Cytotherapy, 2012; 14: 7–11

informa
healthcare

Production of good manufacturing practice-grade cytotoxic T lymphocytes specific for Epstein–Barr virus, cytomegalovirus and adenovirus to prevent or treat viral infections post-allogeneic hematopoietic stem cell transplant

ULUHAN SILI[1,2], ANN M. LEEN[1], JUAN F. VERA[1], ADRIAN P. GEE[1], HELEN HULS[1], HELEN E. HESLOP[1], CATHERINE M. BOLLARD[1] & CLIONA M. ROONEY[1]

[1]*Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children's Hospital, The Methodist Hospital, Houston, Texas, USA, and* [2]*Istanbul University, Cerrahpasa Medical Faculty, Department of Infectious Diseases and Clinical Microbiology, Turkey*

Abstract

Infections with a range of common community viruses remain a major cause of mortality and morbidity after allogeneic hematopoietic stem cell transplantation. T cells specific for cytomegalovirus (CMV), Epstein-Barr virus (EBV) and adenoviruses can safely prevent and infections with these three most common culprits, but the manufacture of individual T cell lines for each virus would be prohibitive in terms of time and cost. We have demonstrated that T cells specific for all three viruses can be manufactured in a single culture using monocytes and EBV-transformed B lymphoblastoid cell lines (LCLs), both transduced with an adenovirus vector expressing pp65 of CMV, as antigen-presenting cells. Trivirus-specific T cell lines produced from healthy stem cell donors could prevent and treat infections with all three viruses, not only in the designated recipient, but in unrelated, partially-HLA-matched third party recipients. We now provide the details and logistics of T cell manufacture.

Key Words: *Cytotoxic T cells (CTLs), viral infections, Immunotherapy, CMV, EBV, adenovirus, HSCT*

Introduction

Opportunistic infections are frequent in allogeneic hematopoietic stem cell transplant (HSCT) recipients and are associated with significant morbidity and high mortality rates. Pharmacologic agents are standard therapy for some infections, but most have substantial toxicities, generate resistant variants and are not effective against all viruses. As the use of antivirals does not improve virus-specific immunity, infections frequently recur after termination of treatment. In contrast, reconstitution of HSCT recipients with antigen-specific T cells can offer an effective nontoxic strategy for providing both immediate and long-term protection. Here we outline the approach used at Baylor College of Medicine (BCM, Houston, TX, USA) to activate and expand antigen-specific T cells that simultaneously target three frequently detected viruses, the endogenous herpes viruses Epstein–Barr virus (EBV) and cytomegalovirus (CMV), and adenovirus (Adv), which is increasingly detected post-transplant (1–4). Since 2004 these cytotoxic T lymphocytes (CTL) have been administered prophylactically to 26 patients at risk of developing CMV, EBV or Adv infections in a phase I/II clinical trial. Fourteen patients were treated during the dose-escalation phase ($1 \times 10^7/m^2$–$1 \times 10^8/m^2$). Then, to obtain further information regarding the immunologic effects after CTL, particularly in patients with low levels of viral re-activation at the time of CTL infusion, additional patients were treated with a fixed dose of $1 \times 10^7/m^2$, chosen because there was no correlation between clinical response and higher cell doses and because of evidence of clinical efficacy seen at this dose in the dose-escalation phase. The clinical outcome of 11 patients has been described elsewhere (5); briefly, the infused cells expanded *in vivo*, correlating with a subsequent reduction in raised levels of all three viruses and with resolution of virus-associated signs and symptoms (5). Below we outline the CTL manufacturing process. We have made the detailed standard operating procedures (SOP) required available with appropriate URL links throughout the manuscript (see the supplementary material). These should facilitate the creation of protocols suitable for

Correspondence: Dr **Cliona M. Rooney**, Center for Cell and Gene Therapy, 1102 Bates Street, Suite 1770 Houston, TX 77030, USA. E-mail: cmrooney@txch.org

ISSN 1465-3249 print/ISSN 1477-2566 online
DOI: 10.3109/14653249.2011.636963

regulatory approval and provide the basis for good manufacturing practice (GMP) processing of trivirus-specific T cells.

