Allogeneic Virus-Specific T cells with HLA Alloreactivity do not Produce GVHD in Human Subjects

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Short Title: Allogeneic virus-specific T cells and GVHD
ABSTRACT
Adoptive transfer of viral antigen-specific memory T cells can reconstitute antiviral immunity but in a recent report by Amir et al, a majority of virus-specific CTL lines showed in vitro cross-reactivity against allo-HLA molecules as measured by gamma interferon secretion. We therefore reviewed our clinical experience with adoptive transfer of allogeneic hematopoietic stem cell transplant donor-derived virus-specific CTLs in 153 recipients including 73 instances where there was HLA-mismatch. There was no de-novo acute GVHD after infusion and incidence of GVHD reactivation was low and not significantly different in recipients of matched or mismatched CTL. However, we found that virus-specific T cell lines recognized up to 10% of a panel of 44 HLA disparate targets, indicating that virus-specific T cells can have cross reactivity with HLA-mismatched targets in vitro. These data indicate that the adoptive transfer of partially HLA-mismatched virus-specific CTL is safe despite in vitro recognition of recipient HLA molecules.
INTRODUCTION

Following stem cell transplantation, there is high morbidity and mortality from viral disease.\(^1\) Such complications are commonest where the donor and recipient are partially HLA mismatched or the donor graft has been depleted of mature T lymphocytes to prevent alloreactivity and GVHD. As a consequence, several investigators have administered donor-derived virus-specific T cells to transplant recipients to reduce the incidence and severity of post-transplant viral disease with apparent clinical benefit.\(^2\)\(^{-9}\) A recent study by Amir et al, however, suggests that transfer of HLA mismatched virus-specific CTL might risk graft-versus-host alloreactions.\(^10\) In that study, T cell lines reactive against Epstein Barr virus (EBV), cytomegalovirus (CMV), varicella zoster virus, and influenza virus were tested against a panel of HLA-typed target cells, and target cells transduced with single HLA molecules.\(^10\) Remarkably, 80% of virus-specific T-cell lines and 45% of virus-specific T-cell clones derived therefrom were cross-reactive against allo-HLA molecules, as measured by gamma interferon secretion.\(^10\) This cross-reactivity was observed in both CD8+ and CD4+ T-cell clones, being directed primarily against HLA class I and II antigens, respectively. These observations raise the concern that virus-specific T cells might mediate graft rejection or GVHD when administered to HLA class I or II mismatched recipients.\(^10\)

Notwithstanding the apparently high level of cross reactivity in the \textit{in vitro} assays reported by Amir et al\(^10\), there are no data to suggest that cross reactivity of virus-specific T cells with HLA specificities leads to clinical complications.\(^3\)\(^{-9}\) None of these studies, however, formally dissected responses in recipients who had received HLA partially mismatched virus-specific CTLs, or examined whether the observed lack of any GVHD was simply due to fortuitous absence of alloreactivity in the administered lines.
We now report that in 73 recipients of virus-specific CTLs from an HLA mismatched donor we have not observed GVHD associated with the cell infusion. In 4 patients the alloreactivity of infused lines was characterized in an in vitro assay against a T cell-APC panel. Our data confirm the presence of in vitro allo-HLA reactivity in infused virus-specific T cells, but do not support the conclusion that such alloreactive CTLs can cause GVHD in vivo.

METHODS

Patient Details

Hematopoietic Stem Cell Transplantation (HSCT) recipients were treated on studies of donor-derived EBV-specific CTLs, bivirus CTLs specific for adenovirus and EBV, and trivirus CTLs specific for CMV, adenovirus and EBV. All studies were approved by the Food and Drug Administration and the Institutional Review Board at Baylor College of Medicine. Clinical details and results of the studies have been previously reported. In these studies one release criterion to exclude alloreactivity was that killing of recipient PHA blasts by the infused CTL line should be less than 10% (with <2% of manufactured lines failing to meet this criterion) and data from the three studies are shown in Figure 1a. 73 of the 153 subjects had a donor that was mismatched at one or more HLA antigens.

In vitro assay of alloreactivity

Four CTL lines from the adenovirus/EBV CTL study underwent analysis for alloreactivity on studies approved by IRBs at the NIH and Baylor. Activated T cells were generated as described under NHLBI IRB-approved protocols, and served as antigen presenting cells (T-APC). A panel of 44 T-APC was composed to cover the most frequent HLA class I and II alleles (Table S1). For the detection of allogeneic targets, the virus-specific T cell lines were labeled with CFSE and stimulated with unlabeled T-APC (table S1) or left unstimulated. After 6 hours the cells were processed as outlined in detail in the supplemental methods section. Responder
cells were identified in the CFSE-positive population by cells that produced both TNFα and IL-2/IFNγ since the background signal for this cytokine combination, i.e. the proportion of T cells producing TNFα and IL-2/IFNγ cultured for 6 hours with secretion inhibitors alone, was lower compared to TNFα or IL-2/IFNγ separately as can be seen in figure 1c. The respective T cell lines cultured under the same conditions but in the absence of T-APC were used as negative controls, and the proportion of cytokine producing cells was subtracted from the panel analysis to arrive at the net allo-HLA response.

(See references and supplemental text for further details).

