Cytokine Gene Polymorphisms Associating With Severe Acute Graft-Versus-Host Disease in HLA-Identical Sibling Transplants

By Peter G. Middleton, Penelope R.A. Taylor, Graham Jackson, Stephen J. Proctor, and Anne M. Dickinson

It is now well known that the initial phase of graft-versus-host disease (GVHD) involves cytokine release during preconditioning of the recipient of an allogeneic bone marrow transplant (BMT). Tumor necrosis factor \( \alpha \) (TNF\( \alpha \)), in particular, has been implicated in pathological damage and is released pretransplant due to irradiation and cytotoxic preconditioning regimens. Interleukin-10 (IL-10), a natural immunosuppressant of TNF\( \alpha \), may be involved in downregulation of these responses, which may be an individual patient-specific effect. In this study, we determined the genotype for polymorphisms associated with TNF\( \alpha \) and IL-10 in 80 potential allo-BMT recipients and correlated the genotype with the severity of GVHD in 49 patients for whom clinical data relating to GVHD was available. The widely studied TNF\( \alpha \) -308 polymorphism does not show any significant associations, but the d3 homozygous allele of the TNFd microsatellite is preferentially associated with grade III/IV GVHD (7 of 11 patients) compared with its occurrence in 8 of 38 patients with grade 0/II GVHD (\( P = .006 \)). Alleles of the IL-10 -1064 promoter region microsatellite polymorphism that possess greater numbers of dinucleotide (CA) repeats also significantly associate with more severe GVHD. This region has been demonstrated to be important in the regulation of the IL-10 promoter. Eighteen of 38 patients with grade 0-II GVHD possessed alleles with greater numbers (12 or more) of dinucleotide repeats, compared with 9 of 11 cases with grade III-IV GVHD (\( P < .02 \)). Of the 38 patients with grade 0-II GVHD, 3 of 38 had a both TNFd/d3 and IL-10 (12-15) genotype, compared with 6 of 11 patients with grade III-IV GVHD (\( P < .001 \)). There was no association of either the TNFd or IL-10 microsatellite polymorphisms with mortality (\( P = .43 \) and .51, respectively). Our results suggest that patient cytokine gene polymorphism genotypes may influence GVHD outcome by affecting cytokine activation during the pretransplant conditioning regimens, and these results are the first to suggest a genetic predisposition to this important transplant-related complication.

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produced high levels of TNFα and low levels of IL-10.\textsuperscript{15,16} A number of polymorphisms of the IL-10 gene have been described, including single base (G/A) polymorphism (\textsuperscript{\textemdash}1082) associated with altered levels of in vitro IL-10 expression\textsuperscript{16} and microsatellite polymorphism (\textsuperscript{\textemdash}1064) mapping near candidate IL-10 gene control elements.\textsuperscript{17} Recent findings suggest a role for some of these polymorphisms in determining rejection in heart transplant recipients.\textsuperscript{15,16}

In this present study we have determined the BMT recipient TNFα and IL-10 genotype for gene polymorphisms associated with either reported cytokine productivity or candidate gene control regions and correlated results with HLA phenotype and clinical outcome, including the incidence and severity of posttransplant GVHD.

MATERIALS AND METHODS

Patients and normal controls for polymorphism studies. Eighty patients being considered for transplant with underlying hematological malignancies predominantly acute leukemia (ALL) or chronic granulocytic leukemia (CGL) were tested for TNFα and IL-10 polymorphism allele frequencies and genotype. Twenty-eight normals (laboratory personnel) were also tested for TNFα and IL-10 polymorphism allele frequencies and genotype. The results are included in Fig 1A and B.

Cytokine gene polymorphism studies were performed on all patients, including the 49 of 80 patients who subsequently underwent BMT from an HLA-- and HLA-DR--matched, mixed lymphocyte culture-negative, sibling donor. The TNFα and IL-10 polymorphism results from these patients were correlated with incidence and severity of GVHD. These patients included 32 males and 17 females (median age, 33; age range, 14 to 47 years). All 49 patients received non-T-cell--depleted marrow grafts. The underlying diseases were CGL (n = 12), acute myeloid leukemia (AML; n = 18), ALL (n = 15), Hodgkin’s disease (HD; n = 1), non-Hodgkin’s lymphoma (NHL; n = 1), and myelodysplastic syndrome (MDS; n = 2).

