Human Cytokine 8-Plex Assay (Biorad, Hercules, CA) and Human Perforin ELISA kit (Abcam, Cambridge, MA). Cells were stimulated with nothing, actin, gag/nef, or PHA overnight in the presence of CD49d/CD28 antibody cocktail (1 μL/mL). Supernatants were collected, spun to remove cell debris, and tested for the presence of IFNγ, TNFα, IL-2, IL-4, IL-6, IL-8, IL-10, and GM-CSF by Bio-plex (Biorad) and perforin by ELISA.

HLA Typing

When samples were available from donor blood used for HIV-specific T cell manufacture, HLA typing was performed (Kashi Clinical Laboratories, Oregon) (Table 1).

Viral Inhibition Assay

CD8-depleted PBMCs were activated in IL-2 (50 U/mL) and PHA (2 μg/mL) before being infected with HIV laboratory strain SF162. Infected target cells were cocultured for 5 days with expanded HIV-specific T cells or unexpanded CD8 T cells that were isolated using magnetic beads added at 1:2 effector to target ratio (Figure 1D) and 20:1 effector to target ratio (Figure 1E). The following conditions were used as controls: uninfected CD8-depleted PBMCs, infected CD8-depleted PBMCs alone, ARV, ARVs plus expanded HIV-specific T cells, and expanded CMV- and EBV-specific T cells. Day 5 supernatants were measured for HIV-1 gag p24 concentration by ELISA.

Statistical Analysis

Data are summarized as mean ± SD or mean (range), unless noted otherwise in the text or figure legends. Two-way ANOVA with multiple comparisons (Dunnet's) were used to determine statistically significant difference in ELISPOT assays, testing between responses to antigen, with 2-tailed *P* values less than .05 indicating significant difference. Multiple *t* tests correcting for multiple comparisons using the Holm-Sidak method were used to determine statistically significant differences between groups as evaluated in ELISA, viral suppression, perforin release, and cytokine secretion assays, using 2-tailed *P* values with less than .05 indicating significant difference.

RESULTS AND DISCUSSION

The immune system of an HIV-seronegative donor has never encountered HIV antigen. Therefore, we sought to determine whether it was possible to prime HIV-specific T cells ex vivo using DC pulsed with HIV pepmixes representing HIV-gag and nef. We chose to target gag and nef to increase T cell specificity towards both late and early antigens, respectively.

T cells expanded to clinically relevant numbers in response to HIV pepmixes (mean, 75.705-fold expansion; range, 17.76 to 272.79; n = 8) (Figure 1A). The majority of the expanded cells were $CD8^+$ T cells expressing the effector