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EBV-Specific CD4⁺ T Cell Clones Exhibit Vigorous Allogeneic Responses

Elise Landais,* Alexis Morice,* Heather M. Long,† Tracey A. Haigh,† Béatrice Charreau,‡ Marc Bonneville,* Graham S. Taylor,† and Elisabeth Houssaint‡

Alloreactive T cells play a key role in mediating graft-vs-host disease and allograft rejection, and recent data suggest that most T cell alloreactivity resides within the CD4 T cell subset. Particularly, T cell responses to herpesvirus can shape the alloreactive repertoire and influence transplantation outcomes. In this study, we describe six distinct EBV-specific CD4⁺ T cell clones that cross-reacted with EBV-transformed lymphoblastoid cell lines (LCLs), dendritic cells, and endothelial cells expressing MHC class II alleles commonly found in the population. Allore cognition showed exquisite MHC specificity. These CD4⁺ T cell clones efficiently killed dendritic cells or LCLs expressing the cross-reactive allogeneic MHC class II molecules, whereas they did not kill autologous LCLs. Endothelial cells expressing the proper allogeneic MHC molecules were poorly killed, but they induced high-level TNF-α production by the EBV-specific CD4⁺ T cell clones. As already proposed, the strong alloreactivity toward LCLs suggest that these cells could be used for selective depletion of alloreactive T cells. The Journal of Immunology, 2006, 177: 1427–1433.

A lloreactive T cells play a key role in mediating graft-vs-host disease and allograft rejection (1, 2). Alloreactive T cells recognize alloantigens via two distinct pathways, direct and indirect. In the direct pathway of allore cognition, which we addressed in this study, responder T cells carrying a TCR originally selected to respond to foreign peptide Ags bound to self MHC recognize intact allogeneic MHC on donor cells. This pathway is characterized by the high precursor frequency of responder T cells, leading to a vigorous T cell allore response that can pose problems in clinical transplantation across HLA differences. Up to 10% of T cells of an individual can recognize foreign MHC class I and class II molecules (3, 4), the magnitude of the allore reactive response being due to the large number of T cells stimulated by the hugely diverse array of self peptides on the nonself MHC molecules. Although alloreactive T cells can recognize structural determinants of the foreign MHC molecules independent of the bound peptides (5–7), it has become clear that, for most alloreactive T cells, recognition depends on peptides within the allo-MHC groove (5, 6, 8, 9). Structural studies on alloreactive TCR complexed with allogeneic MHC molecules have confirmed the role played by peptides in alloreactivity (10, 11). As originally proposed by Matzinger and Bevan (12), it thus appears that alloreactive TCRs exploit the similarities, rather than the differences, between the top of the helices of self- and allo-MHC molecules. The high frequency of alloreactive T cells derives from the presentation on allogeneic MHC molecules of new peptides to which T cells from the recipient were never exposed.

It is now well established that the memory subset of circulating T cells contributes to primary alloresponses (see Ref. 13 for a comment). Recent studies in a mouse model have shown that a critical threshold of memory T cells is needed to promote graft rejection, leading the authors to propose that transplant tolerance is more difficult in humans than mice, because humans are exposed more to environmental Ags (14). Persistent viral infections, like those with herpesviruses, have a profound impact on the T cell repertoire inducing large clonal expansions of virus-specific memory T cells (15, 16). The influence of antiviral T cell responses on the CD8⁺ T cell alloreactive repertoire was first described for EBV (17–19) and, more recently, for CMV (human CMV (HCMV)) (20) or herpes simplex virus (21). In contrast, there is little evidence on the influence of antiviral CD4⁺ T cells on the alloreactive repertoire (22, 23). In this study, we screened for cross-recognition of all of allogeneic EBV-transformed B cell lines (lymphoblastoid cell lines (LCLs)) by EBV-specific CD4⁺ T cell clones (24, 25) and report several examples of cross-reactivity leading to potent killing of allogeneic LCLs expressing a particular MHC class II allele.