Conditioning regimens and GVHD prophylaxis. Before January 1991, standard conditioning for the patients with acute leukemia was with cyclophosphamide (120 mg/kg) and total body irradiation (TBI; 12 Gy in 6 fractions in 3 days at 25 cGy/min). Since January 1991, the TBI dose has remained the same, but melphalan (3 mg/kg) has been substituted for the cyclophosphamide. Patients with MDS were conditioned with cyclophosphamide alone. Patients with CGL were usually conditioned with busulfan (4 mg/kg/d for 4 days) and cyclophosphamide.

The majority of patients were treated with either cyclophosphamide or melphalan plus TBI (Table 1). The number of patients treated with these two types of conditioning regimes were equally distributed between the cohorts of patients developing GVHD grades 0-II and GVHD III-IV (30 of 38 and 9 of 11, respectively; χ\textsuperscript{2} = .043; df = 1; P = .835; Tables 1 and 2).

GVHD prophylaxis and therapy. GVHD prophylaxis was with cyclosporin alone, administered intravenously at a dose of 5 mg/kg until oral treatment could be tolerated, when the same dose was administered by mouth. Once clinical GVHD was diagnosed, standard therapy was administered with high-dose steroids; if there was no immediate response, this was followed by increased cyclosporin (5 to 10 mg/kg).

Ethics committee approval and informed consent. This study was approved by the local ethics committee and informed consent was obtained from all patients and normal controls under study.

HLA typing. All patients in this study were tested by conventional serology for HLA A and B alleles along with low resolution molecular typing of DRB1 using polymerase chain reaction (PCR) sequence-
compared with a control of known genotype (TNF $^2$308 1/2, TNFd1/d3, IL-10 i9/13) to ensure accurate interpretation. Allele designations, eg, d1/d3, 9/13 etc, are as described in the original reports of these polymorphisms.7,10,19

Statistical analyses. Comparisons between patient groups were made using the $\chi^2$ test for heterogeneity.

RESULTS

Incidence and severity of GVHD. GVHD was diagnosed and graded according to previously published criteria.20 Of the 49 patients transplanted, 9 showed no evidence of acute GVHD. Fourteen patients developed grade I GVHD: 10 with skin alone, 3 with skin plus gastrointestinal involvement, and 1 with gastrointestinal involvement alone. Fifteen patients developed grade II GVHD; all had skin involvement, 4 had hepatic involvement (2 with gastrointestinal disease), and 3 had skin and gastrointestinal involvement with no evidence of liver disease. Eleven of 49 patients developed severe (grade III-IV) GVHD. All 9 patients with grade III disease had skin and gastrointestinal GVHD, together with liver involvement in 4 patients. In this whole cohort, only 2 patients died of GVHD (Tables 1 and 2).

Table 1. Patient Characteristics and Cytokine Genotypes With the Development of GVHD Grade 0-II

