Treatmen
t of relapsed Hodgkin’s disease using EBV-specific cytotoxic T cells


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Summary

Donor-derived Epstein–Barr virus (EBV)-specific cytotoxic T lymphocytes (CTL) are successful in the prevention and treatment of Epstein–Barr virus (EBV)-associated lymphoproliferative disease (LPD) in allogeneic bone marrow transplant (BMT) recipients [1, 2]. This finding prompted us to use a similar approach to the treatment of relapsed EBV-positive Hodgkin’s disease [3]. Autologous EBV-specific CTL lines could be generated on the first or second attempt from 11 of 15 patients with Hodgkin’s disease. Peripheral blood TCR i^-chain levels were low, but increased in the activated CTL lines. Three patients have received gene-marked autologous CTL. The first two patients experienced alleviation of stage B symptoms and a drop in peripheral blood EBV load. However, this situation reversed between 6 and 12 weeks after infusion, when chemotherapy and radiation were reinstated. Both patients eventually progressed and died. The third patient had a pleural effusion, which increased after CTL infusion. Analysis of the pleural effusion revealed both tumor cells and levels of marker gene over 100 fold greater than in peripheral blood. The infused CTL line showed activity against LMP2. The patient initially improved and then remained stable for over eight months after CTL infusion, but now has progressive disease. We currently are evaluating methods for introducing the LMP2 gene into dendritic cells and using these to present LMP2 to autologous T cells. Using both retrovirus and herpesvirus vectors to express LMP2 in dendritic cells, LMP2-specific CTL were successfully generated from individuals who were EBV-seronegative or who were non-responsive to LMP2 when presented on autologous LCL. In future protocols, LMP2-specific CTL will be used for treatment.

Key words: cytotoxic T lymphocyte (CTL), dendritic cell, Epstein–Barr virus (EBV), EBV-associated lymphoproliferative disease (EBV-LPD), gene-marking, gene transfer, Hodgkin’s disease, immunotherapy, latent membrane protein 2 (LMP2)

Introduction

Efficacy of prophylactic EBV-specific CTL in BMT recipients

Between November 1993 and November 1997 at St. Jude Children’s Research Hospital, over 50 recipients of T-cell depleted bone marrow from mismatched or unrelated donors received gene-marked, donor-derived EBV-specific CTL for the prevention of EBV-LPD. CTL were stimulated from donor peripheral blood mononuclear cells using the irradiated autologous EBV-transformed B-cell line as antigen-presenting cells (APC) [4]. The safety of the CTL infusions was monitored clinically, and blood was received regularly after infusion for monitoring CTL persistence, and immunological and virological efficacy. None of the patients experienced toxicity that could be ascribed to the CTL. Two patients experienced reactivation of GvHD, but there was no accumulation of marked CTL in biopsy tissues. No patient experienced de novo GvHD. PCR analysis of marker gene expression in EBV-specific CTL expanded in vitro at regular intervals, demonstrated that CTL could persist for up to three years after infusion. EBV-specific CTL could rarely be detected in patients prior to CTL infusion, but within four weeks of infusion CTL precursor frequencies were within the range seen in normal individuals. The CTL also had anti-viral effects. In six patients, peripheral blood EBV DNA levels increased to levels that were highly predictive or indicative of EBV-LPD [5]. These patients had no evidence of disease, other than fevers in some cases. Within three weeks of the first CTL infusion, DNA levels had dropped three to four logs, suggesting that the CTL could control EBV reactivations in vivo. None of the 51 patients who received prophylactic CTL developed EBV-LPD, by contrast with 7 of 62 who did not, suggesting that the CTL were protective.

Efficacy as treatment of active disease

Three patients received CTL as treatment for fulminant disease. Two recovered completely, one without complications, but one with major complications arising from the inflammatory response. A third patient died with active disease. Preliminary results suggest that this failure was because the tumor virus carried a deletion that removed CTL epitopes against which the majority of the CTL were directed. Thus, in highly immunosuppressed
patients in whom EBV-LPD is aggressive and rapidly invasive, it is clearly preferable to prevent rather than treat disease.