Materials and Methods

EBV-specific CD4⁺ T cell clones

EBV-specific CD4⁺ T cell clones were derived from PBL from healthy virus carriers or from synovial fluid from patients suffering from rheumatoid arthritis, as described previously (24, 25). T cell clones were maintained in RPMI 1640 supplemented with 10% pooled human serum, 1 mM L-glutamine, and 150 IU/ml IL-2.

Target cells

LCLs were generated by exogenous transformation of peripheral B cells either with EBV-containing supernatant from the virus-producing B95.8 marmoset cell line or with a B95.8 virus mutant in which the BZLF1 gene

*Institut National de la Sante et de la Recherche Médicale, Unité 601, Institut de Biologie, Nantes, France; †Cancer Research UK Institute for Cancer Studies, University of Birmingham, Birmingham, United Kingdom; and ‡Institut National de la Sante et de la Recherche Médicale, Unité 643, Institut de Transplantation et de Recherche en Transplantation, Centre Hospitalier Universitaire Hôtel-Dieu, Nantes, France

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2 Address correspondence and reprint requests to Dr. Elisabeth Houssaint, Institut National de la Sante et de la Recherche Médicale Unité 601, Institut de Biologie, 9 quai Moncousu, 44093 Nantes Cedex 01, France. E-mail address: chalmeau@nantes.inserm.fr; or to Dr. Graham S. Taylor, Cancer Research UK Institute for Cancer Studies, University of Birmingham, Birmingham, U.K. E-mail address: g.s.taylor@bham.ac.uk

†Cancer Research UK Institute for Cancer Studies, University of Birmingham, Birmingham, U.K. E-mail address: g.s.taylor@bham.ac.uk

‡Institut National de la Sante et de la Recherche Médicale, Unité 601, Institut de Biologie, Nantes, France

‡Cancer Research UK Institute for Cancer Studies, University of Birmingham, Birmingham, United Kingdom

*Institut National de la Sante et de la Recherche Médicale, Unité 643, Institut de Transplantation et de Recherche en Transplantation, Centre Hospitalier Universitaire Hôtel-Dieu, Nantes, France

†Cancer Research UK Institute for Cancer Studies, University of Birmingham, Birmingham, U.K. E-mail address: g.s.taylor@bham.ac.uk

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had been deleted (26). Some LCLs were obtained from the European Collection of Cell Cultures, HLA class II typing was performed by using standard molecular typing techniques at the Etablissement Français du Sang (Nantes, France) and the National Blood Service Laboratories (Birmingham, U.K.). For some of the LCLs, the serologic HLA specificity alone was known. All LCLs were cultured in RPMI 1640 medium supplemented with 10% FCS. For dendritic cell (DC) preparation, PBMCs were isolated from a healthy DRB1*0401 donor by Ficoll density gradient centrifugation. To minimize contamination of PBMC with platelets, the preparation was first centrifuged at 1000 rpm at room temperature. After removal of the top 20–25 ml, which contained most of the platelets, the cells were centrifuged at 1500 rpm at room temperature.

To generate DC, PBMC were depleted from T lymphocytes by adhering to culture Falcon flasks (BD Biosciences) at a density of 5 × 10^6 cells/ml in RPMI 1640 medium supplemented with 5% FCS. Nonadherent cells were discarded by five washes in PBS, and adherent cells were cultured in the presence of IL-4 (100 ng/ml) and GM-CSF (25 ng/ml) in complete RPMI 1640 medium. Cultures were fed on days 2 and 4 with IL-4 (100 ng/ml) and GM-CSF (25 ng/ml) in complete RPMI 1640 medium supplemented with 10% FCS. For dendritic cell (DC) preparation, PBMCs were isolated from a healthy DRB1*0401 donor by Ficoll density gradient centrifugation. To minimize contamination of PBMC with platelets, the preparation was first centrifuged at 1000 rpm at room temperature. After removal of the top 20–25 ml, which contained most of the platelets, the cells were centrifuged at 1500 rpm at room temperature.

