CTL Clones Isolated from an HLA-Cw-Mismatched Bone Marrow Transplant Recipient with Acute Graft-Versus-Host Disease

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CTL Clones Isolated from an HLA-Cw-Mismatched Bone Marrow Transplant Recipient with Acute Graft-Versus-Host Disease

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HLA-Cw disparity in a donor increases the risk of acute graft-vs-host disease (GVHD) after bone marrow transplantation. Acute GVHD is mediated by donor CTLs. However, mismatched HLA-Cw-specific CTLs generated in posttransplant recipients who developed acute GVHD have not been characterized in detail. In this study, CTL clones isolated from a recipient at the onset of acute GVHD who was transplanted from an HLA-A,-B, and -DRB1-matched, HLA-Cw-mismatched (recipient, Cw*0303/Cw*0702; donor, Cw*0801/Cw*0702), unrelated donor were characterized. The seven isolated CTLs, including CD4+, CD8+, and CD4+CD8+ T lymphocytes, lysed recipient cells, HLA-Cw*0303-transfected 721.211 cells, and HLA-Cw*0303-transfected donor cells, but not untransfected 721.211 cells or donor cells. Thus, all CTLs recognized the mismatched Cw*0303 molecule as an alloantigen. The sequences of Cw*0303 and Cw*0801 differ by 16 aas. Stimulation of CTLs by COS cells transfected with Cw*0303 cDNA constructs demonstrated that Cw*0303 mutants in which individual amino acids constituting peptide-binding pockets were substituted with the corresponding Cw*0801 amino acids significantly decreased IFN-γ production by all CTLs, whereas Cw*0303 mutants bearing Cw*0801 amino acids outside the positions constituting peptide-binding pockets stimulated all CTLs to the same degree as the wild-type Cw*0303 construct. These data suggest that all CTLs recognized the Cw molecule in a peptide-dependent manner. ELISPOT revealed that Cw*0303-reactive T cells accounted for one-half of the total of alloreactive T cells in the blood during GVHD. Taken together, non-self Cw-specific CTL clones with a variety of phenotypes and peptide specificities can be generated in posttransplant recipients with acute GVHD.

Materials and Methods

Study patient

A 31-year-old woman with acute lymphoblastic leukemia (ALL) received bone marrow transplantation from an HLA-A,-B, and -DRB1 allele-matched (A*0206/A*2402, B*0702/B*4801, DRB1*0101/DRB1*0901), HLA-Cw allele-mismatched (recipient, Cw*0303/Cw*0702; donor, Cw*0801/Cw*0702), killer Ig-like receptor ligand-matched, unrelated female donor. The preparative regimen consisted of 120 mg/kg cyclophosphamide and 12 Gy total body irradiation. GVHD prophylaxis consisted of 0.03 mg/kg tacrolimus and short-term methotrexate. A neutrophil count >0.5 × 10^9/L and a platelet count >20 × 10^9/L were achieved on days 14 and 22, respectively. The patient developed grade II acute GVHD involving the skin (stage 3), evaluated according to previously published criteria (16). The GVHD resolved gradually without addition of other immunosuppressive agents. The patient continued to be in complete remission 4 years after transplantation.
CTL clones were isolated from a blood sample as described previously (17). Briefly, PBMCs obtained from the recipient at the onset of acute GVHD were stimulated in vitro with aliquots of gamma-irradiated PBMCs that had been obtained from the recipient pretransplant and cryopreserved. After three weekly stimulations, the CTL clones were isolated from the polyclonal T cell culture by limiting dilution. The CTLs were expanded by transfection. Three days after selection, they were used as a target in the cytotoxicity assay. B-LCLs, 721.221 cells, and PBMCs were labeled for 2 h, and fibroblasts were labeled overnight with 51Cr. The 51Cr-labeled cytotoxicity assay. B-LCLs, 721.221, PBMCs, and fibroblasts were used as target cells in the assay (14 days after stimulation or 1 day after thawing a frozen aliquot). The T cells were used in assays with 10^6) were transfected by electroporation (220 V, 500 μF) in 200 μl of potassium-PBS with 15 μg of the pEAK10 plasmid encoding HLA-Cw*0303 cDNA and selected with 0.8 μg/ml puromycin (Edge BioSystems), beginning 48 h after transfection. Three days after selection, they were used as a target in the cytotoxicity assay. The expression of HLA-Cw3 on transfected 721.211 cells was tested by flow cytometric analysis using the anti-class I mAb W6/32 (BioLegend).