<table>
<thead>
<tr>
<th>UPN</th>
<th>Diagnosis</th>
<th>CR</th>
<th>HLA Type</th>
<th>TNF-308</th>
<th>IL-10-1064</th>
<th>TNFd</th>
<th>GVHD Occur.</th>
<th>Survival Post-BMT (mo)</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MDS</td>
<td>C</td>
<td>A1 A32 B8 B18 DR2 DR9</td>
<td>F1/F2</td>
<td>i10/15*</td>
<td>d1/d3</td>
<td>0</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>AML</td>
<td>C/TBI</td>
<td>A28 Aw19 B40 Bw16 DR4</td>
<td>F1/F1</td>
<td>i7/9</td>
<td>d3/d3t</td>
<td>0</td>
<td>163</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ALL</td>
<td>C/TBI</td>
<td>A2 B37 B44 DR7</td>
<td>F1/F1</td>
<td>i7/13*</td>
<td>d3/d5</td>
<td>0</td>
<td>26 Relapse</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>ALL</td>
<td>C/TBI</td>
<td>A1 A3 B8 B7 DR3 DR2</td>
<td>F1/F2</td>
<td>i9/13*</td>
<td>d1/d3</td>
<td>0</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>AML</td>
<td>M/TBI</td>
<td>A1 A3 B37 B5 DR2 DR7</td>
<td>F1/F1</td>
<td>i11/11</td>
<td>d3/d4</td>
<td>0</td>
<td>14 Relapse</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>NHL</td>
<td>C/TBI</td>
<td>A2 A32 B35 B5</td>
<td>F1/F1</td>
<td>i7/9</td>
<td>d3/d4</td>
<td>0</td>
<td>7 Fungal infection</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>CML</td>
<td>C/BU</td>
<td>A3 A24 B44 B15</td>
<td>F1/F1</td>
<td>i9/9</td>
<td>d3/d5</td>
<td>0</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>CML</td>
<td>C/TBI</td>
<td>A3 B7 B6 DR2 DR4</td>
<td>F1/F1</td>
<td>i9/11</td>
<td>d3/d3t</td>
<td>0</td>
<td>38 Relapse</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>ALL</td>
<td>C/TBI</td>
<td>A1 A24 B8 B7 DR2 DR3</td>
<td>F2/F2</td>
<td>i9/9</td>
<td>d1/d1</td>
<td>0</td>
<td>20 Relapse</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>ALL</td>
<td>C/BU</td>
<td>A2 B38 B70</td>
<td>F1/F1</td>
<td>i9/9</td>
<td>d3/d3t</td>
<td>I</td>
<td>10 Relapse</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>ALL</td>
<td>C/TBI</td>
<td>A2 A25 B15 B27 DR2 DR7</td>
<td>F1/F1</td>
<td>i9/9</td>
<td>d3/d4</td>
<td>I</td>
<td>3 Viral infection</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>CML</td>
<td>C/TBI</td>
<td>A1 A2 B8 B17 DRw6 DR7</td>
<td>F1/F1</td>
<td>i9/9</td>
<td>d4/d4</td>
<td>I</td>
<td>156</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>AML</td>
<td>C/BU</td>
<td>A3 A24 B7 DR1 DR4</td>
<td>F1/F1</td>
<td>i9/13*</td>
<td>d3/d3t</td>
<td>I</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>CML</td>
<td>C/BU</td>
<td>Aw33 B44 DR7</td>
<td>F1/F1</td>
<td>i10/13*</td>
<td>d4/d4</td>
<td>I</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>ALL</td>
<td>C/TBI</td>
<td>A1 A2 B7 B6 DR2 DR3</td>
<td>F1/F1</td>
<td>i9/13*</td>
<td>d1/d3</td>
<td>I</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>AML</td>
<td>C/TBI</td>
<td>A1 B8 B44 DR3 DR4</td>
<td>F1/F2</td>
<td>i9/11</td>
<td>d1/d3</td>
<td>I</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>HD</td>
<td>M/V1</td>
<td>A3 B7 DR2</td>
<td>F1/F1</td>
<td>i13/15*</td>
<td>d3/d3t</td>
<td>I</td>
<td>23 Relapse</td>
<td></td>
</tr>
</tbody>
</table>

Characteristics and results on patients with grade 0-II GVHD showing unique patient number (UPN), conditioning regimen (CR) diagnosis, available HLA typing results, genotype for the TNF $^2$308, IL-10 $^2$1064, and TNFd microsatellite polymorphisms, occurrence of acute GVHD, survival, and cause of death.

*Patient who possessed an IL-10 microsatellite allele with greater numbers of dinucleotide repeats (alleles 12-15).
†Patients who were homozygous for the TNFd3 allele (d3/d3).

The results were compared with a control of known genotype (TNF $^2$308 1/2, TNFd1/d3, IL-10 i9/i13) to ensure accurate interpretation. Allele designations, eg, d1/d3, 9/13 etc, are as described in the original reports of these polymorphisms.7,10,19

Statistical analyses. Comparisons between patient groups were made using the $\chi^2$ test for heterogeneity.

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TNF$^\alpha$ $^2$308 polymorphism. Of the 80 patients tested, 3 were homozygous for the uncommon TNF2 allele (the allele frequencies would predict 2.1 homozygotes) and 20 cases were heterozygous (TNF1/2). The allele frequencies of TNF1 and TNF2, respectively, were 0.838 and 0.162. These results were
Table 2. Patient Characteristics and Cytokine Genotypes With the Development of GVHD Grades III-IV

