Interleukin 1 Receptor Antagonist Gene Polymorphism Is Associated with Severe Renal Involvement and Renal Sequelae in Henoch-Schönlein Purpura

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ABSTRACT. ***Objective.*** To assess the influence of interleukin 1 receptor antagonist gene polymorphism (IL1RN) in the incidence of Henoch-Schönlein purpura (HSP) and cutaneous leukocytoclastic angiitis (CLA) and to determine if implications exist with severe systemic complications of HSP, in particular with severe renal involvement and permanent renal dysfunction (renal sequelae).

Methods. Patients from Northwest Spain with primary cutaneous vasculitis classified as HSP or hypersensitivity vasculitis (HV) according to proposed criteria were studied. Patients with HV were included if they had a biopsy proven small size blood vessel leukocytoclastic vasculitis limited to skin and also fulfilled the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis definitions for CLA. All patients were required to have had at least 2 years' followup. Patients and ethnically matched controls were genotyped for IL-1 receptor antagonist intron 2 VNTR polymorphism.

Results. We examined 96 Caucasian patients (58 HSP and 38 CLA) and 109 controls. No allele or genotype differences between the whole group of HSP or CLA patients and controls were observed. We found a significant association between carriage of IL-1 receptor antagonist allele 2 (ILRN*2) and severe renal involvement, manifested as nephrotic syndrome and/or renal insufficiency ($p = 0.016$), and permanent renal involvement (renal sequelae) ($p = 0.012$).

Conclusion. In unselected patients with cutaneous vasculitis, carriage of ILRN*2 alleles influences disease severity rather than susceptibility. (J Rheumatol 2002;29:1404–7)

Key Indexing Terms:
HENOCH-SCHÖNLEIN PURPURA — CUTANEOUS LEUKOCYTOCLASTIC ANGIITIS
INTERLEUKIN 1 RECEPTOR ANTAGONIST GENE — DISEASE SUSCEPTIBILITY
RENAL INVOLVEMENT — RENAL SEQUELAE

Henoch-Schönlein purpura (HSP) and cutaneous leukocytoclastic angiitis (CLA) are primary small size blood vessel vasculitides. Infiltration of the small blood vessels with polymorphonuclear leukocytes and the presence of leukocytoclasia characterize both conditions. In HSP, IgA-dominant immune deposits in the walls of the small vessels and in the renal glomeruli are frequently observed[1]. Granular deposition of C3, IgG, and IgM is seen in 50% of patients with CLA[2]. HSP is common in children and rare in adults. Palpable purpura and joint and gastrointestinal (GI) manifestations are typical of this condition. Renal manifestations, however, constitute the most serious complications, and longterm morbidity and mortality in HSP are mainly due to renal involvement[3]. CLA is an isolated vasculitis limited to skin[1]. Exposure to new medications or infections is often present. Arthralgias or arthritis may be observed. Other features, however, are absent. Thus, for the diagnosis of CLA, systemic involvement must be excluded[2].

Susceptibility to HSP and CLA and associated clinical heterogeneity in HSP may be conferred by a number of genetic loci. The interleukin 1 (IL-1) family of proteins has a major role in the inflammatory response[4]. IL-1 receptor antagonist (IL-1ra) is an endogenous antiinflammatory agent that binds to IL-1 receptor and thus competitively inhibits the binding of IL-1α and IL-1ß[5]. The gene for IL-1ra is mapped to chromosome 2q14-q21[6]. Tarlow, *et al* described variable copy numbers of an 86 base pair tandem repeat (VNTR polymorphism) in intron 2 of this gene. Five alleles of this polymorphism have been reported[7]. Allele 1 and 2 of this polymorphism, which correspond to 4 and 2 repeats, respectively, are more common in the general population and association of allele 2 with various autoimmune diseases has been observed[8-11].