### Table I. Characteristics of isolated CTLs

<table>
<thead>
<tr>
<th>CTL</th>
<th>Recipient-LCL</th>
<th>Donor-LCL</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>60D6</td>
<td>75</td>
<td>1</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>60F1</td>
<td>85</td>
<td>2</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>64A11</td>
<td>84</td>
<td>1</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>11F1</td>
<td>73</td>
<td>0</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>52F11</td>
<td>72</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>42C5</td>
<td>74</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>46D4</td>
<td>73</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* E:T ratio was 10:1.

### Cell culture

CTL clones were isolated from a blood sample as described previously (17). Briefly, PBMCs obtained from the recipient at the onset of acute GVHD were stimulated in vitro with aliquots of gamma-irradiated PBMCs that had been obtained from the recipient pretransplant and cryopreserved. After three weekly stimulations, the CTL clones were isolated from the polyclonal T cell culture by limiting dilution. The CTLs were expanded by transfection. Three days after selection, they were used as a target in the cytotoxicity assay. B-LCLs, 721.221 cells, and PBMCs were labeled for 2 h, and fibroblasts were labeled overnight with 51Cr. The 51Cr-labeled cytotoxicity assay. B-LCLs, 721.221, PBMCs, and fibroblasts were used as target cells in the assay (14 days after stimulation or 1 day after thawing a frozen aliquot). The T cells were used in assays with 10^6) were transfected by electroporation (220 V, 500 μF) in 200 μl of potassium-PBS with 15 μg of the pEAK10 plasmid encoding HLA-Cw*0303 cDNA and selected with 0.8 μg/ml puromycin (Edge BioSystems), beginning 48 h after transfection. Three days after selection, they were used as a target in the cytotoxicity assay. The expression of HLA-Cw3 on transfected 721.211 cells was tested by flow cytometric analysis using the anti-class I mAb W6/32 (BioLegend).

### Table II. Clonotypes of isolated CTLs

<table>
<thead>
<tr>
<th>CTL</th>
<th>TCR Vβ</th>
<th>Nucleotide and Deduced Amino Acid Sequences of the CDR3 Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>60D6</td>
<td>Vβ14</td>
<td>GCC AGC AGT TTT GGG ACA GGG GAC TAC GAG CAG TAC</td>
</tr>
<tr>
<td>60F1</td>
<td>Vβ13.2</td>
<td>GCC AGC AGT TAC CCC CTT CAG CGG CAA TGG GAG ACC CAG TAC TCC</td>
</tr>
<tr>
<td>64A11</td>
<td>Vβ1</td>
<td>GCC AGC AGC CCC GAT GGA CTA GAA CGG CAG GAT GAG CAG CAG TCC</td>
</tr>
<tr>
<td>11F1</td>
<td>ND*</td>
<td>GCC AGC AGC CCC GAT GGA CTA GAA CGG CAG GAT GAG CAG CAG TCC</td>
</tr>
<tr>
<td>52F11</td>
<td>Vβ1</td>
<td>GCC AGC AGT TTT GGG ACA GGG GAC TAC GAG CAG TAC</td>
</tr>
<tr>
<td>42C5</td>
<td>Vβ1</td>
<td>GCC AGC AGC CCC GAT GGA CTA GAA CGG CAG GAT GAG CAG CAG TCC</td>
</tr>
<tr>
<td>46D4</td>
<td>Vβ13.2</td>
<td>GCC AGC AGT TTT GGG ACA GGG GAC TAC GAG CAG TAC</td>
</tr>
</tbody>
</table>

