HLA-DRB1 Associations in Biopsy Proven Erythema Nodosum

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ABSTRACT. ***Objective.*** To examine the HLA-DRB1 associations of patients with erythema nodosum (EN), establish HLA-DRB1 differences among patients with idiopathic and secondary EN, and identify the HLA-DRB1 associations with specific conditions presenting with EN.

Methods. We conducted a retrospective study of 100 patients (83 women) with biopsy proven EN diagnosed for a defined population in Northwest Spain. Patients were classified into idiopathic or secondary if skin nodules occurred in the context of a well defined disease, or there was a precipitating event in close temporal relationship with the onset of EN. Patients and controls were HLA-DRB1 genotyped from DNA using molecular based methods.

Results. At the time of diagnosis no precipitating events or underlying diseases were identified in 35 cases and, due to this, they were considered to be idiopathic. Although strong associations do not appear to exist between HLA-DRB1 and unstratified EN, the idiopathic group showed a significantly lower frequency of HLA-DRB1*04 phenotype compared with controls. Among the patients with secondary EN, the patient subgroup with sarcoidosis exhibited a significantly increased frequency of HLA-DRB1*13 compared with healthy controls.

Conclusion. Idiopathic and some groups of secondary EN seem to have different HLA-DRB1 associations. These differences may have prognostic value in identifying patients with specific conditions associated with this syndrome. (J Rheumatol 2001;28:2660–2)

Key Indexing Terms:
ERYTHEMA NODOSUM ETIOLOGY HLA-DRB1 SARCOIDOSIS

Erythema nodosum (EN) is the most common cause of inflammatory nodules, occurring generally on the lower extremities. It has been associated with a number of heterogeneous, unrelated disorders and various drugs[1-3]. However, in some cases the etiology of EN remains unknown[4,5].

So far, HLA associations in EN have not been well characterized. Some studies have described an association with class I polymorphisms. For example, HLA-B8 was considered to be more common in this syndrome[6,7]. Similarly, an association between HLA-A11 and EN secondary to leprosy has been reported[8]. However, no specific analysis of the HLA-DRB1 pattern of association in unselected series of patients with EN has been reported.

To further investigate the immune–HLA involvement in this syndrome, we examined the HLA-DRB1 phenotypes in an unselected series of patients with EN.

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MATERIALS AND METHODS

Patients were included in this study if they had a skin biopsy showing an acute or granulomatous septal panniculitis with primary inflammation around the veins of the septal system composed of neutrophils, lymphoid cells, and histiocytes, with or without giant cell formation.

All patients were 15 years or older (range 15 to 78) diagnosed in the Department of Medicine of the Hospital Xeral-Calde (Lugo, Northwest Spain). Ethnically matched controls were recruited from the same area[2,9].

On the basis of a recently described protocol[2], EN was classified into idiopathic (primary), when no underlying diseases or precipitating events were found, or secondary to other diseases, when the skin nodules occurred in the context of a well defined disease or there was a precipitating event [drug intake and/or upper respiratory tract infections (URTI) other than streptococcal infection] in close temporal relationship with the onset of EN that might be implicated in the occurrence of the cutaneous lesions.

HLA-DRB1 typing was performed on DNA extracted from anticoagulated blood collected in EDTA using a commercial DNA extraction kit (Bioline™, London, UK). HLA-DRB1 phenotypes were determined using Dynal RELI™ SSO HLA-DRB upgraded typing kit and Dynal RELI™ SSO strip detection reagent kit and the Dynal Auto RELI 48 machine following the manufacturers' instructions (Dynal, Liverpool, UK). Reaction patterns were interpreted using Dynal software.

Statistical analysis. The strength of association between EN and HLA-DRB1 phenotypes was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined using contingency tables by either chi-square test or Fisher exact analysis. Statistical significance was defined as $p \leq 0.05$. Calculations were performed with the statistical package Stata V6.

RESULTS

One hundred unselected biopsy proven EN patients (83 women and 17 men) were included in this study. Thirty-five

were diagnosed as having idiopathic EN. In the remaining cases EN was related to a well defined disease, or there was a history of drug intake and/or nonstreptococcal URTI shortly before the onset of skin lesions (Table 1).

HLA-DRB1 phenotype differences between the EN group as a whole and the control group. No significant HLA-DRB1 phenotype differences between patients and controls were found. (Table 2).

HLA-DRB1 phenotype differences between controls and idiopathic and secondary EN. Patients with EN were subdivided into idiopathic and secondary cases (Table 2). However, no comparisons between the patients with idiopathic EN and those with secondary EN were significant after correcting p values for the number of comparisons. HLA-DRB1*04, however, was significantly decreased in patients with idiopathic EN compared with healthy controls [p = 0.003; OR 0.1 (95% CI 0.01–0.6), corrected p = 0.03]. Phenotype frequencies in patients with EN secondary to sarcoidosis are also shown in Table 2. Compared with healthy controls, patients with EN secondary to sarcoidosis exhibited a significantly increased frequency of HLA-DRB1*13 phenotype [p = 0.002; OR 3.4 (95% CI 1.5–7.4), corrected p = 0.02]. Although HLA-DRB1*03 frequency was increased in EN secondary to sarcoidosis compared to controls, such an increase was not statistically significant after correcting p values for the number of comparisons.