Liu, *et al* observed that the carriage rate of the 2-repeat of IL-1ra allele 2 (IL1RN*2) of the IL-1ra gene was signifi-

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cantly higher in patients with HSP with nephritis than in controls and patients with IgA nephropathy[12]. However, the influence of this polymorphism in the susceptibility to HSP and CLA in unselected series of patients has not been assessed, nor has its possible association with disease severity, manifested by severe renal involvement and renal sequelae, been determined. To address these questions, we studied the frequency of VNTR polymorphism in an unselected series of patients with primary cutaneous vasculitis.

MATERIALS AND METHODS

Patients. Patients were recruited from the Divisions of Pediatrics and Rheumatology of the Hospital Xeral-Calde. Ethnically matched controls were also obtained from the area surrounding Lugo.

Inclusion criteria. Patients with primary cutaneous vasculitis who fulfilled the 1990 American College of Rheumatology classification criteria for hypersensitivity vasculitis (HV) or HSP[13,14] were differentiated using the criteria proposed by Michel, *et al*[15]. They were classified as having HSP if they fulfilled 3 or more of the following criteria: (1) palpable purpura, (2) bowel angina, (3) GI bleeding, (4) macroscopic or microscopic hematuria, (5) age at disease onset ≤ 20 years, and (6) no previous history of medications before the onset of the disease. Patients who met fewer than 3 criteria were classified as having HV. In addition, only patients with HV and biopsy proven small size blood vessel leukocytoclastic vasculitis limited to skin were included. Thus, those patients with HV also fulfilled the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis definitions for CLA, as the term CLA defines an isolated cutaneous leukocytoclastic vasculitis limited to skin[1].

In adults, a skin biopsy showing pathologic evidence of leukocytoclastic vasculitis was always required. In children, a diagnosis of cutaneous vasculitis was considered in most cases without skin biopsy if they had typical nonthrombocytopenic symmetric palpable purpura involving the lower extremities. In these cases other conditions that may present with cutaneous lesions such as connective tissue diseases and infections had to be excluded. However, unlike children with HSP, those children with CLA limited to skin were included in the study if they had a skin biopsy showing leukocytoclastic vasculitis. For the purpose of examining the outcome of HSP and CLA, only patients with at least 2 years' followup were included.

Clinical definitions. Based on former classification criteria[14,15], patients older than 20 years were considered adults and those younger than 21 children. Nephritis (renal involvement) was defined as the presence of any renal event at any time over the whole course of the disease and was graded as follows: (1) mild nephropathy: if hematuria (≤ 10 red blood cells/high power microscopic field) and/or proteinuria (> 500 mg/24 h) without nephrotic range was present; (2) severe nephropathy: if nephrotic syndrome (i.e., in children > 40 mg/m^2 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/m^2 body surface area or > 3.5 g/day proteinuria with plasma albumin ≤ 25 g/l), with or without edema and/or acute nephritic 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 > 150% upper limit of normal) was present; (3) renal sequelae were considered to be present if a patient had any of the renal complications described above at last followup (at least 2 years).

PCR amplification of IL-1ra. DNA from patients and controls was extracted from anticoagulated blood collected in EDTA using a commercial DNA extraction kit (Bioline™).

Polymerase chain reaction (PCR) amplification of the VNTR region was carried out in a total volume of 25 μl containing 100 ng of genomic DNA (5 μl), 10 × KCl buffer (Bioline), 0.2 mM dNTPs (Bioline), 5 pmol of each primer, and 1 unit of Taq DNA polymerase (Bioline). The DNA was denatured at 95°C for 2 min, and temperature cycling was set at 95°C for 45 s, 57°C for 45 s, and 72°C for 45 s for 40 cycles, followed by a final extension at 72°C for 5 min using the following primers: forward 5′-CTC AGC AAC ACT CCT AT-3′ and reverse 5′-TCC TGG TCT GCA GGT AA-3′.

PCR product was visualized on 2% agarose gel stained with ethidium bromide.

The single nucleotide polymorphisms at position 8006, 8061, 9589 were also genotyped using the standard protocol as described[16].