Human arterial endothelial cells (EC) from DR1 donors were isolated from renal artery patches taken before kidney transplantation, as described previously (27). EC were treated with recombinant human IFN-γ from renal artery patches taken before kidney transplantation, as described in Table I. To generate DC, PBMC were depleted from T lymphocytes by adhering to culture Falcon flasks (BD Biosciences) at a density of 5 × 10^6 cells/ml in RPMI 1640 medium supplemented with 5% FCS. Nonadherent cells were discarded by five washes in PBS, and adherent cells were cultured in the presence of IL-4 (100 ng/ml) and GM-CSF (25 ng/ml) in complete RPMI 1640 medium. Cultures were fed on days 2 and 4 with IL-4 (100 ng/ml) and GM-CSF (25 ng/ml), and floating immature DC (iDC) were harvested on day 7. DC maturation was induced by addition of 10 ng/ml TNF-α with IL-4 (100 ng/ml) and GM-CSF (25 ng/ml) in complete RPMI 1640 medium. Cultures were fed on days 2 and 4 with IL-4 (100 ng/ml) and GM-CSF (25 ng/ml), and floating immature DC (iDC) were harvested on day 7. DC maturation was induced by addition of 10 ng/ml TNF-α with IL-4 (100 ng/ml) and GM-CSF (25 ng/ml), and floating immature DC (iDC) were harvested on day 7. DC maturation was induced by addition of 10 ng/ml TNF-α with IL-4 (100 ng/ml) and GM-CSF (25 ng/ml), and floating immature DC (iDC) were harvested on day 7. DC maturation was induced by addition of 10 ng/ml TNF-α with IL-4 (100 ng/ml) and GM-CSF (25 ng/ml), and floating immature DC (iDC) were harvested on day 7. DC maturation was induced by addition of 10 ng/ml TNF-α with IL-4 (100 ng/ml) and GM-CSF (25 ng/ml), and floating immature DC (iDC) were harvested on day 7.

Cytoxicity was tested by standard 4-h chromium-51 release assay, at defined E:T ratios, as described previously (24). When used, synthetic peptides were directly added to chromium-51-labeled targets and incubated for 1 h before excess unbound peptide was washed off. In blocking assays, LCL targets were pre-exposed for 1 h to epitope peptide at 5 µM, washed, and then incubated for a further hour in the presence of mAbs specific for HLA-DR (L243), or HLA-DP (B7.21) before addition of T cells. Assays were performed in triplicate and results are expressed as follows: percent specific [51Cr] lysis = (experimental release − spontaneous release)/(maximal release − spontaneous release) × 100%.

Proliferative activity of responder T cells was determined after 48-h culture of 10^4 responder T cells with 2.5 × 10^5 irradiated LCLs in 100 ml of culture medium supplemented with rIL-2, followed by an overnight pulse with [3H]thymidine. Results are expressed as the means of duplicate samples.

Detection of IFN-γ release was performed by ELISA described previously (25). Cloned T cells (500 T cells/well) were incubated in U-bottom microtest plate wells with standard numbers of LCL cells, which were
either unmanipulated or prepulsed for 1 h with 5 mM peptide (or an equivalent concentration of DMSO solvent as a control) and then washed. The supernatant medium harvested after 18 h was assayed for IFN-γ by ELISA (Endogen) in accordance with the manufacturer’s recommended protocol. In TNF-α release assays, 5 × 10^3 T cells were incubated for 6 h with 3 × 10^5 endothelial cell lines, which were either unmanipulated or prepulsed for 1 h with 5 mM peptide and then washed, and the amount of TNF-α released in the supernatant was estimated after 6 h by the WEHI 164 cytotoxicity assay (28).