* ND, not detected.
ELISPOT

T cells were isolated from PBMCs by negative depletion using the Pan T Cell Isolation Kit II (Miltenyi Biotec) and used as responder T cells. Responder T cells at a concentration of 2 × 10^5 per well were plated in individual wells of the 96-well Multiscreen-IP filter plates (Millipore) coated with anti-human IFN-γ Ab (5 μg/ml; Mabtech) and tested in triplicate against a total of 2 × 10^5 stimulator cells: recipient B-LCL; donor B-LCL; and HLA-Cw*0303-transfected donor B-LCL. The plates were incubated for 24 h at 37°C, washed, and incubated with biotinylated anti-human IFN-γ Ab (1 μg/ml; Mabtech) for 2 h at room temperature. After addition of streptavidin (Fitzgerald Industries International) to the wells, the plates were developed with 3-amin-9-ethylcarbazol substrate kit (Vector Laboratories). Spots were counted using a microscope, and mean numbers were calculated from triplicate wells after subtraction of the number of spots obtained with medium alone.

Results

Isolation of CTLs

Seven CTLs were isolated from the peripheral blood of the recipient just after the onset of grade II acute GVHD involving the skin. All seven isolated CTLs lysed recipient B-LCL but failed to lyse donor B-LCL (Table I). Flow cytometric analysis revealed that four CTLs were CD8⁺, one was CD4⁺, and two were CD4⁺/CD8⁻. We compared the sequences of the HLA-Cw cDNA of each CTL clone with the canonical sequences of the recipient Cw*0303, the donor Cw*0801, and the recipient and donor Cw*0702 obtained from the GenBank DNA sequence database. All clones had Cw*0801 and Cw*0702 (data not shown), demonstrating that all isolated clones originated from the donor.

Clonotyping of isolated CTLs

The TCR Vβ usage of each CTL was studied, and the nucleotide sequences of the amplified PCR products of the TCR Vβ gene were determined (Table II). The CD8⁺ 60D6 CTL used VB14. The CD8⁺ 60F1 and CD4⁺ CD8⁻/⁻ 46D4 CTLs used the same VB13.2, but neither nucleotide sequences nor amino acid sequences in the CDR3 regions were identical in these two CTLs, indicating that these CTLs were different clones. Three of the CTLs, including CD8⁺ CTL 64A11, CD4⁺ CTL 52F11, and CD4⁺/CD8⁻/⁻ CTL 42C5, had the same nucleotide sequences in the CDR3 regions of their TCR Vβ1, suggesting that three of these CTLs with variable phenotypes originated from a single clone. The TCR Vβ usage of 11F1 could not be determined because this clone did not react with any of the anti-TCR Vβ Abs used in flow cytometric analysis. Thus, based on these data, the seven isolated CTLs appear to have been derived from five independent clones.

All CTL clones recognized the mismatched HLA-Cw molecule but not minor histocompatibility Ags

To identify the genes encoding the Ag recognized by the isolated CTLs, the CD8⁺ clones, 60D6 and 60F1, were initially studied. Both clones lysed all 15 B-LCL lines from unrelated individuals who shared HLA-Cw*0303 with the recipient, but not all B-LCL lines from 17 unrelated individuals who shared class I HLA molecules other than HLA-Cw*0303 (data not shown). Thus, both CTL clones recognized the HLA-Cw*0303 molecule as an alloantigen.

Next, 721.221 cells, which lack HLA class I expression on their cell surface, were transfected with a full-length HLA-Cw*0303 cDNA construct and used as a target in the cytotoxicity assay. Both 60D6 and 60F1 clones clearly lysed HLA-Cw*0303-transfected 721.221 cells, but not untransfected 721.221 cells (Fig. 1, A and B). Then, whether some of the other CTL clones recognized the HLA-Cw*0303 molecule was determined. Unexpectedly, all of the other CTLs, including CD4⁺ clones, lysed HLA-Cw*0303-transfected 721.221 cells (Fig. 1, C–G). Thus, all isolated CTLs with variable phenotypes recognized the mismatched HLA-Cw*0303 molecule as an alloantigen.