<table>
<thead>
<tr>
<th>UPN</th>
<th>Diagnosis</th>
<th>CR</th>
<th>HLA Type</th>
<th>TNF – 308 Genotype</th>
<th>IL-10 Genotype</th>
<th>TNFd Genotype</th>
<th>GVHD Occurrence</th>
<th>Survival Posttransplant (mo)</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>CGL</td>
<td>Cy/TBI</td>
<td>A2 A9 (24) B7 Bu22 (55) Cw3</td>
<td>F1/F1</td>
<td>i13/13*</td>
<td>d3/d3†</td>
<td>III</td>
<td>152</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>AML</td>
<td>M/TBI</td>
<td>A2 A30 (19) B13 B39 (16) DR17 DR8</td>
<td>F1/F1</td>
<td>i9/13*</td>
<td>d3/d3†</td>
<td>III</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>ALL</td>
<td>M/TBI</td>
<td>A2 A30 (19) B27 B44 (12) DR1501-1503 DR0701</td>
<td>F1/F1</td>
<td>i11/13*</td>
<td>d3/d3†</td>
<td>III</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>CGL</td>
<td>Bu/M</td>
<td>A1 A11 B5 B57 (17) DR4 DR71 (3)</td>
<td>F1/F1</td>
<td>i12/12*</td>
<td>d4/d4</td>
<td>III</td>
<td>2</td>
<td>Fungal infection</td>
</tr>
<tr>
<td>43</td>
<td>AML</td>
<td>M/TBI</td>
<td>A25 A31 B18 B40 DR2 DR4</td>
<td>F1/F1</td>
<td>i8/15*</td>
<td>d3/d3†</td>
<td>III</td>
<td>1</td>
<td>Allotoxicity</td>
</tr>
<tr>
<td>44</td>
<td>CGL</td>
<td>Cy/TBI</td>
<td>A2 A3 B8 B5 DR3 DR5</td>
<td>F1/F2</td>
<td>i8/13*</td>
<td>d1/d3</td>
<td>III</td>
<td>2</td>
<td>GVHD and viral infection</td>
</tr>
<tr>
<td>45</td>
<td>AML</td>
<td>M/TBI</td>
<td>A2 A3 B7 DR2 DR4</td>
<td>F1/F1</td>
<td>9/14*</td>
<td>d3/d3†</td>
<td>III</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>ALL</td>
<td>CGL</td>
<td>A1 A3 B17 B15 DR v DR7</td>
<td>F1/F1</td>
<td>i9/9</td>
<td>d4/d4</td>
<td>III</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>AML</td>
<td>M/TBI</td>
<td>A1 A3 B8 B35 DR1 DR17 (3)</td>
<td>F1/F2</td>
<td>i9/13*</td>
<td>d1/d4</td>
<td>III</td>
<td>122</td>
<td>Nontransplant?? related death</td>
</tr>
<tr>
<td>48</td>
<td>MDS</td>
<td>Bu/Cy</td>
<td>A11 A32 B7 B4 DR4 DR7</td>
<td>F1/F1</td>
<td>i9/9</td>
<td>d3/d3†</td>
<td>IV</td>
<td>2</td>
<td>GVHD</td>
</tr>
<tr>
<td>49</td>
<td>AML</td>
<td>M/TBI</td>
<td>A2 A29 (19) B44 (12) DR4 DR7</td>
<td>F1/F1</td>
<td>i10/13*</td>
<td>d3/d3†</td>
<td>IV</td>
<td>2</td>
<td>VOD</td>
</tr>
</tbody>
</table>

Characteristics and results on patients with grade III-IV GVHD showing unique patient number (UPN), diagnosis, conditioning regimes available HLA typing results, genotype for the TNF-308, IL-10 –1064, and TNFd microsatellite polymorphisms, occurrence of acute GVHD, survival, and cause of death. 

Abbreviations: VOD, veno-occlusive disease; Cy/TBI, cyclophosphamide/TBI; M/TBI, melphalan/TBI. C/Bu, Busulphan; M/VP16/B, melphalan/ atoposide/BCNU.

*Patients who possessed an IL-10 microsatellite allele with greater numbers of dinucleotide repeats (alleles 12-15).

†Patients who were homozygous for the TNFd3 allele (d3/d3).