Application

Increasing numbers of viral pathogens have been implicated in infectious complications after HSCT, largely because of the extension of this procedure to higher risk patients who require more intensive and prolonged post-transplant immunosuppression and often receive more extensively manipulated products. Several groups have produced and infused antigen-specific T cells, most commonly targeting either CMV or EBV (6–9). The goal of our study was to broaden the spectrum of viruses targeted in a single CTL product to include the three most common viral pathogens of stem cell recipients, namely EBV, CMV and Adv.

Patient eligibility

Trivirus-specific CTL are generated from CMV-seropositive HSCT donors. For the generation of CTL lines, blood is procured at the earliest appropriate time, usually prior to stem cell collection in order to allow sufficient time for the generation of an EBV-transformed lymphoblastoid cell line (EBV-LCL) that is an essential antigen-presenting cell (APC), and subsequently for trivirus-specific CTL production and cryopreservation. The protocol allows multiple samples to be drawn, so that sufficient T cells can be obtained for the generation of EBV-LCL and CTL, which may be generated from fresh or frozen aliquots. However, in general a single blood draw of 60 mL is sufficient for manufacturing purposes because the CTL are produced from healthy donors. The protocol is discussed with eligible stem cell donors and patients, and the informed consent required for participation in the study is obtained from both parties. The protocol is approved by the Recombinant DNA Advisory Committee (RAC), the Food and Drug Administration (FDA) and BCM's Institutional Review Board (IRB). The National Marrow Donor Program (NMDP) has an IRB protocol and consent form that are used when obtaining blood for cell line preparation from an unrelated donor.

Manufacturing antigen-specific cytotoxic T cells using good manufacturing practices (GMP)

All cell culture and gene transfer manipulations are carried out in the Center for Cell and Gene Therapy GMP facility (BCM, The Methodist Hospital and Texas Children's Hospital) using current SOPs (available online; see the supplementary material).

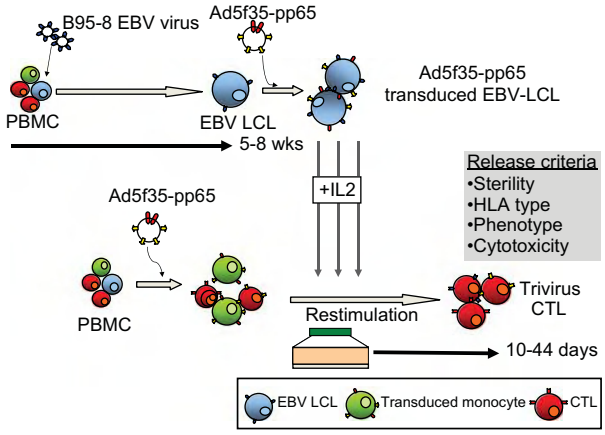

Figure 1. Generation of trivirus-specific CTL for clinical applications.

Blood procurement for CTL and APC generation

The generation of trivirus-specific CTL lines requires the production of several different components from peripheral blood mononuclear cells (PBMCs). The CTL line is initiated from the stem cell donor by transduction of adherent PBMC with an Adv vector expressing CMV-pp65. Within PBMCs, monocytes, which have been activated following overnight adherence, are preferentially transduced monocytes stimulate the T-cells component with both CMV-pp65 and Adv virion proteins. For the second and subsequent stimulations, autologous EBV-LCLs transduced with the same Ad5f35-pp65 vector are used to expand the trivirus-specific T cells (Figure 1).

A maximum of 60 mL peripheral blood $\times 2$ for a total maximum amount of blood of 120 mL is collected from the stem cell donor, who must be at least 12 kg (24 pounds) in weight. For donors <18 years, a maximum of 3 mL/kg blood is taken in an 8-week period. PBMC are isolated on Ficoll (Lymphoprep, Cosmo Bio USA, Carlsbad, CA, USA) gradients. Each component, T cells, EBV-LCL and monocytes, can be prepared from fresh or cryopreserved PBMC.

CTL initiation

CTL specific for CMV-pp65, Adv and EBV are prepared according to GMP SOP D03.31 (see the supplementary material).

For donor-derived trivirus-specific CTL, donor PBMC are plated overnight in 24-well plates in X-Vivo 15 media (BioWhittaker; Walkersville, MD, USA) at a concentration of 2×10^6 cells/well. This overnight adherence results in the transient up-regulation of the co-stimulatory molecules CD80 and CD83 on monocytes, which is crucial for optimal T-cell activation (10). The following day, the PBMC are harvested and activated monocytes are scraped