Cytotherapy, 2011; 13: 518–522

informa
healthcare

Good manufacturing practice-grade cytotoxic T lymphocytes specific for latent membrane proteins (LMP)-1 and LMP2 for patients with Epstein–Barr virus-associated lymphoma

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Key Words: *T-cell immunotherapy, standard operating procedures*

Introduction

In the last decade, virus-specific cytotoxic T lymphocyte (CTL) manufacture has become a more widespread and reproducible technology. A number of clinical-grade techniques have been described. Here we outline the approach used at Baylor College of Medicine (BCM, Houston, TX, USA) since 2004 to treat more than 35 patients with Epstein–Barr virus (EBV)-positive lymphoma using T cells specific for the latent membrane proteins (LMP)-1 and LMP2 of EBV in phase I/II clinical trials. Clinical outcomes have been described elsewhere (1,2) but briefly, objective tumor responses were achieved in 11/16 patients and complete remissions in eight. The CTL manufacturing process is outlined here, and we have made the detailed standard operating procedures (SOP) required available, as supplementary material can be found at: http://www.informahealthcare/cyt/10.3109/14653249.2011.561983. These should facilitate the creation of protocols suitable for regulatory approval and provide the basis for GMP manufacture of LMP1- and LMP2-specific T cells.

Application

In EBV-associated lymphomas and lymphoproliferative disorders that develop in the immunocompetent host, the EBV antigens LMP1, LMP2 and Epstein Barr nuclear antigen (EBNA1) are expressed in malignant cell populations. These include the Hodgkin Reed Sternberg cells in Hodgkin's lymphoma and B or natural killer (NK)/T cells in B or NK/T cells non-Hodgkin's lymphomas, respectively. These antigens therefore represent a potential source of target antigens for adoptive T-cell immunotherapy. In our phase I dose-escalation proof-of-principle studies, we have demonstrated the anti-tumor efficacy of autologous LMP1- and LMP2-specific cytotoxic T lymphocytes for patients with type II latency EBV-positive lymphomas, who had failed standard therapies for lymphoma (3,4). CTL have minimal toxicity; indeed, they have reduced B symptoms and provide an effective strategy for treating tumors without the devastating side-effects of standard chemoradiotherapies.

Patient eligibility

LMP-specific CTL are generated from patients with relapsed EBV-positive lymphoma expressing type II latency. For each patient, the expression of Epstein-Barr virus Encoded small nuclear RNA (EBER) and/or LMP is determined by *in situ* hybridization and immunohistochemistry, respectively, performed on paraffin-embedded diagnostic biopsy specimens. For the generation of CTL lines, blood is procured at the earliest appropriate time, usually immediately before a chemotherapy cycle. The protocol allows for multiple samples to be drawn, so that we can obtain sufficient T cells for the generation of dendritic cells (DC), EBV-transformed lymphoblastoid cell lines (LCL) and CTL, all of which may be generated from fresh or frozen aliquots. This is important because patients may have low lymphocyte counts, and it allows for re-initiation of cell lines in the case of failure. The protocol is discussed with eligible patients, and informed consent, required for participation in the study, is obtained for the generation of the autologous cell lines. The protocol is approved by the Recombinant DNA Advisory Committee (RAC, Bethesda, MD, USA), the Food and Drug Administration (FDA,

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(Received 29 January 2011; accepted 4 February 2011)

ISSN 1465-3249 print/ISSN 1477-2566 online
DOI: 10.3109/14653249.2011.561983

Silver Spring, MD, USA) and the BCM's institutional review board (IRB). In addition, for patients who have received allogeneic hematopoietic stem cell transplants, the CTL product is manufactured from the stem cell donor. In this setting, consent is obtained from the allogeneic donor to procure blood for CTL manufacture. The National Marrow Donor Program (NMDP, Minneapolis, MN, USA) has an IRB protocol and consent form that is used when obtaining blood for cell line preparation from unrelated donors.

