Interleukin 1β Gene Polymorphism Association with Severe Renal Manifestations and Renal Sequelae in Henoch-Schönlein Purpura

MAHSA M. AMOLI, MARIA C. CALVIÑO, CARLOS GARCIA-PORRUA, JAVIER LLORCA, WILLIAM E.R. OLLIER, and MIGUEL A. GONZALEZ-GAY

ABSTRACT. Objective. To assess the influence of the interleukin (IL)-1β gene (-511 C/T) in the incidence of Henoch-Schönlein purpura (HSP) and determine its possible implication in severe systemic complications of HSP, in particular severe renal involvement and permanent renal dysfunction (renal sequelae).

Methods. Patients from Northwest Spain with primary cutaneous vasculitis classified as HSP according to proposed criteria were studied. All patients were required to have had at least 2 years’ followup. Patients and ethnically matched controls were genotyped for IL-1β gene (-511 C/T) polymorphism by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results. Forty-nine Caucasian patients (38 of them younger than 21 years) who fulfilled classification criteria for HSP and 148 controls were examined. No allele or genotype differences between the whole group of HSP and controls were observed. However, all 5 patients who developed severe nephropathy during the course of disease carried the rare T allele compared with only 16 of the remaining 44 patients (pcorr = 0.01). A significant association between carriage of the -511(IL-1β) T allele and renal sequelae (p = 0.02; OR: 3.6, 95% CI: 1.3-10.0) was also found.

Conclusion. In unselected patients with cutaneous vasculitis who fulfill classification criteria for HSP, carriage of IL-1β (-511) T allele appears to influence severity of renal involvement.

Key Indexing Terms: HENOCH-SCHÖNLEIN PURPURA, IL-1β GENE (-511 C/T) POLYMORPHISM, DISEASE SUSCEPTIBILITY, SEVERE NEPHRITIS, RENAL SEQUELAE

Henoch-Schönlein purpura (HSP) is a primary small-sized blood vasculitis. Although the classic clinical triad of HSP consists of palpable purpura, joint symptoms, and abdominal pain, renal involvement represents the most serious complication.

Interleukin 1 (IL-1) is a prototypic proinflammatory cytokine localized to chromosome 2q13-q21 within a 430 kb region of a gene cluster close to the IL-1 receptor antagonist gene (IL-RN). IL-1β elicits the production of various cytokines in endothelial cells including colony-stimulating factor and chemokines and stimulates production of other cytokines such as tumor necrosis factor (TNF)-α and IL-6. IL-1β is a potent stimulator of endothelial cells for IL-1 expression in an autocrine fashion.

IL-1 promotes leukocyte extravasation by inducing the expression of adhesion molecules such as endothelial leukocyte adhesion molecule (ELAM)-1 (E-selectin) and intercellular adhesion molecule (ICAM) and induces the production of IL-8 by endothelial cells.

High IL-1β expression in the skin biopsy specimens of patients with HSP has been observed. Furthermore, high IL-1β serum concentration was also observed in HSP patients with nephritis.

Several single nucleotide polymorphisms have been found in the IL-1β gene. A biallelic polymorphism in the IL-1β gene at position -511 has been described. This (-511 C/T) polymorphism in the IL-1β gene is thought to influence IL-1β production and has been examined in several diseases. We examined IL-1β gene (-511 C/T) polymorphism in patients with cutaneous vasculitis who fulfilled classification criteria for HSP. Due to previous reports of increased IL-1β levels, special interest was focused on the possible association of this polymorphism with severe renal manifestations and renal sequelae in HSP.
Clinical manifestations of patients with Henoch-Schönlein purpura. All patients presented with palpable purpura involving the legs. Forty patients suffered gastrointestinal bleeding and/or bowel angina. Thirty-six had joint manifestations. All 31 patients with nephritis had hematuria with or without proteinuria. Five patients (4 younger than 21 years) developed severe nephropathy (all of them nephrotic without proteinuria. Five patients (4 younger than 21 years) developed severe nephropathy (all of them nephrotic without proteinuria. Five patients). Five patients who developed severe nephropathy during the course of the disease (Table 1). However, all 5 patients who developed severe nephropathy during the course of the disease carried the rare T allele (p<0.01). As a consequence, none of the patients who carried a CC genotype had nephrotic syndrome or renal insufficiency during the course of the disease (Table 1). A statistically significant association between T allele and the development of severe nephropathy during the course of the disease was found (p<0.003; OR: 7.9, 95% CI 1.9-33.5). Moreover, a statistically significant association between carriage of the -511(IL-1ß) T allele and renal sequelae was found (p=0.02; OR: 3.6, 95% CI 1.3-10.0). Patients carrying CT or TT genotype had an increased risk of developing renal sequelae (OR: 4.2, 95% CI 1.0-18.7). However, due to the small number of patients who developed renal sequelae, this difference was not significant when a Bonferroni correction was applied (p=0.1).