**Results**

**Screening of EBV-specific CD4 T cell clones for recognition of allogeneic LCLs**

We recently characterized CD4+ T cell clones specific to EBV epitopes, derived from viral proteins produced during latency or lytic cycle (24, 25). Nineteen of these clones, with different antigenic specificities, were screened for alloreactivity toward a large panel of LCLs expressing allogeneic MHC class II molecules (40 LCLs were used). For all of these clones, except clone A4.6, the antigenic specificity and the HLA context of recognition had been defined previously (Tables I and II) (24, 25). Clone A4.6 was found to proliferate against the LCL donor as well as allogeneic LCLs sharing the DRB1*0101 allele with the donor LCL. This suggested that clone A4.6 could recognize an EBV epitope in the HLA-DRB1*0101 context. Recognition of an EBV lytic epitope was confirmed by the fact that clone A4.6 produced IFN-γ upon stimulation with a DRB1*0101+ LCL transformed with wild-type B95.8 (WT-JT LCL), whereas it did not respond to an LCL line raised from the same donor by using a B95.8 virus mutant in which the BZLF1 gene had been deleted (26) (BZLF1-KO JT LCL) and so was unable to enter lytic cycle (Fig. 1). However, we did not succeed to define the lytic epitope recognized by clone A4.6. Overlapping peptides, covering the sequence of several EBV lytic proteins (BZLF1, BMLF1, BRLF1, BCRF1, BHRF1, BMRF1, gpl10), were tested and they scored negative (data not shown).

Among the 19 EBV-specific CD4+ T cell clones that were tested for alloreactivity in a cytotoxic assay, 6 were found to cross-react with LCLs expressing allogeneic MHC class II molecules (Table II). The more striking result was that, although none of these clones spontaneously killed the autologous LCLs, until they were pulsed with the synthetic cognate EBV peptide, they all exhibited vigorous killing of allogeneic LCLs bearing particular cross-reactive HLA-DR molecules (Fig. 2A). In contrast, LCLs that express non-cross-reactive allogeneic MHC class II molecules were not killed (Fig. 2A and Table I). For example, clone P4.2, specific to the EBNA3C100–111/DRB1*16 epitope, was found to kill the autologous LCL and DRB1*16-matched LCLs provided they were loaded with the cognate peptide, and to also kill unpulsed allogeneic LCLs expressing DRB1*0101 molecules. Clone AL78, specific to the EBNA1475–489/DPB1*1001 epitope, killed DRB1*0102+ LCLs, even when they were peptide unloaded. The clone AL78 was tested in a cytotoxicity assay on different LCLs expressing or not the DPB1*1001 restricting allele and expressing or not the DRB1*0102 allogeneic allele. As shown in Fig. 2B, killing of the DPB1*1001+ LCLs loaded with the EBNA1 peptide was blocked by an anti-DP mAb, whereas killing of DRB1*0102+ LCLs was blocked by an anti-DR mAb.

**IFN-γ production and proliferation against the cross-reactive LCLs** were also tested for some of the alloreactive CD4+ T cell clones. Fig. 3 shows a representative experiment. For example, clone P4.2, which recognized the EBNA3C100–111 epitope in the DRB1*16 context, proliferated and produced IFN-γ against allogeneic DRB1*0101+ LCLs. In contrast, this clone did not respond to allogeneic LCLs that express neither DR16 nor DRB1*0101. The clone PRSc5, originating from a DRB3*0202 donor, specific to EBNA2276–295/DRB3*0202 (PRS epitope) (25), was found to be alloreactive to HLA-DRB1*0101+ LCLs (Fig. 3). Of note, two other PRS-specific clones (clones SL93 and JS10) were isolated from two different DRB3*0202 donors, but none of them was alloreactive to DRB1*0101+ LCLs.