Statistical analysis. Strength of association between HSP or CLA and alleles or genotypes of IL-1ra gene was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined using contingency tables by either chi-square or Fisher's exact analysis. The same methods were used to examine the strength of association between HSP subgroups with or without renal manifestations and IL-1ra alleles. P values (p_{corr}) were corrected using the Bonferroni method. They were calculated by multiplying the p value by the number of alleles compared. For the analysis of HSP genotypes, when categorical variables had more than 2 mutually exclusive categories (i.e., HSP with or without hematuria or HSP with or without renal sequelae), a p value as an overall measure of statistical significance was provided. Statistical significance was defined as $p \leq 0.05$. Calculations were performed with the statistical package Stata V6.

RESULTS

Ninety-six patients with cutaneous vasculitis and 109 healthy controls were examined in this study.

Clinical manifestations of patients with HSP and CLA. Fifty-eight patients fulfilled classification criteria for HSP and 38 met definitions for CLA.

The main epidemiological and clinical data of the patients with HSP are shown in Table 1. Hematuria with or without proteinuria and severe GI manifestations were frequently observed in the group of patients with HSP. However, after a minimum of 2 years' followup (median 8 yrs) only 12 of the 58 patients had persistent renal involvement (renal sequelae), mainly hematuria.

Table 1. Main features of patients with primary cutaneous vasculitis.

	HSP (n = 58)	CLA (n = 38)
Children*/adults	46/12	2/36
Male/female	1/1	1.2/1.0
Age at the onset of the disease (yrs) median (range)	6.5 (2–62)	56.5 (17–77)
Duration of followup (yrs) median	8	4
Drug intake history** (%)	17 (29)	13 (34)
Palpable purpura and/or maculopapular rash (%)	58 (100)	38 (100)
Arthralgia and/or arthritis (%)	40 (69)	9 (24)
Gastrointestinal bleeding (%)	24 (41)	—
Bowel angina (%)	44 (76)	—
Renal manifestation (%)		
Hematuria	38 (66)	—
Proteinuria	20 (34)	—
Nephrotic syndrome	7 (12)	—
Renal insufficiency	2 (3)	—
Renal sequelae (persistent renal involvement) %	12 (21)	—

* Age less than 21 yrs. ** Within a week before the onset of the vasculitis. HSP: Henoch-Schönlein purpura; CLA: cutaneous leukocytoclastic angiitis.

Patients with CLA presented with maculopapular or purpuric skin lesions. Drug intake (generally analgesics or antibiotics) within a week prior to the onset of the vasculitis was observed in one-third of the cases. However, apart from joint manifestations (generally arthralgia) during the course of the vasculitis, no systemic manifestations were observed in these patients after a minimum of 2 years' followup (Table 1).

Allele and genotype frequencies of IL-1ra gene in patients with HSP or CLA and controls. In our population, only allele 1 and 2 of the IL-1ra VNTR polymorphism were detected. The single nucleotide polymorphisms at position 8006, 8061, 9589 were also genotyped using the standard protocol[16], and complete linkage disequilibrium was observed between these markers and VNTR polymorphism. Consequently, only VNTR alleles and genotypes were analyzed further. The allele and genotype frequencies of VNTR polymorphism were compared to those of controls. No significant differences in frequency were found between the different disease groups and controls (Table 2).

Allele and genotype frequencies of IL-1ra gene in patients with HSP and renal manifestations. Although HSP patients with GI manifestations or hematuria had no different IL-1ra allele or genotype distributions from those without these clinical manifestations, allele 2 appeared to be a marker for severe renal involvement or persistent renal dysfunction. In this regard, patients with nephrotic syndrome or with renal insufficiency during the course of the disease exhibited a significantly increased frequency of allele 2. Similarly, allele 2 was significantly increased in HSP patients who developed renal sequelae (Table 2). As a consequence, HSP patients with severe renal manifestations or renal sequelae showed a different genotype distribution (Table 2).

