Cytokine Gene Polymorphism in Sympathetic Ophthalmia

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PURPOSE. Sympathetic ophthalmia (SO) is a prototypical autoimmune disease in which injury to one eye causes sight-threatening inflammation in the otherwise normal contralateral eye. Previous work found that human leukocyte antigen alleles HLA-DRB1*04 and DQA1*03 are markers of increased susceptibility and severity in British and Irish patients. Evidence is accumulating that single nucleotide polymorphisms (SNPs) in cytokine genes can also influence the development of autoimmune disease through their effect on levels of cytokine production. The purpose of this study was to determine whether polymorphisms in the cytokine genes are important markers for disease severity and outcome in patients with SO.

METHODS. Twenty-six British and Irish patients meeting well-defined criteria for the diagnosis of SO were compared with 48 matched controls. Genotyping of SNPs in the TNFα, TNFβ, and IL-10 genes was performed using the polymerase chain reaction and sequence-specific primers (SSP-PCRs) and of the CTLA-4 and TNF receptor 2 genes using restriction length polymorphism-PCR (RFLP-PCR).

RESULTS. Significant associations were found between the IL-10 –1082 SNP and disease recurrence from previously stable disease and the level of steroids required for maintenance therapy. In addition, the GCC IL-10 promoter haplotype (IL-10 –1082G, –819C, –592C) was found to be protective against disease recurrence.

CONCLUSIONS. These results show that cytokine gene polymorphisms are markers for the severity of disease in SO. They were found to be associated with recurrence of previously stable disease and with the level of maintenance steroid treatment required to control inflammatory activity. (Invest Ophthalmol Vis Sci. 2005;46:4245–4250) DOI:10.1167/iovs.05-0126

Sympathetic ophthalmia (SO) is a prototypical autoimmune disease in which injury to one eye causes sight-threatening inflammation in the otherwise normal contralateral eye.1 Although the precise immunopathogenesis is not known, the initiating injury to the “exciting” eye is thought to disrupt uveoscleral tissue and to compromise the relative immune privilege of the eye.2 Subsequent sensitization to previously sequestered ocular antigens leads to posterior uveitis associated with granuloma formation at the retinochoroidal interface mediated by major histocompatibility complex (MHC) class 2-restricted CD4+ T cells, affecting both the exciting eye and the contralateral “sympathizing” eye with potential blinding.3,4 Although rare—the reported incidence is 0.03 in 100,000 persons5—SO may result from accidental trauma or elective surgery to the eye and is consequently a feared complication of any ocular surgery.

Associations with specific human leukocyte antigen (HLA) alleles are well established for several autoimmune diseases, including SO, in which HLA DRB1*04 and DQA1*03 are significantly associated with disease susceptibility and severity in British and Irish patients.6 Polymorphisms in the HLA genes influence the initial presentation of disease-inducing peptides to T cells through their effect on HLA-peptide binding affinity and the development of the T cell repertoire during thymic maturation when autoreactive T cells are deleted.7 In addition, recent evidence has implicated single nucleotide polymorphisms (SNPs) in cytokine genes in modulating the susceptibility and severity of a number of autoimmune diseases.8–11 Studies seeking an etiological role for cytokine SNPs have found that many influence gene transcription rather than the molecular structure of the cytokines. Polymorphisms that result in the upregulation of proinflammatory cytokines, such as TNF (TNFβ2, TNFa–308A),12,13 or the downregulation of anti-inflammatory cytokines, such as IL-10 (IL-10 –1082A),14 are predicted to create a more proinflammatory environment in the eye and consequently to worsen the severity of inflammation.

It is not possible to determine who will develop SO after ocular injury, nor is it possible to determine the outcome of disease based on clinical signs observed at presentation. This can result in inadequate treatment of the patient with sight-threatening disease or in overtreatment of the patient whose disease would otherwise have a benign course and in whom treatment through systemic immunosuppression may be complicated by potentially life-threatening adverse effects. However, patients with SO respond well to treatment, and, if adequate immunosuppression is initiated promptly in appropriate patients, the prognosis in SO can be very good.5,15,16

The purpose of this study was to determine whether polymorphisms of the cytokine genes are associated with severity and outcome in patients with SO. We chose to genotype SNPs that functionally influence transcription levels and that are known to be associated with other autoimmune diseases: TNFa–308G/A (dbSNP ID rs1800629), TNFa–238G/A (rs361525), TNFβ1/2 (rs909253), IL-10 –1082G/A (rs1800896), IL-10 –819C/T (rs1800871), IL-10 –592C/A (rs1800872), TNF receptor 2 +196R/M (rs1061622, rs17883437), and CTLA-4 +49A/G (rs231775).