Although only 8 cases from this unselected series of EN were confirmed to be due to a previous streptococcal infection, 7 of these (87.5%) exhibited the HLA-DRB1*15/16 phenotype. Moreover, no statistically significant differences between the cases of nonstreptococcal URTI (n = 14) and idiopathic EN were found. No obvious HLA-DRB1 associations were observed in the 7 patients with EN secondary to medication (data not shown).

Table 1. Clinical features in patients with biopsy proven erythema nodosum.

	Number of Cases, N = 100	Sex Female/Male	Age (yrs) at Time of Diagnosis Mean ± SD
Idiopathic	35	29/6	46.1 ± 15.6
Secondary	65	54/11	42.7 ± 16.8
Sarcoidosis	32	24/8	44.8 ± 16.0
Non-streptococcal URTI*	14	13/1	38.4 ± 15.3
Group A ß-hemolytic Streptococcus	8	8/0	38.0 ± 15.7
Drugs*	7	6/1	43.6 ± 16.1
Tuberculosis	3	2/1	28.3 ± 13.0
Sweet's syndrome	3	3/0	67.3 ± 8.0
Inflammatory bowel disease†	2	2/0	37.0 ± 24.0

URTI: upper respiratory tract infection.
* Four patients (all women) had a history of non-streptococcal URTI and had also taken antibiotics or nonsteroidal antiinflammatory drugs, within a week prior to the onset of the skin lesions.
† A patient was diagnosed with ulcerative colitis and another with Crohn's disease.

DISCUSSION

This study constitutes the first attempt to examine the HLA class II phenotypes of unselected patients with biopsy proven EN. The idiopathic group showed a significantly lower frequency of the HLA-DRB1*04 phenotype compared with controls. Previous reports have shown an increase of HLA-DRB1*03 phenotype in sarcoidosis[10,11]. Swider, *et al* recently described that HLA-DR3 phenotype was significantly higher in patients with Löfgren's syndrome compared with patients who had sarcoidosis without Löfgren's syndrome[12].

In our series, patients with EN secondary to sarcoidosis also exhibited an increased frequency of HLA-DRB1*03 allele compared with idiopathic EN and controls. However,

Table 2. HLA-DRB1 phenotype differences between patients with EN and healthy controls. Values in parentheses are percentages.

HLA-DRB1	Controls, N = 145	Cases, N =100	Idiopathic, N =35	Secondary, N =65	Sarcoidosis, N = 32
01	25 (17.2)	27 (27.0)	9 (25.7)	18 (27.7)	7 (21.9)
15 or 16	38 (26.2)	29 (29.0)	10 (28.6)	19 (29.2)	5 (15.6)
03	29 (20.0)	23 (23.0)	8 (22.9)	15 (23.1)	12 (37.5)
04	38 (26.2)*	16 (16.0)	1 (2.9)*	15 (23.1)	8 (25.0)
11 or 12	33 (22.8)	15 (15.0)	8 (22.9)	7 (10.8)	3 (9.4)
13	40 (27.6)**	37 (37.0)	12 (34.3)	25 (38.5)	18 (56.3)**
14	11 (7.6)	3 (3.0)	1 (2.9)	2 (3.1)	1 (3.1)
07	39 (26.9)	27 (27.0)	12 (34.3)	15 (23.1)	3 (9.4)
08	11 (7.6)	2 (2.0)	2 (5.7)	0 (0)	0 (0)
09	5 (3.4)	0 (0)	0 (0)	0 (0)	0 (0)
10	6 (4.1)	1 (1.0)	0 (0)	1 (1.5)	0 (0)

* HLA-DRB1*04 was significantly decreased in idiopathic EN compared with controls [p = 0.003; OR = 0.1 (95% CI: 0.01–0.6), and corrected p = 0.03].
** HLA-DRB1*13 was significantly increased in EN secondary to sarcoidosis compared with controls [p = 0.002; OR = 3.4 (95% CI: 1.5–7.4), and corrected p = 0.02].

this difference was not statistically significant after correcting p values for the number of comparisons. Of note, Odum, *et al* observed an HLA-DR6 (HLA-DRB1*13 and HLA-DRB1*14) increase that was most pronounced in their cases with severe sarcoidosis. These authors suggested that HLA-DR6 might confer susceptibility and HLA-DR3 resistance to severe, long-standing disease[13]. In our series, in contrast, we observed a significantly higher frequency of DRB1*13 allele in acute, with generally good outcome, sarcoidosis, Löfgren's syndrome, compared to healthy controls. Based on this, we feel that HLA-DRB1*13 allele may also have prognostic value as a marker of acute sarcoidosis.

Also, the absence of statistically significant differences between cases of idiopathic EN and those associated with nonstreptococcal URTI may suggest a possible overlap between these 2 conditions.

Idiopathic erythema nodosum and some groups of secondary EN seem to have different HLA-DRB1 associations. These differences may have prognostic value in identifying patients with specific conditions associated with this syndrome. Additional studies in other unselected series of EN are required to further establish associations between HLA-DRB1 alleles and EN.

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