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HLA ANTIGENS IN JUVENILE DERMATITIS HERPETIFORMIS

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Abstract. The incidence of histocompatibility antigens HLA-A, B, C, DR was studied in 32 Italian children with dermatitis herpetiformis. A significantly increased relative risk was found for B8 (R.R.=6.2), which was present in 50% of the patients as against 14% of the controls and for DR3 (R.R.=11.7), present in 69% of the patients as against 16%. DR7 also appeared to be increased, but only among DR3-negative patients. There was no significant difference in the incidence of DR3 between children with abnormal and normal jejunal biopsy, though there does seem to be a difference in expressivity of the intestinal involvement between DR3 apparent homozygous, DR3 heterozygous and DR3-negative patients.

Key words: Dermatitis herpetiformis; Coeliac disease; HLA-A, B, C, DR antigens

The present data concern the association between HLA markers and dermatitis herpetiformis (D.H.) in children. Dermatitis herpetiformis in childhood is a chronic skin disease characterized by itching papulo-vesicular eruption, occurring chiefly on the extensor surface of the extremities, buttocks, shoulders, face and scalp; D.H. responds promptly to treatment with sulphones, whereas oral iodine administration induces a flare-up of the eruption.

Early skin lesions consist in dermal papillary accumulation of neutrophils and fibrin and separation between the tips of the papillae and epidermis. In addiction, IgA granular deposits are always present in dermal papillae, often along with the C3 fraction of complement. Most of these patients have a gluten-sensitive enteropathy, usually asymptomatic.

Several studies have already reported a relationship between HLA A, B, C markers, D.H. and BCBDC in adults and in children belonging to Caucasian populations other than the Italian (4, 5, 7, 13). An association with HLA-Dw3 and possibly with DW7 (LD-12a) has been reported by Solheim et al. (10) and Thomsen et al. (12), while the association with HLA-DR antigens and other markers controlled by the HLA region (such as Bf polymorphism) has not been sought up to now.

The purpose of the present study was to investigate the correlation between D.H. and all the HLA serologically detectable markers in a group of affected children belonging to the Italian population. Properdin B factor (Bf) and glyoxalase I (GLO-I) polymorphisms were also investigated.

MATERIAL AND METHODS

Patients

32 unrelated children with D.H. (24 females and 8 males) aged 3–16 were studied. Age at onset of disease ranged between 2 and 16 years.

Diagnosis of D.H. was achieved on following criteria:

- presence of itching erythemato-papulo-vesicular eruption, and occasionally blisters, on the typical sites;
- subepidermal multilocular blisters with prominent neutrophilic infiltration and papillary microabscesses: at electron-microscopy, these blisters appeared to be localized between the discontinuous basal lamina and the dermis;
- granular IgA and C3 deposits shown by direct immunofluorescence in the dermal papillae; IgA deposits were also detected by immunoelectron-microscopy (with peroxidase-conjugated anti-human IgA) on the microfibrils of the papillary dermis;
- sensitivity to oral administration of iodine that induced a flare-up of the eruption on the usual sites in all patients;
- good response to therapy with sulphones;
- gluten-sensitive enteropathy, shown by jejunal biopsy in 23 patients. An improvement of the enteropathy and, usually, partial resolution of the cutaneous lesions was achieved with a gluten-free diet.

Controls

431 healthy, adult blood donors from the Italian population were used as control group for HLA-A, B, C antigens while 125 random donors extensively typed with Torino sera and 8th Histocompatibility Workshop sera were used as controls for DR specificities. GLO-I and Bf phenotype and gene frequencies were analysed on a sample of 264 and 613 healthy individuals, respectively (6).

HLA-A, B, C Typing

Thirty-two patients were typed for HLA-A, B, C using the standard NIH lymphocytotoxicity technique with a set of well standardized sera. All defined specificities (8th Histocompatibility Workshop) were tested.

HLA-DR Typing

Twenty-nine patients were typed for the following DR specificities: DR1l 2l 3l 4l 5l 7, w8 with the 7th Histocompatibility Workshop standard technique. DRw6 could not be assigned reliably, due to the lack of monospecific anti-DRw6 sera. DR typing was performed on B-lymphocyte enriched suspension from peripheral blood (2) with sera from the 8th Histocompatibility Workshop and with local sera.

Bf and GLO polymorphisms

Analysis of Bf polymorphism was made in 21 patients by the immunoprecipitation technique after high voltage electrophoresis (1), while GLO-1 determination was done following the standard technique (8) with modifications.

Statistical analysis

The strength of association with D.H. was estimated for each of the typed specificities and the relative risk (R.R.) calculated from the phenotype frequencies in the patients and controls (11) following Woolf's formula. Haldane's modifications were used when one of the compared values was nil. The significance of the association was evaluated by Fisher's exact test (P). Because of the large number of comparisons ($N=48$) only the association with $P<0.001$ can be considered significant, unless it had been reported previously.