RESULTS AND DISCUSSION

GVHD in Mismatched Patients

Of the 73 recipients of HLA mismatched virus-specific CTLs, 34 received EBV-specific CTLs from an unrelated donor mismatched at one or more HLA antigens, while 13 received EBV-specific CTLs produced from an HLA haploidentical family member. Five received bivirus-specific CTLs from unrelated donors mismatched at 1 or 2/10 HLA antigens and 6 from haploidentical donors. Six subjects received trivirus CTLs from an unrelated donor mismatched at 1-3/10 antigens and 9 from a haploidentical family member. The overall incidence of acute GVHD was 6.5%, with all episodes representing reactivations and no significant difference in the incidence between recipients of matched or mismatched CTLs (Table 1). Since GVHD only occurred in patients with previous episodes of the disease, the absence of de-novo GVHD after CTL infusions and the lack of correlation with the degree of mismatching of the CTLs indicated that mismatched CTLs did not induce GVHD.

In vitro alloreactivity

To exclude the possibility that the observed absence of GvHD was due to the fortuitous choice of CTLs lacking recipient-specific alloreactivity, we analyzed the HLA reactivity of four infused
bivirus-specific lines. All CTLs responded to a number of T-APC (Fig.1b, c; Fig. S1; table S2). Some T-APC were recognized strongly, and strong alloreactivity appeared to be confined to the CD4+ T cell subset (e.g. CTL C2910; C3000; Fig. S2), whereas most stimulators induced only weak or undetectable cytokine signals (Fig. S1). The proportion of stimulators recognized ranged from broad (CTLs C2910, C3000, C3311) to restricted (C3183), suggesting polyclonal versus oligoclonal responses. We then determined whether the CTLs recognized T-APC expressing the HLA alleles of the recipient (Table S3). In the four lines tested, virus-specific CD4+ and CD8+ T cells displayed moderate reactivity with 1-5 T-APC expressing the recipient’s HLA allele (Fig.1B and S1).

Lack of in-vitro / in-vivo correlation

Since most patients were off immunosuppressive treatment at the time of T cell infusion, other explanations must be sought to explain the lack of GVHD in recipients of T cell lines recognizing the HLA type of the recipient. It is possible that the lack of reactivity with GVHD-susceptible tissues in the recipient could be explained by a discordance in antigen expression between the T-APC and GVHD targets, as might be the case in the study by Amir et al.10 While it is possible that GVHD-reactive T cell lines had only a limited capacity to survive and expand in vivo14, our gene marking studies suggest that these infused virus-specific cells are long lived.2 Since GVHD may be primarily mediated by naïve T cells15 the predominance of memory-effector cells in these infusions may have been protective. Alternatively the polarity of the infused T cells may have protected against GVHD16 but the Th1/Th2 characteristics of these lines was not studied. Irrespective of the ultimate explanation, our conclusion from the data is that in vitro alloreactivity of virus-specific T cell lines against hematopoietic APC does not correlate with the risk of developing GVHD and that alloreactive T cell lines can be safely infused into both MHC class I and II mismatched recipients.
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Authorship:

JJM performed laboratory studies of alloreactivity, interpreted data and contributed to writing the manuscript. AML developed the methodology for multivirus specific CTLs, performed laboratory analyses and reviewed the manuscript. CMB was PI of the multivirus CTL studies and reviewed the manuscript. MFQ and DAP assisted in large scale screening experiments. CMR developed the standard operating procedures for CTL production, developed the clinical trials and reviewed the manuscript. MKB developed the clinical trials and reviewed the manuscript. AJB interpreted data from studies of alloreactivity and contributed to writing the manuscript. HEH developed the clinical trials, was PI of the EBV CTL study, analyzed the data and contributed to writing the manuscript.

The authors have no conflict of interest to disclose.
Reference List


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FIGURE LEGEND

Figure 1  Alloreactivity of Infused CTLs

Before infusing the donor CTLs, we characterized their cytotoxicity against PHA blasts obtained from the transplant recipient in a standard chromium release assay. The release criterion was that cytotoxicity should be less than <10% and Figure 1A shows that mean cytotoxicity was 2.07% for EBV CTLs, 2.24% for trivirus CTLs and 3.07% for bivirus CTLs. Each symbol represents a cell line infused into a single subject.

Figure 1B shows the response of CD8+ T cells in CTL line C3183, to the panel of T-APC. Only cells producing both IL-2/IFNγ and TNFα were scored positive, and the responses are ranked according to their frequency. CD8+ T cells responding to APC that expressed an HLA class I allele shared with the recipient of this cell line are indicated as blue bars. Since allo-HLA reactive CD8+ T cells can recognize HLA class II-presented antigens we have displayed their reactivities with T-APC expressing a recipient-matching HLA class II mismatch as greyed bars. One T-APC expressed both an HLA class I and II mismatch shared with the recipient of C3183; the response to this APC is displayed as a blue bar with bold striped grey border.

Figure 1C shows the response of CTL line C2910 to autologous EBV-LCL and three representative T-APC, plus the negative control: CTL line without APC.
Figure 1

A. \( \% \) specific Killing

B. %CD8+ cmpt dend

C. none 0.33 0.01 0.04 EBV-LCL 0.70 16.9 13.4

T-APC09 1.80 2.90 3.41 T-APC10 0.41 1.49 1.04 T-APC13 0.33 0.01 0.07
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