Donor B-LCL was then transfected with an HLA-Cw*0303 cDNA construct and examined in the cytotoxicity assay. All CTL clones lysed HLA-Cw*0303-transfected donor B-LCL (Fig. 1). Thus, none of the isolated CTLs recognized the recipient’s minor histocompatibility Ags.

T cell recognition of the HLA-Cw*0303 molecule with a variety of peptide specificities

Various forms of direct T cell recognition of the allogeneic MHC, ranging from peptide independent to peptide dependent, have been
TABLE III. Summary of IFN-γ production of CTLs stimulated by Cw*0303 mutants

<table>
<thead>
<tr>
<th>Cw*0303 Mutant</th>
<th>Substituted Amino Acid</th>
<th>Position</th>
<th>Cw<em>0303 → Cw</em>0801</th>
<th>Pocket(s)*</th>
<th>IFN-γ Production of CTLs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cw*0303–1</td>
<td>Gly</td>
<td>1</td>
<td>Cys</td>
<td>–</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–21</td>
<td>His</td>
<td>21</td>
<td>Arg</td>
<td>–</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–35</td>
<td>Arg</td>
<td>35</td>
<td>Gin</td>
<td>B</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–91</td>
<td>Arg</td>
<td>91</td>
<td>Gly</td>
<td>–</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–94</td>
<td>Ile</td>
<td>94</td>
<td>Thr</td>
<td>–</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–95</td>
<td>Ile</td>
<td>95</td>
<td>Leu</td>
<td>–</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–103</td>
<td>Val</td>
<td>103</td>
<td>Leu</td>
<td>–</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–114</td>
<td>Asp</td>
<td>114</td>
<td>Asn</td>
<td>D, E</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–116</td>
<td>Tyr</td>
<td>116</td>
<td>Phe</td>
<td>F</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–152</td>
<td>Glu</td>
<td>152</td>
<td>Thr</td>
<td>E</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–163</td>
<td>Leu</td>
<td>163</td>
<td>Thr</td>
<td>A</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–173</td>
<td>Lys</td>
<td>173</td>
<td>Glu</td>
<td>–</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–177</td>
<td>Glu</td>
<td>177</td>
<td>Lys</td>
<td>–</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–219</td>
<td>Trp</td>
<td>219</td>
<td>Arg</td>
<td>–</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–275</td>
<td>Glu</td>
<td>275</td>
<td>Gly</td>
<td>–</td>
<td>↓</td>
</tr>
<tr>
<td>Cw*0303–304</td>
<td>Val</td>
<td>304</td>
<td>Met</td>
<td>–</td>
<td>↓</td>
</tr>
</tbody>
</table>

* Peptide-binding pocket(s) constituted by each amino acid position.

** Significant decrease (P < 0.05) of IFN-γ production of CTLs stimulated by Cw*0303 mutants compared with the wild-type Cw*0303.

demonstrated (20). If allorecognition of isolated CTLs is dependent on peptides bound to HLA molecules, changing HLA-Cw*0303 molecules by amino acid substitutions at residues constituting peptide-binding pockets should affect the presentation of peptides and allorecognition of CTLs. The sequences of the recipient Cw*0303 and the donor Cw*0801 differ by 16 aas. Sixteen variants of Cw*0303 mutants, in which individual amino acids were substituted with the corresponding amino acid in Cw*0801 (Table III), were generated. COS cells were then transfected with each mutant and examined using the CTL stimulation assay. The expression level of HLA class I on the surface of COS cells transfected with all 16 types of HLA-Cw*0303 mutant cDNA was almost the same as that with wild-type Cw*0303 cDNA.