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METHODS

Patients and Controls

Twenty-seven patients (14 male, 13 female) with newly diagnosed and established SO and 48 controls were recruited from throughout the United Kingdom and Ireland, as previously described.6,7 Seventeen were patients with newly diagnosed SO who had been recruited through the British Ophthalmological Surveillance Unit (BOSU) of the Royal College of Ophthalmologists between July 1997 and September 1998 inclusive, and 10 were patients with established SO who had been recruited from uveitis clinics at Grampian University Hospitals, Aberdeen, and the Mater Hospital, Dublin.3,6 One patient (female) was excluded from the present study because her DNA had degraded and could not be used for reliable genotyping.

All patients had bilateral posterior uveitis or panuveitis with a definite history of ocular trauma followed by contralateral posterior uveitis with features clinically consistent with SO (anterior chamber activity with mutton-fat keratic precipitates, vitreitis, macular and disc edema, Dalen-Fuchs nodules, choroidal neovascularization, exudative retinal detachment or extraocular signs including CSF pleocytosis and hair and skin changes), and five patients demonstrated histologic features consistent with SO in enucleated excised eyes.6

Clinical phenotype and markers of disease severity were assessed, including visual acuity, time interval between SO onset and last ocular injury, biomicroscopic features of intraocular inflammation, and immunosuppression therapy, as previously described.6 Disease recurrence was defined as an increase in disease activity with worsening of visual acuity, BIO score, or both requiring an increase in immunosuppression therapy from previously stable maintenance therapy for at least 2 months. Patients whose activity increased after a reduction of immunosuppression therapy were not defined as having disease recurrence. Disease recurrences were identified within the follow-up period of the original study, which had a median of 12 months (range, 6–354 months).6 Maintenance therapy was defined as the degree of immunosuppression required to control inflammation.6

Controls were matched by age, sex, and region. They were healthy, unrelated volunteers without any history of uveitis or other autoimmune disease who attended the same hospitals or primary care clinics as the patients.

Informed consent was obtained from patients and controls after explanation of the nature and possible consequences of the study. The local regional ethics committees granted approval, and the study was conducted in accordance with the tenets of the Declaration of Helsinki.

Genotyping

DNA was extracted using the salt extraction method, as previously described.6,17

Sequence-Specific Primer PCR Genotyping of TNFa, TNFB, and IL-10 SNPs

The 25 μL PCR reaction mixtures contained 50 ng sample DNA, buffer (GeneAmp PCR Buffer II; Applied Biosystems, Warrington, Cheshire, UK), optimized MgCl2 (Table 1), 200 mM each dNTP (Applied Biosystems), 1 mM forward and reverse primers (Eurogentec; Romsey, Hampshire, UK)(Table 1), 0.2 mM control primers (Eurogentec), and 0.5 U polymerase (AmpliTaq Gold DNA polymerase; Applied Biosystems).

Control primers were 5’-TGCCAAGTGGGACACCCTA-3’ and 5’-GACATCTGCTCGGAGCAT-3’, which recognize an intron sequence in the HLA-DRB1 gene.18

The following cycling conditions were used on a thermal cycler (Peltier Thermal Cycler, model 220; MJ Research Systems, Watertown, MA): 94°C for 5 minutes, 30 cycles of 94°C for 1 minute, optimized Tm°C for 1 minute (Table 1), 72°C for 1 minute, and 72°C for 5 minutes.

Agarose Gel Electrophoresis

PCR products were run on a standard 2% agarose gel in 0.5× TBE running buffer for 45 minutes at 100 V and 250 mA in an electrophoresis tank (Horizon 11.14 Horizontal Gel Electrophoresis Tank; Gibco/BRL Life Technologies Inc. Gaithersburg, MD) and were visualized after staining with ethidium bromide under UV light.

Method Reliability

For a subset of patients, the results of genotyping using the gold standard technique of DNA sequencing were compared with the results of SSP-PCR genotyping to ensure method reliability and accuracy.