**EBV in Hodgkin's disease as a target for CTL**

The EBV proteins expressed in the tumor cells of about 50% of cases of Hodgkin's disease provide antigenic targets for EBV-specific cytotoxic T cells (CTL). Thus CTL might provide a salvage therapy for patients who fail or relapse after induction therapy and if successful might provide a less toxic form of primary therapy [6, 7]. However, there are potential problems with the success of such therapy.

**Generation of CTL lines from heavily treated cancer patients**

The immune system of patients with multiply relapsed disease is not robust. T cell receptor z chain levels are low and we anticipated difficulties in activating and expanding CTL [3, 8]).

**Generation of CTL lines with appropriate antigen specificity**

EBV-transformed B cells express nine latent cycle proteins EBNAs 1, 2, 3a, 3b, 3c and LP, LMP1 and 2 and BARFO. These proteins display a hierarchical immunodominance, with the EBNA3 proteins being the most immunogenic to CTL and EBNA1 the least [9]. Hodgkin tumor cells express only four of the latency proteins, EBNA1, LMP1, LMP2 and BARFO, and these are poorly immunogenic. EBNA1 is not processed for HLA class I recognition, CTL clones specific for BARFO have not been detected and although LMP1 and 2-specific CTL have been identified these are relatively low frequency [9, 10]. Further LMP1 is heterogeneous between virus strains and LMP1-specific CTL raised against the B95-8 strain of EBV might not recognize the LMP1 tumor viruses [11]. Therefore CTL lines generated using LCL as antigen-presenting (APC) cells may contain few clones with specificity appropriate for the treatment of Hodgkin's disease.

**Results**

**Generation and use of EBV-specific CTL in patients with Hodgkin's disease**

Stimulation with the autologous LCL induced EBV-specific CTL lines from 11 of 15 patients, including six with relapsed disease. TCR 3 chain levels on peripheral T cells were low, but increased after activation in vitro, although levels reached normal only on lines from remission patients. CTL lines from patients expanded slowly compared to lines from normal donors, and usually required additional mitogenic stimulation after day 21 to reach the doses required for infusion.

Three patients have received two doses of $2 \times 10^7$ cells per m². Marked CTL persisted in peripheral blood for a median of 12 weeks. This was similar to persistence in BMT recipients. EBV DNA levels dropped from high to undetectable levels in two patients (DNA levels in the third patient were within the normal range), and the EBV-specific CTL precursor frequency increased by 10-fold in the two patients analyzed. The first two patients experienced alleviation of stage B symptoms, but these reappeared and DNA levels increased after both patients received chemotherapy and radiation, six weeks after CTL infusion. The third patient had a pleural effusion at the time of CTL infusion. The pleural effusion increased in volume after infusion and was tapped, revealing tumor cells and an accumulation of marked T cells that was over 100 fold that in peripheral blood. This indicated that CTL had homed to and accumulated or expanded in the effusion. Interestingly, the only antigen specificity that could be detected in the infused CTL line was for LMP2. This patient remained stable for about eight months, but now had progressive disease.

**Generation of LMP2-specific CTL**

To ensure that we infuse CTL lines with appropriate antigen specificity in all patients, we have explored the feasibility of using autologous dendritic cells modified to express the appropriate antigens, as APC.

**Choice of antigen**

We selected LMP2a as the stimulating antigen, because this protein is highly conserved between virus strains and CTLs specific for LMP2 have been identified [12].

**Choice of antigen-presenting cell**

Dendritic cells are the most potent APC known. They can induce primary immune responses in vitro and overcome non-responsiveness to tumor antigens [13].