**Table II. Cross-recognition of allogeneic LCLs by EBV-specific CD4 T cell clones**

<table>
<thead>
<tr>
<th>CD4 T Cell Clones</th>
<th>EBV Reactivity in Self HLA Context</th>
<th>Reactivity to Allogeneic HLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL78</td>
<td>EBNA1475–489/DRB1*1001</td>
<td>DRB1*0102</td>
</tr>
<tr>
<td>CD59</td>
<td>EBNA1515–529/DRB1*0103</td>
<td>None</td>
</tr>
<tr>
<td>DD4</td>
<td>EBNA1529–543/DRB1*14</td>
<td>None</td>
</tr>
<tr>
<td>PRSc5</td>
<td>EBNA2276–295/DRB3*0202</td>
<td>DRB1*0101</td>
</tr>
<tr>
<td>SL93</td>
<td>EBNA2276–295/DRB3*0202</td>
<td>None</td>
</tr>
<tr>
<td>JS10</td>
<td>EBNA2276–295/DRB3*0202</td>
<td>None</td>
</tr>
<tr>
<td>AL17</td>
<td>EBNA2301–3205/DRB1*0301</td>
<td>None</td>
</tr>
<tr>
<td>GT132</td>
<td>EBNA3A364–372/DRB1*1501</td>
<td>None</td>
</tr>
<tr>
<td>c6</td>
<td>EBNA3A364–372/DRB1*1501</td>
<td>None</td>
</tr>
<tr>
<td>c67</td>
<td>EBNA3A380–395/DRB1*0101</td>
<td>None</td>
</tr>
<tr>
<td>GT76</td>
<td>EBNA3A380–395/DRB1*0101</td>
<td>None</td>
</tr>
<tr>
<td>P4.2</td>
<td>EBNA3C100–111/DRB1*16</td>
<td>DRB1*0101</td>
</tr>
<tr>
<td>c165</td>
<td>EBNA3C136–149/DRB1*0105</td>
<td>None</td>
</tr>
<tr>
<td>c91</td>
<td>LMP2291–305/DRB1*0901</td>
<td>None</td>
</tr>
<tr>
<td>c142</td>
<td>LMP2291–305/DRB1*0901</td>
<td>DRB1*0406</td>
</tr>
<tr>
<td>c162</td>
<td>LMP2291–305/DRB1*0901</td>
<td>DRB1*0901</td>
</tr>
<tr>
<td>A4.6</td>
<td>Undefined lytic epitope/DRB1*0101</td>
<td>DRB1*0101</td>
</tr>
<tr>
<td>G22</td>
<td>BHRF1111–1122/DRB1*0401</td>
<td>None</td>
</tr>
<tr>
<td>GF17</td>
<td>BHRF1111–1122/DRB1*0401</td>
<td>None</td>
</tr>
</tbody>
</table>
This is in accordance with previous data demonstrating the clonal inactivation of potentially self-reactive T cells in humans (22, 29).

Recognition of DCs and endothelial cells expressing allogeneic MHC alleles by EBV-specific CD4\(^+\) T cell clones

To address the possibility that the alloreactive EBV-specific CD4\(^+\) T cell clones exhibiting alloreactivity toward LCLs could also recognize other target cells, we tested the cytotoxic potential of three clones toward different target expressing the relevant allogeneic MHC class II allele. All targets were found to be labeled by an anti-DR mAb (data not shown). Interestingly, iDC and mDC were efficiently killed by clone AL78, provided they express HLA-DRB1*0102 molecules recognized by this alloreactive CD4 T cell clone (Fig. 4). Similarly, clone PRSc5 killed DRB1*0101\(^+\) iDC and mDC, whereas it did not kill DRB1*0102\(^+\) DC (Fig. 4). In contrast, a DRB1*0101\(^+\) melanoma cell line (M101), and a DRB1*0101\(^+\) CD8\(^+\) T cell clone were not killed by clone P4.2, which exhibited strong alloreactivity to DRB1*0101\(^+\) LCLs (Fig. 4).

Endothelial cell lines, derived from three different DR1\(^+\) donors, EC1 (DRB1*0102\(^+\)), EC2 (DRB1*0101\(^+\)), and EC3 (DRB1*0101\(^+\)), were also tested for their capacity to activate the CD4\(^+\) T cell clone PRSc5, alloreactive to DRB1*0101 LCLs. As shown in Fig. 5, clone PRSc5 exhibited rather poor killing toward the DRB1*0101\(^+\) EC2 and EC3 cell lines, whether they were pulsed or not with the EBNA2\(_{276-295}\) peptide, whereas it did not kill the DRB1*0102\(^+\) EC1 cell line. In contrast, clone PRSc5 produced high-level TNF-\(\alpha\) in response to EC2 and EC3, but not to EC1. These data are in accordance with the recognition of DRB1*0101\(^+\), but not DRB1*0102\(^+\) nor DRB1*0103\(^+\) LCLs by clone PRSc5 (Table I).