Restriction Fragment Length Polymorphism Genotyping of the TNFR 2 and CTLA-4 Genes

TNFR 2 + I696T/G. The 25 μL PCR reaction mix contained 50 ng DNA, buffer (GeneAmp PCR Buffer II; Applied Biosystems), 1.0 mM MgCl2, 200 mM each dNTP, 1 mM forward (5’-ACTCTTGCTCTGCTGTGG-3’ and reverse (5’-TGCCAAGTGGGACACCCTA-3’) primers, and 0.5 U AmpliTaq Gold DNA polymerase, based on the method described by Al-Ansari et al.10
RESULTS

Comparison of SNP Genotype and Allele Frequency between SO Patients and Controls

There were no statistically significant differences in genotype or allele frequencies in any of the SNPs between patients and controls that were independent of the HLA class 2 alleles described previously. In addition, there was no association with IL-10 promoter haplotype.

Comparison of SNP Genotype and Allele Frequency with Clinical Features of SO and Markers of Disease Severity

Clinical Features of SO. There were no statistically significant associations among SNP genotype, allele frequency, and IL-10 haplotype with any clinical features of uveitis.

Severity of SO. IL-10. There was a statistically significant association between IL-10 -1082 SNP and disease recurrence. Disease recurrence was associated with the AA and AG genotypes (P = 0.031) or with carrying an A allele at this locus (P = 0.020; Table 2). In addition, the GCC IL-10 promoter haplotype (IL-10 -1082G, -819C, -592C) was found to be protective against disease recurrence (P = 0.033; Table 3).

All SO patients had been taking at least one maintenance immunosuppression drug. All patients with recurrent disease had been taking more than one immunosuppression drug (four patients taking prednisolone + azathioprine, 10 patients taking prednisolone + cyclosporin A, 10 patients taking prednisolone + cyclosporin A [though tacrolimus was substituted for cyclosporin A in one patient], and three patients taking prednisolone + cyclosporin A + either azathioprine, mycophenolate mofetil, or alemtuzumab (Campath-1H; Therapeutic Antibody Centre, Oxford, UK). Only 2 of 9 patients who did not have recurrent disease were taking more than one immunosuppression agent (both were taking prednisolone + cyclosporin A). In addition, 10 of 17 patients with recurrent disease were taking more than 10 mg/d prednisolone maintenance treatment despite alternative immunosuppression.

Overall, 11 of 12 patients taking more than 10 mg/d prednisolone were also taking more than one immunosuppression agent (seven patients taking prednisolone + cyclosporin A [one patient was later changed to tacrolimus], two patients taking prednisolone + azathioprine, two patients taking prednisolone + cyclosporin A + either azathioprine or mycophenolate mofetil), compared with 8 of 14 patients taking less than 10 mg/d prednisolone (five patients taking prednisolone + cyclosporin A, two patients taking prednisolone + azathioprine, and one patient taking prednisolone + cyclosporin A +

Table 3. IL-10 Promoter Haplotype and Disease Recurrence

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Yes</th>
<th>No</th>
<th>Odds Ratios</th>
<th>Confidence Intervals</th>
<th>P</th>
<th>Corrected P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCC</td>
<td>16</td>
<td>15</td>
<td>0.18</td>
<td>0.03-0.84</td>
<td>0.011*</td>
<td>0.033*</td>
</tr>
<tr>
<td>ACC</td>
<td>13</td>
<td>2</td>
<td>4.95</td>
<td>0.86-37.06</td>
<td>0.040</td>
<td>0.12</td>
</tr>
<tr>
<td>ATA</td>
<td>5</td>
<td>1</td>
<td>2.93</td>
<td>0.28-72.07</td>
<td>0.326</td>
<td>0.978</td>
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</table>

* Significant value.
found that they are associated with disease recurrence and the level of maintenance steroid treatment required to control the inflammatory process. These results are important because at this time it is not possible to determine who will develop SO after surgical or accidental trauma and how severe the disease is likely to be once established. The clinician is faced with the dilemma of how heavily to immunosuppress a patient with a potentially sight-threatening though treatable disease with drugs that can have life-threatening effects.