IFN-γ production of the 60D6 CTL was significantly decreased when stimulated by Cw*0303-116 and Cw*0303-152 mutants, in which amino acids at position 116 (tyrosine) and 152 (glutamic acid) were substituted with phenylalanine and threonine, respectively, compared with the wild-type Cw*0303 construct (Fig. 2A). Amino acids at positions 116 and 152 constitute peptide-binding pockets F and E, respectively, of the HLA class I molecule (Table III and Refs. 21 and 22). IFN-γ production of the 60F1, 11F1, and 46D4 CTLs was significantly decreased when stimulated by Cw*0303 mutants in which amino acids at positions 116/152/163 for 60F1, 35/116/152 for 11F1, and 152 for 46D4 were substituted with the corresponding Cw*0801 amino acids, compared with the wild-type Cw*0303 construct (Fig. 2, B, D, and G). All of these amino acid positions also constitute peptide-binding pockets (Table III). The 64A11, 52F11, and 42C5 CTLs, which presumably originated from a single clone, demonstrated the same pattern; IFN-γ production was significantly decreased when stimulated by Cw*0303-114, -116, or -152 mutants constituting peptide-binding pockets (Fig. 2, C, E, and F, and Table III). Finally, the Cw*0303 mutants bearing Cw*0801 amino acids outside the positions constituting peptide-binding pockets stimulated all CTLs to the same degree as the wild-type Cw*0303 construct (Fig. 2). These data suggest that all CTLs recognized the Cw molecule in a peptide-dependent manner. In addition, the fact that the seven isolated CTLs consisting of five CTL clones had five different recognition patterns for the mutated HLA-Cw*0303 molecules (Table III) suggests that CTLs for a variety of peptide/non-self Cw molecule complexes are expanded in post-HSCT recipients with acute GVHD.

**Correlation between HLA-Cw*0303-reactive T cells and acute GVHD and graft-vs-leukemia effect**

We determined the frequencies of Cw*0303-reactive T cells in the posttransplant recipient blood samples using the IFN-γ ELISPOT (Fig. 3A). Cw*0303-reactive T cells accounted for about one-half of the total of allreactive T cells on day 28 after transplant, when skin acute GVHD developed, and on day 104 after transplant, when acute GVHD subsided under immunosuppressant use, but no IFN-γ-producing Cw*0303-reactive T cells were detected on day 153 after transplant, when skin acute GVHD completely disappeared with a minimal immunosuppressant (Fig. 3B). Additionally, all isolated CTLs lysed recipient-derived dermal fibroblasts as well as recipient PBMCs, but not Cw*0303-negative fibroblasts (Fig. 4). These data are consistent with the participation of Cw*0303-reactive T cells in the development of skin acute GVHD in this patient.

We could not examine the cytotoxicity of CTL clones against autologous leukemic cells because the patient’s ALL cells were not cryopreserved. Instead, we determined whether each CTL clone lysed PBMCs containing >90% ALL cells obtained from Cw*0303-positive (n = 3) and Cw*0303-negative (n = 3) unrelated patients. All clones lysed all Cw*0303-positive ALL cells, but failed to lyse all Cw*0303-negative ALL cells (Fig. 5). These data suggest that the expression level of HLA-Cw molecules on the surface of at least some ALL cells is enough to be recognized by CTLs, and that the isolated Cw*0303-specific CTL clones might be involved in the graft-vs-leukemia effect as well as acute GVHD.

**Discussion**

In this study, several CTL clones that lysed recipient cells but not donor cells were isolated from a recipient at the onset of acute GVHD who had received bone marrow from an HLA-A, -B, and -DRB1-matched, HLA-Cw-mismatched, killer Ig-like receptor ligand-matched, unrelated donor. All isolated CTL clones recognized the mismatched HLA-Cw molecule as an alloantigen, whereas no clone recognized minor histocompatibility Ags. A question left unresolved was whether this finding is unique to this single patient or can be duplicated from additional Cw-mismatched patients. In addition, we cannot exclude the possibility that this finding could be affected by our in vitro culture conditions.
Previous statistical studies have shown that HLA-Cw matching plays an important role in the outcome of allogeneic HSCT. Analyses of transplants performed under the auspices of the Japan Marrow Donor Program identified that HLA-Cw incompatibilities were significantly associated with a higher incidence of acute GVHD and lower leukemia relapse (2, 4, 23). Analyses of transplants through the National Marrow Donor Program also demonstrated that HLA-Cw mismatch adversely affected engraftment, acute GVHD, and mortality after transplants (3, 24). Another study from the European Blood and Marrow Transplant Group showed an increased mortality trend in patients who received bone marrow transplants from HLA-Cw-mismatched unrelated donors (25). Alternatively, the impact of HLA-Cw mismatches on T cell responses in post-HSCT recipients remains largely unexplored. A few studies have shown that the frequency of CTL precursors is closely correlated with the occurrence of acute GVHD (26–29), and no data are available on the characteristics of individual T cell clones generated in patients who actually received an allogeneic HSCT from an HLA-Cw-mismatched donor and developed acute GVHD.