DISCUSSION
Upregulated expression of proinflammatory cytokines such as TNF, IL-6, and IL-1 has been reported in vasculitis syndromes. Our results suggest that IL-1ß gene (-511 C/T) may be directly implicated in the severity and outcome but not in the susceptibility of unselected patients with cutaneous vasculitis. We have reported an association between IL-1ra VNTR polymorphism with the development of severe renal manifestations and renal sequelae in HSP. A moderate degree of linkage disequilibrium across a ~400-bp stretch of chromosome region 2q13 that contains the IL-1 cluster has been described. Carriage of IL-1ß (-511) T allele has been associated with increased IL-1 production. In contrast to IL-1, IL-1 receptor antagonist (IL-1ra) has antiinflammatory effects. IL-1ra protein is an active competitive inhibitor of the binding of IL-1 to the T-cell/fibroblast form of the IL-1 receptor. The IL-1ra allele 2 (IL-1RN*2) was reported to increase production of IL-1ra. The enhancing effect of IL-1RN*2 on plasma IL-1ra levels requires the presence of IL-1ß allele, which implies that the IL-1ß gene participates in the regulation of IL-1ra production. Interestingly, there patients had persistent renal involvement (renal sequelae), mainly hematuria. Anti-neutrophil cytoplasmic antibody (ANCA) tests were negative in the 6 adults on whom they were tested.

Allele and genotype frequencies of IL-1ß gene (-511 C/T) polymorphism in patients with HSP and controls. In controls no evidence of departure from Hardy-Weinberg equilibrium was observed. No allele or genotype differences between the whole group of HSP and controls were observed (Table 1). No differences were observed when HSP patients were stratified by the presence of joint or gastrointestinal manifestations (data not shown). This was also the case when patients were stratified for the presence of renal manifestations (hematuria) during the course of the disease (Table 1).

Inclusion criteria. Patients and ethnically matched controls included in this study were from the Lugo region of Northwest Spain. Only patients with cutaneous vasculitis and at least 2 years' followup who fulfilled the criteria for HSP proposed by Michel, et al were assessed for the IL-1ß gene (-511 C/T) polymorphism. As described, in adults (age ≥ 21 years) a skin biopsy showing leukocytoclastic vasculitis was required. In children, a diagnosis of cutaneous vasculitis was considered if they had typical non-thrombotic (symmetric palpable purpura involving the lower extremities and if other conditions such as connective tissue diseases and infections had been excluded. Nephritis (renal involvement) was defined as the presence of any renal event that happened at any time over the course of the disease. Nephritis was graded as follows: (a) mild nephropathy if hematuria was present (≥ 10 red blood cells/high power microscopic field) and/or proteinuria (> 500 mg/24 h) without nephrotic range; (b) severe nephropathy if nephrotic syndrome was present (i.e., in children > 40 mg/m² body surface/h or > 50 mg/kg/day or > 2 g/day proteinuria with plasma albumin ≤ 25 g/l, and in adults 1 g/day/m² body surface area or > 3.5 g/day proteinuria with plasma albumin ≤ 25 g/l), with or without edema and/or acute nephrotic syndrome (i.e., hematuria with at least 2 of the following: hypertension, raised plasma urea, or creatinine and oliguria) or renal insufficiency (plasma creatinine concentration above 150% the upper limit of normal); and (c) renal sequelae were considered present if a patient had any of the renal complications described at last followup (at least 2 years).

Genotyping. DNA from HSP patients and controls was extracted from anticoagulated blood collected in EDTA tubes. The polymerase chain reaction (PCR) was carried out in a volume of 25 µl containing 100 ng of genomic DNA, 10 KCl buffer (Bioline, London, UK), 0.2 mM dNTPs (Bioline), 1.5 mM MgCl₂, 0.1 µM of each primer: forward 5′-TGGCATTGATCTGGTTCATC-3′ and reverse 5′-GTTAGGAATCTTCCCACTT-3′, 5 units of Taq DNA polymerase (Bioline), 1 unit of AvaI (New England Biolab, Hitchin, UK), which cuts the product of the C allele to 190 bp and 114 bp. The digestion was incubated overnight at 37°C and the product was performed by enzyme digestion using 3 units of AvaI (New England Biolab, Hitchin, UK), which cuts the product of the C allele to 190 bp and 114 bp. The digestion was incubated overnight at 37°C and the products of the digest were then visualized on a 3% agarose gel stained with ethidium bromide.

Statistical analysis. Strength of association between HSP and alleles or genotypes of the IL-1ß gene was estimated using odds ratios (OR) and 95% confidence intervals (CI). P values (p) were corrected using the Bonferroni method. Levels of significance were determined by chi-square or Fisher exact analysis. Statistical significance was defined as p < 0.05.

RESULTS
Forty-nine Caucasian patients (38 younger than 21 years) classified as having HSP and 146 controls were available for study.