Table II. *HLA-DR phenotype distribution in 29 patients with DH*

N	%	DR 1	2	3	4	5	7	8	HLA-B locus antigens
7	24.1			+					B8, w21
				+					B8, w35
				+					B8, 5
				+					B8, 17
				+					B8, 15
				+					B8, 17
				+					B5, 14
13	44.8		+	+					B8, 12
				+		+			B8, w35
				+		+			B8, 15
			+	+					B8, 22
			+	+					B8, w16
		+		+					B8, 14
		+		+					B8, 15
			+	+					B18, 12
			+	+					Bw35, 12
		+		+					B18, 14
		+		+					B18,
				+			+		B8, 12
				+			+		B18, 12
7	24.1						+		12
						+	+		B13, 12
			+				+		B17, 12
						+	+		B5, 12
						+	+		B5, 12
						+	+		B5, 14
					+		+		B5
2	6.9	+				+			B5, w35
		+							B5

RESULTS

HLA-A, B, C, DR

B8 and DR3 were significantly increased among the patients (see Table I). The frequency of B12 was higher than expected, but not significantly so after correction of the P value. Among the other specificities tested, A9, B7, Bw16, Bw35, DR4 and DR5 were decreased but the significance did not apply after correction for the number of comparisons. No other specificity tested showed significant correlation.

Table I. *Significant correlation between HLA-A, B, C, DR and dermatitis herpetiformis*

Specificity	% Patients ($N=32$)	% Controls ($N=431$; 125)	P	R.R.
B8	50.0	13.9	$<10^{-5}$	6.2
B12	40.6	19.5	$<10^{-2}$	2.8
DR3	69.0	16.0	$<10^{-7}$	11.7

The DR phenotype and complete HLA-B typing of the patients are shown in Table II. Among the 20 DR3-positive patients, 13 were heterozygous DR3/X (where X is a second detected DR specificity), while in 7, DR3 was the only one detected among the DR antigens tested. The latter are therefore either heterozygous DR3/blank or DR3 homozygous. Among the 9 DR3-negative patients, 7 were DR7-positive. As the phenotype frequency of DR7 in the Italian non-DR3 population is 22.7%, the expected number of DR7 patients (among the DR3-negative) is about 2.0%, which is significantly ($p<0.05$) lower than that observed. Thus DR7 appears to be increased among DR3-negative patients. Analogous considerations can be made for B12. In fact in the B8-negative patients B12 is significantly more frequent than expected ($p<.01$). Only 2 patients were negative for both DR3 and DR7 (R.R. for absence of DR3 and 7=0.04, $p<10^{-7}$).

Table III. *HLA antigens associated with coeliac disease and dermatitis herpetiformis*

Specificity	C.D. ($N=104$)[a] R.R.	P	D.H. ($N=32$) R.R.	P
Aw30	4.4	$<10^{-6}$		N.S.
B8	3.0	$<10^{-4}$	6.2	$<10^{-5}$
B12	2.0	$<10^{-2}$	2.8	$<10^{-2}$
B13	3.5	$<10^{-3}$		N.S.
DR3	11.7	$<10^{-9}$	11.7	$<10^{-7}$
DR7	3.7	$<10^{-4}$		N.S

[a] From De Marchi et al. (3) and unpublished data.

Bf and GLO

No significant difference of the frequency of any of the Bf and GLO alleles was observed between the DH patients and the control population.

DISCUSSION

The above results confirm the association of HLA-B8 and DR3 (which is strongly correlated with the previously reported Dw3) with Dermatitis Herpetiformis in Italian paediatric patients. The associations found in this study are somewhat lower than those described, since B8 is present in 50% of the patients as against 16%, while in other studies B8 showed frequencies up to 87% (9) and Dw3 up to 92% (12).

Nevertheless, these values are similar to those found in coeliac disease among Italian paediatric patients (B8=38%; DR3=60%) (3) and may reflect a different genetic background of a southern European population. Due to the well known relationship of dermatitis herpetiformis with coeliac disease, it may be of interest to compare the results of DH and CD patients, coming from the same population and of approximatively the same age (see Table III). B8, B12 and DR3 resulted increases in both CD and DH with similar R.R. values, predominantly high for DR3 (R.R.=11.7 both in DH and CD). The genetic "factor" shared by the two diseases might be DR3 itself or a DR3-associated disease gene. Further antigens are associated with CD: Aw30, B13, DR7. While Aw30 and B13 seem to be completely neutral to DH, it has been shown (Table II) that DR7 is significantly increased among DR3-negative DH patients.

In addition, Thomsen et al. (12) have reported a significant increase in Dw7 (LD-12a) among adult patients with both DH and CD (identified by jejunal biopsy). In that report, 22 out of 23 patients with DH and symptoms of CD were Dw3-positive; of these, 8 were also Dw7: thus most (or all) Dw7-positive patients must have been Dw3/7 heterozygous. On the other hand, only 2 DR3/7 heterozygotes have been found among our patients, which is not significantly different from the frequency of heterozygotes among controls.

A correlation between HLA phenotypes and an intestinal involvement has been sought among our patients; the frequency of DR3 does not differ significantly between children with subtotal or partial villous atrophy (16/21) and those with a normal jejunal biopsy (2/5), though the apparently DR3 homozygotes all have a subtotal atrophy and include the only 2 patients with clinical CD, while most DR7 patients have a partial atrophy or normal biopsy. Clinical signs of enteropathy were found in 66% of the apparently DR3 homozygous patients (including the 2 children with CD), 33% of DR3 heterozygotes, 16% of DR7 (not DR3) and neither of the 2 DR3, 7-negative patients. (Note that the risk for DH of these four phenotype classes ranks in the same order.) Due to the small sample size, this distribution is not statistically significant, but might indicate a different importance of DR specificities to the intestinal involvement.

Our data confirm the well known relationship between DH and CD, since the two diseases seem to have a common genetic background such as the DR3 and maybe DR7 linked "disease gene(s)". However, other non-HLA associated factors, either genetic or/and environmental, must be postulated in order to explain the different clinical expression of the two diseases.

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