Isolated CTL clones included CD8⁺, CD4⁺, and CD4⁺CD8⁺ cells. In contrast to precursor T cells in the thymic cortex that simultaneously coexpress CD4 and CD8 molecules, mature peripheral blood T cells released in the circulation following a selection process generally express either CD4 or CD8. However, double-positive T cells expressing both CD4 and CD8 have been

![Figure 2](image-url)
producing cells is shown against recipient B-LCL (A) and CD4+ differentiated from CD4+ Double-positive T cells in the peripheral blood may be terminally differentiated from single-positive T cells, or, alternatively, re-derived from the thymus as mature cells (31). The thymus of HSCT recipients is severely damaged by preconditioning regimens consisting of high-dose anticancer drugs and/or irradiation. Whether circulating double-positive T cells in post-HSCT recipients at the onset of acute GVHD are terminally differentiated from single-positive T cells or directly derived from the thymus, and whether the higher numbers of double-positive T cells in target organs correlate with a poor GVHD prognosis are of considerable interest. Also, further studies to isolate CTL clones from additional HLA-mismatched HSCT recipients could provide the frequency information relating to the distribution of CD8+ T cells in post-HSCT recipients in a cytotoxicity assay. The lysis of recipient PBMC, recipient-derived dermal fibroblasts, HLA-Cw*0303-negative unrelated patient-derived dermal fibroblasts, and donor B-LCL is shown as the means of triplicate cultures at various E:T ratios.

FIGURE 3. Detection of HLA-Cw*0303-specific CTLs in recipient PBMCs after transplantation. A. Representative ELISPOT wells show triplicate results of PBMCs at 28 days after HSCT stimulated by recipient B-LCL, donor B-LCL, and HLA-Cw*0303-transfected donor B-LCL. B. The frequency of CTLs in PBMCs that recognized HLA-Cw*0303 at different time points (28, 104, and 153 days after HSCT) was measured by IFN-γ ELISPOT analysis. The frequency of IFN-γ-producing cells is shown against recipient B-LCL (I), donor B-LCL (II), and HLA-Cw*0303-transfected donor B-LCL (III). Data are the means of triplicate determinations.

FIGURE 4. Cytotoxicities of CTLs against recipient-derived dermal fibroblasts for CTL clones 60D6 (A), 60F1 (B), 64A11 (C), 11F1 (D), 52F11 (E), 42C5 (F), and 46D4 (G) in a cytotoxicity assay. The lysis of recipient PBMC, recipient-derived dermal fibroblasts, HLA-Cw*0303-negative unrelated patient-derived dermal fibroblasts, and donor B-LCL is shown as the means of triplicate cultures at various E:T ratios.

described in several pathological conditions, including autoimmune, neoplastic, and chronic inflammatory disorders, as well as in normal individuals (30, 31). These data suggest the existence of non-self HLA-specific, CD4+ CD8+ CTLs in the peripheral blood of an allogeneic bone marrow transplant recipient. Isolated CD4+ CD8+ CTL 42C5 shared TCR Vβ with CD8+ CTL 64A11 and CD4+ CTL 52F11, although we cannot rule out the possibility that this finding could be the result of in vitro culture conditions. Double-positive T cells in the peripheral blood may be terminally differentiated from CD4+ or CD8+ T cells, or, alternatively, released from the thymus as mature cells (31). The thymus of HSCT recipients is severely damaged by preconditioning regimens consisting of high-dose anticancer drugs and/or irradiation. Whether circulating double-positive T cells in post-HSCT recipients at the onset of acute GVHD are terminally differentiated from single-positive T cells or directly derived from the thymus, and whether the higher numbers of double-positive T cells in target organs correlate with a poor GVHD prognosis are of considerable interest. Also, further studies to isolate CTL clones from additional HLA-mismatched HSCT recipients could provide the frequency information relating to the distribution of CD8+ T cells in post-HSCT recipients in a cytotoxicity assay. The lysis of recipient PBMC, recipient-derived dermal fibroblasts, HLA-Cw*0303-negative unrelated patient-derived dermal fibroblasts, and donor B-LCL is shown as the means of triplicate cultures at various E:T ratios.