Objective assessment of the severity of uveitic disease is difficult, and there is no consensus on how assessments should be graded. No single clinical feature suffices, and a scoring system that grades disease activity at the time of examination—e.g., the Uveitis Scoring System—is unsuitable because it is not designed to give a cumulative score for severity and because it gives an impression of the degree of activity at only a single time point, irrespective of time since onset, degree of immunosuppression, or visually significant complications. Hence, the severity of inflammation without validated composite scoring can be defined indirectly by markers such as amount of maintenance immunosuppression required to control inflammation, recurrence of inflammation despite previously stable maintenance treatment, and visual acuity. Given these limitations, our results, in conjunction with those from previous work on HLA risk factors in SO, allow a more complete genetic profile of a patient to be built. Polymorphisms of HLA class 1 and 2 genes affect MHC-peptide binding affinity and, therefore, predictably are linked to disease initiation and susceptibility in SO. During the chronic phase of disease, there is a gradual decline in antigen-induced proliferative responsiveness, when antigen specificity becomes less relevant and in- fluamtion is mediated purely by cytokines and bystander recruitment. The cytokine milieu becomes more important in sustaining the inflammatory response and maintaining pathogenic T cells, so polymorphisms in the cytokine genes are more likely to influence disease severity rather than susceptibility. Indeed, in the present study, we found no significant link between any of the cytokine polymorphisms and.

Table 4. IL-10–1082 SNP and Maintenance Steroid Treatment

<table>
<thead>
<tr>
<th>Prednisolone Maintenance Dose (mg)</th>
<th>IL-10–1082 Genotype (n = 26)</th>
<th>IL-10–1082 Allele (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AG</td>
</tr>
<tr>
<td>&gt;10</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>&lt;10</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td><em>P</em></td>
<td>0.052</td>
<td></td>
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</tbody>
</table>

* Significant value.

Table 5. TNFα–308 SNP and Onset of SO

<table>
<thead>
<tr>
<th>Onset of Disease from Last Ocular Injury (mo)</th>
<th>TNFα–308 Genotype (n = 26)</th>
<th>TNFα–308 Allele (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AG</td>
</tr>
<tr>
<td>≤5†</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>&gt;5‡</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td><em>P</em></td>
<td>0.057</td>
<td></td>
</tr>
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</table>

* Mean time to onset from last ocular injury in this group was 1.42 months, with a median of 1.5 months (range, 0.3–3 months).
† Mean time to onset from last ocular injury in this group was 24.33 months, with a median of 18 months (range, 4–75 months).
‡ Significant value.
susceptibility to SO, but we did find that cytokine polymorphisms are linked to markers of severity.

IL-10 is a potent anti-inflammatory cytokine produced by T cells, macrophages, and retinal cells. It strongly inhibits antigen-specific T cell proliferation, cytokine production, and MHC class 2 expression and is important in the induction of antigen-specific anergy or tolerance. Functional studies of IL-10 promoter polymorphisms have found that the IL-10 -1082A allele and the ACC/ATA promoter haplotypes are linked to the downregulation of IL-10 production, whereas the IL-10 -1082G allele and the GCC haplotype are linked to upregulation. We found that the IL-10 -1082 A allele and the AA/AG genotypes were associated with disease recurrence, even though the patients with recurrent disease were on more than one immunosuppressant drug. In addition, we found the GCC IL-10 promoter haplotype to be protective against disease recurrence. Furthermore, patients with the IL-10 -1082G allele required lower doses of steroids as maintenance treatment, though many of these patients (6/14) were on prednisolone alone. Thus, our results are in keeping with the experimental evidence for the importance of IL-10 in dampening the inflammatory process during the effector phase of SO.

The role of TNF in the pathogenesis of uveitis is somewhat more complex. TNF is a multifunctional cytokine secreted by monocytes (TNFα), lymphocytes (TNFβ) and resident retinal cells. It has potent proinflammatory effects since blocking its action in experimental models of uveitis reduces structural damage to the retina whereas the administration of TNF leads to worsening of disease. In contrast, chronic TNF exposure suppresses the cytokine and proliferative responses of T cells and drives Fas-dependent apoptosis.

Because of the proximity of the TNF genes to class 1 and 2 genes within the MHC region of chromosome 6 and the linkage disequilibria that exist between them, independent associations with disease can be difficult to define. Certain polymorphisms may be considered as part of an extended haplotype that includes the HLA class 1 and 2 genes. However, independent associations have also been demonstrated. We have previously found that SO patients who are HLA DRB1*04-DQA1*05 positive experienced earlier onset of disease, whereas the administration of TNF leads to worsening of disease. In contrast, chronic TNF exposure suppresses the cytokine and proliferative responses of T cells and drives Fas-dependent apoptosis.

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References