In controls, no differences were observed when HSP patients were stratified for the presence of renal manifestations (hematuria) during the course of the disease (Table 1). No differences were observed when HSP patients were stratified by the presence of joint or gastrointestinal manifestations (data not shown). This was also the case when patients were stratified for the presence of renal manifestations (hematuria) during the course of the disease (Table 1). However, all 5 patients who developed severe nephropathy during the course of the disease carried the rare T allele (p=0.01). As a consequence, none of the patients who carried a CC genotype had nephrotic syndrome or renal insufficiency during the course of the disease (Table 1). A statistically significant association between T allele and the development of severe nephropathy during the course of the disease was found (pcorr = 0.003; OR: 7.9, 95% CI 1.9-33.5). Moreover, a statistically significant association between carriage of the -511(IL-1ß) T allele and renal sequelae was found (pcorr = 0.02; OR: 3.6, 95% CI 1.3-10.0). Patients carrying CT or TT genotype had an increased risk of developing renal sequelae (OR: 4.2, 95% CI 1.0-18.7). However, due to the small number of patients who developed renal sequelae, this difference was not significant when a Bonferroni correction was applied (pcorr = 0.1).

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Carriage of IL-1ß (-511) T allele has been associated with increased IL-1 production. In contrast to IL-1, IL-1 receptor antagonist (IL-1ra) has antiinflammatory effects. IL-1ra protein is an active competitive inhibitor of the binding of IL-1 to the T-cell/fibroblast form of the IL-1 receptor. The IL-1ra allele 2 (IL-1RN*2) was reported to increase production of IL-1ra. The enhancing effect of IL-1RN*2 on plasma IL-1ra levels requires the presence of IL-1ß allele, which implies that the IL-1ß gene participates in the regulation of IL-1ra production. Interestingly, there...
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Table 1. Frequency of IL-1ß gene (-511 C/T) polymorphism in patients with HSP with and without renal manifestations and controls. T allele was increased in HSP patients with severe renal involvement: \( p_{corr} = 0.003; \text{OR: } 7.9 \text{ (95\% CI 1.9–33.5).} \) T allele was increased in HSP patients with renal sequelae: \( p_{corr} = 0.02; \text{OR: } 3.6 \text{ (95\% CI 1.3–10.0).} \) Patients with severe renal manifestations showed an increased frequency of TT and CT genotypes compared with those without severe renal manifestations: \( p_{corr} = 0.01. \)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls ((n = 148))</th>
<th>HSP ((n = 49))</th>
<th>HSP with Nephritis ((n = 31))</th>
<th>HSP with Severe Renal Manifestations* ((n = 5))</th>
<th>HSP with Renal Sequelae** ((n = 44))</th>
<th>HSP with Renal Sequelae** ((n = 39))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td>Yes ((n = 31))</td>
<td>No ((n = 18))</td>
<td>Yes ((n = 5))</td>
<td>No ((n = 44))</td>
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<td>28</td>
<td>27</td>
<td>28</td>
<td>70</td>
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* During the clinical course of the disease. ** Renal sequelae: persistent renal involvement at the end of the study (at least 2 years’ followup).

is evidence to suggest that IL-1 induces the pathological changes observed in glomerulonephritis.

HSP is an IgA- and complement-mediated process affecting small vessels of the involved organs. IgA immune complexes in patients with IgA-related renal disease compared with controls have been implicated in the pathogenesis of the HSP nephritis. HSP and IgA nephropathy are closely related conditions. Several polymorphisms seem to be implicated in the pathogenesis of the IgA nephropathy. Angiotensin-converting enzyme polymorphism has been implicated in the outcome in the pediatric IgA nephropathy. Association of the uteroglobin gene polymorphism with IgA nephropathy in mutant homozygous or heterozygous has been described. Cytokine polymorphisms have also been implicated in the pathogenesis of this nephropathy. A recent study supports a role for TNF-α polymorphisms in the occurrence but not in the progression of the nephritis. Another recent study has shown an association between carriage of IL-82, IL-1RN*2 and noncarriage of TNF2 in the susceptibility to IgA nephropathy. Also, in Japanese, polymorphisms of both T helper 1 and 2 cytokines, interferon gamma, and IL-4 gene polymorphisms, respectively, seem to influence disease susceptibility and progression in IgA nephropathy.

The etiology of HSP remains unknown. Infection was reported to be a precipitating factor in more than 50% of the cases. Cytokines produced during the antigenic challenge may play a role in the susceptibility and severity of HSP. In this regard, genetic factors may determine the immune and inflammatory response to unknown antigens. The polymorphism of cytokine genes may influence the level of expression of cytokines. IL-1ß gene participates in the regulation of IL-1ra production. Polymorphism in these genes may mediate an abnormal inflammatory response that may lead to the development of severe renal involvement and renal sequelae in HSP. In addition, abnormal expression of proinflammatory cytokines may mediate in the proliferation and differentiation of lymphoid cells. It may lead to an increased number of circulating IgA secreting cells in a patient with HSP. It is possible that the effect of different cytokine polymorphisms on HSP renal susceptibility and severity might be due to the interdependent effects of more than one polymorphism rather than to the effect of individual polymorphisms.

In conclusion, our observations may contribute to the existing knowledge in terms of stratification of patients with primary cutaneous vasculitis. However, studies in other populations and, in particular, on larger numbers of patients are required to confirm the association between IL-1ß and IL-1ra gene polymorphisms in the development of severe renal manifestations and renal sequelae in HSP.

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