Manufacturing LMP-specific cytotoxic T cells using good-manufacturing practices

All cell culture and gene transfer manipulations are carried out at the Center for Cell and Gene Therapy (CAGT, Baylor College of Medicine, Houston, TX, USA) good-manufacturing practice (GMP) facility using current SOP (available online).

Blood procurement for CTL and antigen-presenting cell generation

Generation of LMP-specific CTL lines requires the generation of several different components from peripheral blood mononuclear cells (PBMC). The CTL line is initiated from patient (or donor) PBMC by stimulation with antigen-presenting cells (APC) expressing the LMP1 and LMP2 antigens in the presence of interleukin (IL)-15, followed by expansion with IL-2. The APC used to stimulate and expand the LMP1-specific T cells are DC or monocytes and EBV-transformed B LCL derived from patient (or donor) mononuclear cells and B lymphocytes (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 patient or stem cell donor (subjects must be at least 12 kg or 24 pounds). For donors or patients $<$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, LCL, monocytes and DC, can be prepared from fresh or cryopreserved PBMC.

CTL initiation

CTL specific for the EBV tumor-associated antigens LMP1 and LMP2 are prepared according to GMP SOP D03.16 (4) (supplementary material can be found at: http://www.informahealthcare/cyt/10.3109/14653249.2011.561983). For patient-derived LMP-specific CTL, autologous DC are used as APC for the first stimulation. DC are manufactured according to SOP D03.15 (supplementary material can be found at: http://www.informahealthcare/cyt/10.3109/14653249.2011.561983) by culture of adherent PBMC-derived monocytes with cytokines [granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-4], followed by transduction on day 5 or 6 with a replication-incompetent adenovirus vector expressing inactive LMP1 and LMP2 separated by an internal ribosomal entry site (Ad5f35-ΔLMP1-I-LMP2; produced by the Gene Vector Laboratory of the Center for Cell and Gene Therapy, BCM) (5,6) at a viral particle (vp):cell ratio of 30 000:1, and maturation with a cytokine cocktail containing IL-1β, IL-6, tumor necrosis factor (TNF)-α and prostaglandin-E (PGE1). After 48 h, mature, transduced DC are used to stimulate LMP CTL according to SOP D03.15 (supplementary material can be found at: http://www.informahealthcare/cyt/10.3109/14653249.2011.561983). Transduced DC are harvested, washed and irradiated (30 Gy) and then co-cultured with autologous PBMC at an effector:target (E:T) ratio of 20:1 in the presence of IL-15. In this case, DC are prepared from about 40 mL blood and the T cells are derived from approximately 20 mL blood. If the DC are established from fresh blood, then non-adherent PBMC can be cryopreserved as the source of CTL.

For donor-derived LMP-specific CTL, donor PBMC are adhered overnight in 24-well plates in X-Vivo 15 media (BioWhittaker; Walkersville, MD, USA) at a concentration of 2×10^6 cells/well. The following day, the activated monocytes are scraped from the wells and transduced with the adenovirus vector (Ad5f35-LMP1/2) at a vp:cell ratio of 30 000:1 for 2 h. This procedure is critical because adenovirus infection of fresh monocytes results in monocyte death (7). After this time, the cells are resuspended at a concentration of 1×10^6/mL in CTL media containing advanced RPMI (Invitrogen, Carlsbad, CA, USA), Clicks Eagle's Hanks's aminoacids medium (EHAA, Irvine Scientific, Santa Ana, CA, USA), 10% fetal calf serum (FCS; Hyclone, Logan, UT, USA) and glutamine (Invitrogen). With this strategy, the transduced monocyte fraction of PBMC will express and present LMP1 and LMP2 peptide epitopes to the LMP-specific T-cell fraction of the PBMC. This step requires 20–40 $\times 10^6$ PBMC from about 40 mL blood. Monocytes are effective in the reactivation of LMP-specific T cells from healthy donors, but not from lymphoma patients, probably because their T cells are anergized, low frequency or fragile.

Expansion of LMP-specific CTL

For the second and subsequent stimulations, autologous LCL transduced with Ad5f35-LMP1–2 are used