All CTLs recognized the Cw molecule in a peptide-dependent manner. To assess the importance of peptides presented by non-self HLA-Cw molecules in allorecognition of isolated CTLs, HLA-Cw*0303 mutants were constructed, and the residue that affects recognition of CTLs was examined. IFN-γ production was decreased when stimulated by Cw*0303 mutants in which an amino acid constituting a peptide-binding pocket was substituted with a Cw*0801 amino acid, whereas the Cw*0303 mutants bearing Cw*0801 amino acids outside the positions constituting peptide-binding pockets stimulated all CTLs to the same degree as the wild-type Cw*0303 construct. These data demonstrate that peptides bound to HLA molecules play an important role in the recognition of these CTLs. These mutations appear to alter the affinity of peptides with the HLA-Cw*0303 molecule, resulting in presentation of different peptide repertoires or no peptides and subsequent loss of CTL recognition. Another possibility is that the mutations may change the conformation of the peptide-HLA molecule complex so that T cell receptors can no longer effectively recognize them. Although the peptides recognized by isolated CTLs are uncertain, each CTL clone showed a different recognition pattern for the mutated HLA-Cw*0303 molecules. Thus, it is reasonable to conclude that polyclonal CTLs for a variety of peptides presented by non-self HLA-Cw molecules are expanded in post-HSCT recipients from Cw-mismatched donors, although the possibility that some T cell clones are dominant cannot be excluded (32). Further efforts to identify the peptides recognized by isolated CTLs should help to elucidate the mechanisms of immunoreactions, such as acute GVHD in HLA-Cw*0303-mismatched HSCT.

A recent statistical analysis showed a significant association between some specific amino acid substitutions of recipient and donor HLA class I molecules and the occurrence of acute GVHD after unrelated bone marrow transplantation (33). Substitutions of amino acids at positions 9 and 116 in the HLA-A molecule and positions 9, 77, 80, 99, 116, and 156 in the HLA-Cw molecule
FIGURE 5. Cytotoxicities of CTLs against ALL cells for CTL clones 60D6 (A), 60F1 (B), 64A11 (C), 11F1 (D), 52F11 (E), 42C5 (F), and 46D4 (G) in a cytotoxicity assay. PBMCs containing >90% ALL cells were obtained from Cw*0303-positive (n = 3; patients 1–3) and Cw*0303-negative (n = 3; patients 4–6) unrelated patients and used as a target in the cytotoxicity assay. Data are means of triplicate cultures at various E:T ratios between recipient and donor were identified as risk factors for severe acute GVHD. Because amino acids at positions 77 and 80 in the HLA-Cw molecule are epitopes for killer Ig-like receptor (34), these substitutions may be associated with alloreactivity of donor NK cells. Amino acids at other positions, 9, 99, 116, and 156, in the HLA-class I molecule constitute peptide-binding pockets. Therefore, higher T cell responses may explain the increased occurrence of acute GVHD in recipients who have these amino acid substitutions. However, no immunobiological evidence to support this hypothesis exists. The present study clearly demonstrated that recognition of Cw*0303-specific CTLs generated in the recipient with acute GVHD was affected by substitutions of amino acids constituting peptide-binding pockets in the Cw*0303 molecule; in particular, recognition of most CTL clones was affected by substitution of an amino acid at position 116.

In conclusion, T cells recognizing recipient Cw molecules in a peptide-dependent manner are important in the alloreaction that occurs in post-HSCT recipients from HLA-Cw-mismatched donors.

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Disclosures

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References