The importance of the cytokine cascade in both the initial preconditioning and posttransplant phases of GVHD is well established. TNFα secretion is of particular importance during the irradiation and cytotoxic treatment of the recipient before transplant giving rise to initial endothelial cell damage and aiding T-cell activation by upregulation of class I, class II, and adhesion molecules. Several reports have dealt with the measurement of serum TNFα and its role in predicting GVHD. One problem that arises is that independent of the problems associated with serum measurements of cytokines is that high levels of TNFα are also associated with other transplant-related complications, including infection and veno-occlusive disease. 

comparable with those quoted for a normal Caucasian population (0.84 and 0.16, respectively, measured on 40 individuals [80 alleles]7). In common with other studies, a strong association of the TNF2 allele with HLA haplotypes containing DR3, and a weaker association with DR4 was observed. (All but 4 of the observed TNF2 alleles were carried by individuals possessing a DR3 or a DR4 haplotype.)

The rare allele TNF2 did not associate with incidence or severity of GVHD. Thirteen of 38 patients with GVHD grade 0-II and 2 of 11 patients with grade III/IV GVHD possessed a TNF2 allele. There was no association of GVHD with HLA type.

Allele frequencies of the TNFd and IL-10 microsatellite polymorphisms. The allele frequencies of TNFα and IL-10 microsatellites found in the 80 patients tested, along with those previously reported in other studies, and a panel of 28 unselected normals are shown in Fig 1A and B. The observed allele frequencies were similar to those reported elsewhere, indicating that the appropriate allele designations were made for patient and normal control cohorts. The frequency of predicted homozygotes (as predicted by Hardy-Weinberg equilibrium) for the more common alleles compare with the observed frequencies, suggesting that homozygous genotypes were being appropriately assigned in the study group (TNFd3/d3 predicted v observed; 23.1 v 26; IL-10 i9/9 predicted v observed; 14.45 v 15; i9/13 predicted v observed; 12.75 v 13).

TNFd and IL-10 microsatellite polymorphisms and association with GVHD. The d3 allele of the TNFd microsatellite was preferentially associated with grade III/IV GVHD, with 7 of 11 patients possessing the d3/d3 genotype, compared with its occurrence in 8 of 38 patients with grade 0-II (χ² = 7.598; df = 1; P < .006; Tables 1 and 2).

Alleles of the IL-10 –1064 promoter region microsatellite polymorphism that possessed greater numbers of dinucleotide repeats (alleles 12, 13, 14, or 15, as described by Eskdale and Gallagher15) also significantly associated with more severe GVHD. Eighteen of 38 patients with grades 0-II GVHD possessed high repeat number alleles (12-15; Table 1), compared with 9 of 11 patients with grades III-IV GVHD only (χ² = 5.443; df = 1; P < .02; Table 2).

If both TNFd and IL-10 genotypes were considered together, there appears to be a retrospective association with GVHD severity and occurrence. Of the 38 patients with grade 0-II GVHD, 3 of 38 had a TNFd3/d3/IL-10 (12-15) genotype, compared with 6 of 11 patients with grade III-IV GVHD (χ² = 14.11; df = 1; P < .001).

TNFd and IL-10 microsatellite polymorphisms and association with death and relapse. The TNFd3/d3 genotype was not associated with increased mortality; 8 of 15 patients with the homozygous genotype died, compared with 14 of 34 patients who did not have this allele (χ² = .62; df = 1; P = .43). Possession of an IL-10 genotype with alleles containing greater numbers of dinucleotide repeats (alleles 12, 13, 14, or 15) was not associated with mortality; χ² analysis of its association failed to reach significance (χ² = .42; df = 1; P = .51). Of the 9 patients who died from disease relapse, 8 had grade 0-I GVHD, compared with 1 relapse from the group of patients with grade II-III-IV GVHD (χ² = 7.79; df = 1; P < .006).