Allorecognition by EBV-specific CD4\(^+\) T cell clones shows exquisite MHC specificity

It is known that alloreactivity usually maps to a single MHC allele. The clones P4.2, A4.6, PRSc5, and AL78 were screened on a large panel of allogeneic LCLs, and for all of them allorecognition showed exquisite MHC specificity (Table I and Fig. 2A). Clones P4.2 and PRSc5 exhibited allogeneic killing toward all of the DRB1*0101\(^+\) LCLs tested, but not toward LCLs expressing other DR1 subtypes (DRB1*0102 or DRB1*0103), even though DRB1*0102 and DRB1*0103 differ from DRB1*0101 by only 2 or 3 aa, respectively. Similarly, clone AL78, alloreactive to DRB1*0102, did not respond to DRB1*0101 nor DRB1*0103. Clone A4.6, alloreactive to DRB1*0404 did not react to DRB1*0401\(^+\) LCLs, nor to DRB1*0402, *0405, *0406, and *0407 LCLs (Table I). The strict MHC specificity of allorecognition by the EBV-specific CD4\(^+\) T cell clones was observed not only for LCLs but also for DCs (Fig. 4) or endothelial cells (Fig. 5).

**Discussion**

Herpesvirus infections have long been recognized as one of the most important infectious causes of morbidity and mortality in immunocompromised transplant recipients (30, 31). Recent data suggest that most T cell alloreactivity resides within the CD4\(^+\) T cell clones toward allogeneic LCLs. A. The EBV-specific CD4\(^+\) T cell clones P4.2, A4.6, PRSc5, AL78, c142, and c162 were tested in a 4-h chromium release assay against either autologous or allogeneic LCLs, which were either unpulsed or pulsed with the cognate peptide. For all six clones, the autologous LCLs were killed only if they were peptide loaded, whereas allogeneic LCLs, expressing the relevant MHC class II allele, were killed without any previous peptide loading. E:T ratio, 10:1. These data are representative of several experiments. B. The CD4\(^+\) T cell clone AL78, specific to EBNA1/DPB1*1001 and alloreactive to DRB1*0102, was tested in a 4-h chromium release assay against different LCLs expressing or not the DPB1*1001 restricting allele and expressing or not the DRB1*0102 allogeneic MHC molecule, which were either pulsed or unpulsed with the cognate peptide, in the presence or in the absence of mAb to HLA-DR or HLA-DP. Data are presented as the mean percentage lysis. E:T ratio, 10:1. These data are representative of three different experiments.
cell subset (23), in which herpesvirus-specific T cells are well represented (16, 32, 33). By screening a panel of EBV-specific CD4 T cell clones for alloreactivity, we found that about one-third of these clones exhibited cross-reactivity toward allogeneic LCLs expressing MHC class II molecules commonly found in the population. These memory CD4 T cell clones exhibited strong cytotoxicity toward allogeneic LCLs, whereas they did not kill the autologous LCL. DCs, which represent physiologically relevant allotargets, were also efficiently killed by EBV-specific CD4 T cell clones cross-reactive to allogeneic MHC class II alleles. This is in accordance with previous data, obtained with CD8 T cells, which showed that allogeneic responses are considerably stronger than syngeneic responses (17–19). In contrast to naive T cells, memory T cells are programmed to activate quickly, with a reduced requirement for costimulatory signals (34). Upon cross-reactivity, these memory T cells strongly contribute to the alloresponse, due to their lack of requirement for costimulation, easy and rapid activation, and vigorous effector functions. Along this line, it has been shown that the pretransplant frequency of donor-specific memory lymphocytes correlates with the posttransplant risk of developing acute rejection (35).