DISCUSSION

Genetic susceptibility to HSP has previously been reported. This condition is associated with HLA-DRB1*01 in both Italian and Spanish patients[17,18]. However, patients with severe manifestations or with persistent renal involvement did not have any specific HLA-DRB1 association other than the underlying association with HLA-DRB1*01. Further, in patients from Northwest Spain intercellular adhesion molecule-1 (ICAM-1) polymorphisms alone were not associated with development of HSP, but patients not carrying the codon 469 K/E genotype were at decreased risk of developing severe GI complications[19].

Carter, *et al*[20] found that IL-1ra protein was an active competitive inhibitor of the binding of IL-1 to the T cell/fibroblast form of the IL-1 receptor. This protein inhibited IL-1 bioactivity, particularly on T cells and endothelial cells *in vitro*, and was a potent inhibitor of IL-1 induced corticosterone production *in vivo*. The 2-repeat of IL1RN*2 has been associated with discoid rash and photosensitivity in systemic lupus erythematosus[9]. Also, a significant increase in IL1RN*2 frequency and carriage rate was observed in Graves' disease, although this was not seen in patients with Hashimoto's thyroiditis and in the control group[10]. Carriage of IL1RN*2 was also significantly increased in patients with diabetes mellitus who developed nephropathy[11].

There is some evidence that IL-1 induces the pathological changes in glomerulonephritis[21]. High plasma levels of IL-1ra have been observed in patients with chronic renal failure. This may be due to inadequate clearance of IL-1ra and possible increased production[22].

In Chinese patients with nephropathy, the carriage rate of allele 2 of VNTR was higher in HSP than in those with IgA nephropathy or with acute postinfectious glomeru-

Table 2. Frequency of IL–1ra VNTR polymorphism in CLA and HSP patients with and without renal manifestations. HSP patients with severe renal manifestations showed an increased frequency of 2.2 and 1.2 genotypes; p = 0.03. HSP patients with renal sequelae showed an increased frequency of 2.2 and 1.2 genotypes; p = 0.02.

	Controls	CLA	HSP With Hematuria		HSP With Severe Renal Manifestations*		HSP With Renal Sequelae**	
			Yes	No	Yes	No	Yes	No
No. patients	109	38	38	20	7	51	12	46
Allele								
1	72	68	70	77.5	43	76.5	50	78
2	28	32	30	22.5	57***	23.5***	50†	22†
Genotype								
11	54	42	53	55	14	59	25	61
22	10	5	13	0	29	6	25	4
12	36	53	34	45	57	35	50	35

CLA: cutaneous leukocytoclastic angiitis. HSP: Henoch-Schönlein purpura. * During the clinical course of the disease. ** Renal sequelae: Persistent renal involvement at the end of the study (at least 2 years' followup).
*** Allele 2 was increased in HSP with severe renal involvement: p = 0.008; p_c= 0.016; OR 4.3 (95% CI 1.4–13.7). † Allele 2 was increased in patients with renal sequelae: p = 0.006; p_c = 0.012; OR 3.6 (95% CI 1.4 –9.2).

lonephritis[12]. However, there was no significant difference between patients with nephropathy due to HSP and those with IgA nephropathy that had recurrent hematuria[12]. These observations support a possible role of this allele in the severity of the nephritis in these closely related conditions.

This study constitutes the first attempt to establish the influence of IL-1ra gene in the susceptibility and severity for cutaneous vasculitis. Our data suggest that IL-1ra gene polymorphism is directly implicated in the severity and outcome but not in the susceptibility of unselected patients with cutaneous vasculitis. While no association of this polymorphism was observed in vasculitis limited to skin, the carriage of ILRN*2 allele in HSP patients was associated with a higher risk of severe renal manifestations and renal sequelae.

Additional studies in other types of leukocytoclastic vasculitis are required to further delineate the role of IL-1ra in the severity of the disease and specifically in the risk of developing severe renal involvement.

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