DISCUSSION

The importance of the cytokine cascade in both the initial preconditioning and posttransplant phases of GVHD is well established. TNFα secretion is of particular importance during the irradiation and cytotoxic treatment of the recipient before transplant giving rise to initial endothelial cell damage and aiding T-cell activation by upregulation of class I, class II, and adhesion molecules. Several reports have dealt with the measurement of serum TNFα and its role in predicting GVHD. One problem that arises is that independent of the problems associated with serum measurements of cytokines is that high levels of TNFα are also associated with other transplant-related complications, including infection and veno-occlusive disease.3
In this present study, the d3 allele of the TNF\(\alpha\) polymorphism was found to be associated with severe GVHD grades III/IV but not with overall clinical survival. This result suggests that the effect seen in the d3/d3 TNF\(\alpha\) genotype may be susceptible to immunosuppression as suggested by Turner et al\(^2\) in studies on heart transplant recipients in which the effect was related to the degree of immunosuppression. The increased incidence of GVHD in the homozygous d3/d3 cohort was associated with increased GVHD, but also this subpopulation was possibly more responsive to the GVHD therapy in the form of increased cyclosporin and methotrexate and therefore did not succumb to fatal GVHD. A similar effect may be the explanation for the lack of correlation with GVHD mortality and IL-10 (12-15) allele polymorphisms. This hypothesis can only be tested in larger cohorts of patients with and without increased GVHD prophylaxis and altered therapy. Our studies have also shown that the \(-308\) polymorphism was not associated with GVHD severity. Mayer et al\(^4\) have recently completed a study on 53 CML allograft recipients indicating a possible role of the \(-308\) polymorphism in severe GVHD. That study was confined to CML patients and included matched unrelated donor transplants as well as HLA-identical siblings (personal communication, 1998) and therefore may not be directly compared with this present study. Our study is also too small to correlate TNF\(-308\) polymorphism with incidence or severity of GVHD in particular cohorts of leukemia patients.

A large number of studies have looked unsuccessfully for associations between TNF\(\alpha\) genotype and disease incidence, severity, or susceptibility in a range of immunoregulatory disorders. These include studies on rheumatoid arthritis,\(^21,22\) systemic lupus erythematosus,\(^23\) inflammatory bowel disease,\(^24\) lichen sclerosus,\(^25\) and ankylosing spondylitis.\(^26\) An association between the TNF\(\alpha\) allele and the incidence of insulin-dependent diabetes mellitus has been reported, but this association is not independent of the HLA type and probably reflects linkage disequilibrium between the TNF\(\alpha\) polymorphism and the ancestral HLA haplotype.\(^27\) A similar association has been seen in rheumatoid arthritis patients with systemic lupus erythematosus.\(^28\) An association between disease incidence and the TNF\(\alpha\) allele that is independent of the HLA type has been reported in studies on asthma.\(^29\) Taken together, these findings indicate that the TNF\(\alpha\) genotype may not be important in immunoregulatory disorders and that other candidate loci or genetic elements must be considered.

A proven association of cytokine gene polymorphisms with GVHD would undoubtedly enable the clinician to modify therapy in those patients predicted to develop GVHD by virtue of their underlying cytokine genotype. The study reported here documents the possible role of IL-10 gene polymorphism in GVHD. Analysis of both the TNF\(\alpha\) and IL-10 polymorphisms and GVHD severity was based on knowledge that grade 0-II GVHD is more susceptible to therapeutic intervention than grades III-IV, which are often fatal.\(^18\) This increase in risk of severe GVHD if possessing risk-associated alleles for both the TNF\(\alpha\) d3/d3 and IL-10 (12, 13, 14, and 15) genotype was highly significant (\(P < .001\)). However, possession of either genotype alone was also significant. These results suggest both an interacting role for TNF\(\alpha\)s and IL-10 and a role for both as independent indicators of severity of GVHD. This further suggests that other factors undoubtedly regulate TNF\(\alpha\) and IL-10 production and play a role in the ultimate degree of GVHD.

The extension of these studies to include other indicators/predictors of GVHD in HLA-identical siblings, such as minor histocompatibility testing,\(^30\) HTL\(\alpha\)p frequency analysis,\(^31\) cytokine production,\(^32\) and/or predicting GVHD using a skin explant model,\(^33\) may allow the development of an individual risk index for GVHD. Such a risk index would allow for improved management for GVHD, which is still the major cause of morbidity and mortality after allogeneic BMT.

Recent approaches to therapy include engineering a shift in cytokine profiles before BMT as a way to attenuate inflammatory processes of GVHD.\(^3,5\) These approaches include the possible use of anti-TNF\(\alpha\) antibodies and recombinant IL-10, a strong inhibitor of TNF\(\alpha\). The finding of 8 of 9 deaths from disease relapse in the grade 0-I GVHD group suggests that prior knowledge of a patient’s risk status for cytokine production might be of value not only in attenuating the effects of GVHD in high-risk individuals, but also in potentially upregulating the response in GVHD low-risk individuals to manipulate the graft-versus-leukemia (GVL) effect. Such management options would only become reality if accurate GVHD/GVL predictions for an individual patient could be made.

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Cytokine Gene Polymorphisms Associating With Severe Acute Graft-Versus-Host Disease in HLA-Identical Sibling Transplants

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