The human immune repertoire is constantly being shaped through exposure to environmental Ags, resulting in generation of memory T cells primed to respond rapidly upon re-exposure to the

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**FIGURE 3.** Proliferation and production of IFN-γ by EBV-specific CD4 T cell clones in response to LCLs expressing allogeneic MHC class II molecules. Stimulation of EBV-specific CD4 T cell clones by autologous LCLs, allogeneic cross-reactive and allogeneic non-cross-reactive LCLs (for the allogeneic LCLs, data are presented for only one LCL cell line representative from the panel of LCLs used in the experiment). A, The production of IFN-γ by clones P4.2, A4.6, and PRSc5 against the autologous LCL, which was either pulsed or unpulsed with the cognate peptide, or allogeneic LCLs was tested. For clone A4.6, peptide loading was not done (NT) because the peptide had not been defined. The supernatant was harvested after 18 h and assayed for IFN-γ by ELISA. IFN-γ production by 500 T cells is indicated in picograms per milliliter. B, The proliferative activity of clones P4.2 and A4.6 was determined after 48-h culture of 10⁵ responder T cells with 2.5 × 10⁵ autologous or allogeneic LCLs in 100 ml of culture medium supplemented with rIL2, followed by an overnight pulse with [³H]thymidine. Results are expressed as follows: (cpm of T cells + LCLs) – (cpm of T cells alone + cpm of LCLs alone). Results are the mean of triplicate. NT, Not tested.

**FIGURE 4.** Allorecognition of DCs by EBV-specific CD4 T cell clones. Clones AL78 (specific to EBNA1/DPB1*1001, alloreactive to DRB1*0102), P4.2 (specific to EBNA3C/DRB1*16, alloreactive to DRB1*0101), and PRSc5 (specific to EBNA270s/DRB3*0202, alloreactive to HLA-DRB1*0101) were tested in a 4-h chromium release assay (E:T ratio of 10:1) against autologous or allogeneic LCLs, allogeneic iDC or mDC, an HLA-DR melanoma cell line and an HLA-DR CD8 T cell clone, which were either pulsed or unpulsed with the cognate peptide. mDC and iDC, expressing the relevant MHC class II molecule, were efficiently killed by the EBV-specific CD4 T cell clones that were used in the assay, whereas the melanoma cell line and the CD8 T cell clone were not. These data are representative of three different experiments.

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![Image](https://via.placeholder.com/150)
creased number of complications in HLA-B8.

against EC1, EC2, or EC3, which were pulsed or not with the EBNA2276–295 study documented that total HCMV-specific T cell responses rep-
ruses maintain a potent specific memory T cell response. A recent infections. In particular, it is now well documented that herpesvi-
rejection is most probably due to cross-reactivity of herpesvirus-
38). The association between hepesvirus infection and allograft transplants appeared to be a risk factor for HCMV-associated complica-
tive clonotype was shown to be alloreactive against HLA-B*4402
HLA-B35 (17, 18). The public TCR expressed by this cross-reac-
nonameric EBV peptide, FLRGRAYGL, which is alloreactive
with a higher incidence of both acute and chronic rejection (37,
graft-vs-host disease and in solid organ transplant settings, it is associated
organs from HLA-B44 donors. Similarly, it has recently been

original stimulus. Some of these primed T cells cross-react with alloantigens, in part accounting for the high frequency of alloreac-
tivity (reviewed in Ref. 36). The association between episodes of
infection and allograft rejection or graft-vs-host disease is well docu-
mented. A range of acute viral infections, most particularly herpesvirus infections, has been linked with initiating the clinical complications that often follow transplantations. In bone marrow transplanted recipients, HCMV infection is associated with graft-vs-
host disease, and in solid organ transplant settings, it is associated with a higher incidence of both acute and chronic rejection (37, 38). The association between hepesvirus infection and allograft rejec-
tion is most probably due to cross-reactivity of herpesvirus-
specific T cells toward allogeneic MHC molecules. Even in the absence of posttransplant acute viral infection, the incidence and severity of rejection episodes or graft-vs-host disease could be in-
creased by alloreactive T cell expansions induced by past viral infections. In particular, it is now well documented that herpesvi-
ruses maintain a potent specific memory T cell response. A recent study documented that total HCMV-specific T cell responses re-
present ~10% of both the CD4+ and CD8+ memory compartments in blood (16). The most striking example of alloreactive T cell expansions was described in EBV-exposed HLA-B8+ individuals, which showed a highly dominant CTL response against the nonamer EBV peptide, FLRGRAYGL, which is alloreactive against distinct members of the HLA-B44 family, HLA-B14 and HLA-B35 (17, 18). The public TCR expressed by this cross-reac-
tive clonotype was shown to be alloreactive against HLA-B*4402
and HLA-B*4405 but not B*4403, which differ by only 1 aa (17). Interestingly, HLA-B44 was identified as a “false mismatch” for HLA-B8+ renal transplant recipients (39), due to a greatly in-
creased number of complications in HLA-B8+ patients receiving organs from HLA-B44 donors. Similarly, it has recently been