**Gene modification of dendritic cells**

For optimal anti-virus activity and persistence, we aim to infuse a combination of CD8+ and CD4+ T cells. Therefore the majority of the antigen should be presented on class I molecules, which classically process endogenously-expressed antigens. This could be achieved by transfection or transduction. We were unable to find a reliable and efficient means of transfecting dendritic cells with DNA and therefore turned to viral vectors. A potential problem with the use of viral vectors to generate immune responses to a weak immunogen is that virally expressed proteins or virion proteins may compete for HLA binding grooves and quench the response to the transgene. Although herpesvirus vectors did induce LMP2-specific CTL, we prefer to use retrovirus vectors, because they express no competing viral proteins, and because patients should have no immunological memory to the virion proteins, a potential problem with adenovirus or herpesvirus vectors.
Use of dendritic cells transduced with an LMP2-containing retrovirus vector to induce LMP2-specific CTL

Peripheral blood dendritic cells were prepared by culture in GM-CSF and IL-4 using standard methodology [14]. The efficiency of transduction was improved by using a reverse flow through method, in which about 30 ml of retrovirus supernatant was concentrated onto an anchor cell membrane using a vacuum [15]. The dendritic cells were then gently applied to the membrane using vacuum, to increase the virus-cell contact. After incubation overnight in medium containing GM-CSF and IL-4, the DC were irradiated and used to stimulate autologous peripheral blood mononuclear cells. After weekly antigenic stimulation with DC/LMP2 and expansion in medium containing interleukin 2 from day 14, the CTLs were tested for their antigen-specificity. Target cells were autologous fibroblasts infected with vaccinia recombinants expressing EBV latent cycle proteins, as well as autologous and HLA-mismatched EBV-transformed B-cell lines. CTL lines that had specificity only for LMP2 were generated from both seroprivate individuals, who did not respond to LMP2 presented on the LCL and from seronegative individuals who did not produce CTL responses at all when the autologous LCL was used for stimulation. Importantly, the LMP2-specific CTL lines also killed the autologous LCLs, which are biological targets for EBV.

Discussion

We have previously shown that EBV-specific CTL generated from allogeneic bone marrow donors can persist and function long term in marrow recipients, protecting them from EBV-associated lymphoproliferative disease. EBV-specific CTL were also effective in treating bone marrow recipients who had developed frank EBV lymphoma. We have now shown that it is also possible to generate EBV-specific CTL from patients with multiply relapsed EBV-positive Hodgkin's disease, and to grow these CTL to numbers sufficient for autologous therapy. After infusion into patients, the CTL persisted and functioned to reduce virus load, increase cellular immunity to EBV and produce transient clinical improvements. It is not known whether the high levels of EBV DNA in peripheral blood are a measure of circulating tumor cells, or reflect poor control of EBV-infected normal B cells. Therefore it was not clear whether the clinical and virological improvements seen after CTL infusion were due CTL-mediated anti-tumor effects, or due to increased control of general EBV persistence. However, in one patient (at least), the CTLs infused had activity against one of the viral proteins expressed in the tumor cells. Further, in this patient, gene-marked CTL were detected in pleural fluid at levels over two logs greater than in peripheral blood, providing evidence of homing to tumor sites. That this patient remained stable for so long was remarkable and suggested that the CTL may have had some anti-tumor activity. Nonetheless, improvements in CTL specificity and activity are clearly required. Improvements in specificity may be achieved by using CTL specific only for those antigens, such as LMP1 and LMP2, expressed in Hodgkin tumor cells. Improvements in activity may be achieved by increasing cell doses, by using CTL specific for combinations of antigens or by genetically modifying the CTL.

If immunotherapy approaches prove to be effective in the treatment of patients with relapsed disease, the cytotoxic T cells may become a useful adjunct to conventional treatment for Hodgkin's disease, allowing the use of less aggressive and toxic regimens.

Acknowledgements

This work was supported by NIH grants CA2164, CA71426, CA 21765 (CORE), The Assisi Foundation of Memphis and ALSAC (American Lebanese Syrian Associated Charities).

References


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