shown that HLA-DR7-restricted HCMV-specific CD4+ CTL can display a dual specificity toward a glycoprotein-B epitope and the alloantigen HLA-DR4 (22). This could be related to the observation that presence of the HLA-DR7 allele in patients who receive

Peptide involvement in T cell allorecognition has long been de-
bated. Circumstantial evidence indicates that most alloreactive T cells are peptide specific, but successful attempts to identify the peptides recognized are few (reviewed in Ref. 41). Although we did not address in this study the influence of the peptide in the allo-cross-reactivity of the CD4+ T cell clones, the recognition of allogeneic DCs and, to a lesser extent, of allogeneic endothelial cells by the EBV-specific CD4+ T cell clones described in this study indicates that an EBV peptide is not necessarily involved in the allorecognition. Moreover, our data indicated that the allogeneic LCLs, expressing the relevant allogeneic MHC class II allele, were killed even though they were not loaded with the cognate peptide. This is in accordance with previous data indicating that degenerate peptide recognition by TCR may play an important role in the vigorous response of self-MHC-restricted T cells to alloantigens (18).

Although there is clear evidence that hemopoietic cells of donor origin, such as DCs and macrophages, are potent activators of alloreactive CD4+ T cells, it remains controversial whether non-
hemopoietic cells, such as vascular endothelium possess Ag-pre-
senting capacity to activate alloreactive CD4+ T lymphocytes (42). This issue is important in transplantation, because, unlike hemopoietic APCs, allogeneic vascular endothelium remains in the allograft indefinitely. It was shown in a mouse model that the vascular endothelium does not play an important role in CD4+
direct allorecognition and does not contribute to the vigor of acute rejection (43), although it can activate alloreactive CD8+ T lymphocytes both in vitro and in vivo (44). Our data indicated that human endothelial cells induced high-level TNF-α production by an EBV-specific CD4+ T cell clone cross-reactive to allogeneic MHC molecules expressed by the endothelial cells, although they were poorly killed by this clone. It should be underlined, however, that these data were obtained in vitro upon strong stimulation of MHC class II expression that may not reflect in vivo conditions. It appears from our data that LCLs and DCs could be killed by allo-
reactive CD4+ T cell clones, thus indicating that the major targets of MHC class II-restricted allosresponses are APCs that pro-
cess exogenous Ags (45). In this regard, it is interesting to note that LCLs have proven superior to PBMCs from the same individual when used as stimulator cells for the selective depletion of allo-
reactive T cells (46), a promising approach to overcome clinical problems associated with alloreactivity.

Disclosures

The authors have no financial conflict of interest.

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FIGURE 5. Allorecognition of human endothelial cells by the EBV-
specific CD4+ T cell clone PRSc5. A, Clone PRSc5 (specific to EBNAB276–295/DRB3*0202 and alloreactive to DRB1*0101) was tested in a 4-h chromium release assay (E/T ratio of 15:1) among three HLA-DR1+ endothelial cell lines (EC1 DRB1*0102, EC2 DRB1*0101*, and EC3 DRB1*0101*). The autologous LCL or against an HLA-DRB1*0101+ LCL. The different targets were pulsed or not with the EBNAB276–295 peptide at 5 μM. The data are representative of two different experiments. B, The production of TNF-α by clone PRSc5 alone (5 × 10⁶ T cells/well) or against EC1, EC2, or EC3, which were pulsed or not with the EBNAB276–295 peptide at 5 μM (3 × 10⁶ EC/well), was tested. The amount of TNF-α released in the supernatant was determined after 6 h by the WEHI 164 cytotoxicity assay production and is indicated in picograms per milliliter.