PAPERS

HLA antigens and necrobiosis lipoidica diabetorum—a comparison between insulin-dependent diabetics with and without necrobiosis

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Summary

To investigate whether HLA-A, -B, -C, and -DR alloantigen frequencies are different in diabetic patients with and without necrobiosis lipoidica diabetorum we studied 37 insulin-dependent (Type I) diabetics, 15 with and 22 without necrobiosis, and 96 normal control subjects. Compared to controls Type I diabetics had increased frequencies of B8, CW3, and DR4 and decreased frequencies of DR5 and DR7. Diabetics with necrobiosis differed from diabetics without necrobiosis only in that HLA-A2 was significantly less frequent in patients with necrobiosis. It is suggested that the lack of major differences between patients with and without necrobiosis argues in favour of the role of metabolic and/or vascular rather than genetic factors in the aetiology of necrobiosis.

KEY WORDS: HLA antigens, diabetes mellitus, necrobiosis.

Introduction

Several lines of evidence indicate that insulin-dependent diabetes mellitus (Type I) is a heterogeneous disorder (Cahill and McDevitt, 1981; Dornan *et al.*, 1982). Numerous studies also suggest that susceptibility to the disease is inherited and definite associations have been demonstrated between insulin-dependent diabetes and certain alleles of the HLA major histocompatibility complex, notably B8, B15, CW3, DR3, and DR4 (Solow *et al.*, 1979; Farid *et al.*, 1979; Platz *et al.*, 1981). Some of these associations hold regardless of the age of onset of insulin-dependent diabetes (Pittman *et al.*, 1982). However, there is variability in the HLA associations of the disease in different populations (Nakao *et al.*, 1977) and researchers disagree as to whether certain HLA phenotypes predispose diabetics to complications of the disease (Bodansky *et al.*, 1982; Dornan *et al.*, 1982).

Necrobiosis lipoidica is a chronic skin lesion, long associated with diabetes mellitus (Hildebrand, Mont-

gomery and Rynearson, 1940; Muller and Winkelmann, 1966). Different studies have shown that 0.3 to 3.0% of diabetics have necrobiosis and that women are affected four to five times as frequently as men (Melin, 1964). Clinically, the lesion has a characteristic appearance and histologically there is evidence of focal degeneration of collagen and significant deposits of lipid surrounded by histiocytes, lymphocytes, epithelioid cells and even giant cells (Ullman and Dahl, 1977). Although microangiopathy (Muller and Winkelmann, 1966), immune complex vasculitis and increased platelet aggregability (Eldon, Diaz and Naparstek, 1978) have all been proposed as aetiological factors, the issue remains unresolved.

We studied the HLA-A, -B, -C and -DR alloantigens among Type I diabetics with and without necrobiosis lipoidica to determine whether this skin complication has any HLA antigen associations which may distinguish these two groups of diabetics.

Material and methods

Patients

Thirty-seven Caucasian ketosis-prone insulin-dependent diabetics (Type I) were studied. The study was planned in a way that the age, sex distribution and age of onset of diabetes for patients with and without necrobiosis was similar. Fifteen patients (13 females and 2 males) had necrobiosis and twenty-two patients (19 females and 3 males) did not have necrobiosis. Ninety-six normal Caucasian subjects living in the same geographical area and including equal numbers of males and females served as controls. The diagnosis of necrobiosis was made clinically in all instances and, in six patients, also confirmed histologically, and was first detected at a mean age of 15.3 ± 5.7 years. Among patients with necrobiosis, the mean patient age was 27.4 ± 5.5 years and the mean age of onset of diabetes was 9.7 ± 4.5 years. For patients without necrobiosis, the mean

patient age was 29.4 ± 5.4 years and the mean age of onset of diabetes 10.2 ± 4.2 years. Other complications of diabetes affected 10 out of 15 patients with necrobiosis (10 retinopathy, 6 neuropathy, and 4 nephropathy) and 9 out of 22 patients without necrobiosis (9 retinopathy, 2 neuropathy and 4 nephropathy).

Methods

Tissue typing for HLA-A, -B, and -C antigens was carried out using the standard Terasaki microlymphocytotoxicity technique (Mittal *et al.*, 1968) and antisera obtained from U.C.L.A. and N.I.H. and local sources. The alleles tested for included 13 of the A locus, 17 of the B locus, 4 of the C locus and 10 of the DR locus. For DRW typing, B cells were obtained from whole heparinized blood treated with carbonyl iron (50 mg/10 ml) at 37°C for 10 min, then decanted and diluted 10% with Hanks balanced salt solution and layered and centrifuged at 1500 *g* for 20 min over lymphocyte separating medium (LSM—Litton Bionetics). The monocyte depleted small lymphocytes from the LSM interface were incubated in prewarmed pasteur pipette columns of polymeth-methacrylate beads previously coated with mouse-antimouse Ia antibody cross reactive with human DR (Cedarlane ATH anti ATL). After 30 min incubation, non-adherent cells were washed through the column and adherent B cells were recovered from the beads by agitation, using a modification of the method of Milford *et al.* (1980). Sera to detect DRW antigens were obtained from U.C.L.A., N.I.H. and local sources and the microlymphocytotoxicity test was used with 1-hr serum and 2-hr complement incubations.

Statistical analysis was performed using Chi square and Fisher's exact test, the former in 2×2 comparisons with large numbers and the latter in situations where frequencies of five or less were expected in a given cell (Zar, 1974).

Results

The frequencies of 13 HLA-A locus, 17 HLA-B locus and 4 HLA-C locus antigens were compared between all 47 Type I diabetics, without regard to necrobiosis, and 96 normal Caucasian controls from the same geographic area. The frequencies of HLA B8, B15 and CW3 were significantly increased in the diabetic group ($P < 0.05$, corrected for number of antigens detected). No other antigen frequencies in the A, B and C loci differed significantly. The frequencies of 10 DR antigens were compared between the diabetic group and a control group of 62 normal Caucasian subjects. The DR4 frequency was significantly increased in diabetics and both DR5 and DR7 were significantly less frequent in diabetics

($P < 0.05$, corrected for number of antigens detected). In fact, DR5 was not detected in this diabetic population.

The frequencies of the HLA-A, B, C and DR antigens were then compared in the 15 diabetics affected with necrobiosis lipoidica and in the 22 diabetics who were not so affected. The HLA phenotypes for the two groups of patients are shown in Table 1. No differences were noted between the two diabetic subgroups in the frequencies of HLA B8, B15, CW3, DR5 and DR7, antigens already shown to have a significantly different frequency in diabetics compared to controls. Although using Fisher's exact test there were antigen differences between diabetics with and without necrobiosis with respect to HLA-A2, A11, DR1 and DR4, when corrected for the number of antigens tested, only HLA-A2 remained significantly different (Table 2). Patients with necrobiosis had a significantly decreased frequency of HLA-A2 compared to patients without necrobiosis.

Discussion

The results of the present comparison of HLA antigen frequencies between insulin-dependent diabetics and controls are in most respects in agreement with other reports. While confirming increased frequencies of B8 (Moerloose *et al.*, 1978) and of B15 and CW3 and DR4 (Solow *et al.*, 1979), we did not find the previously reported increased frequency of DR5 in diabetics (Farid *et al.*, 1979). The latter may be accounted for by the predominantly female composition of our diabetic sample (Orchard *et al.*, 1982). On the other hand, we confirmed that there is a significant reduction in the frequency of DR7 (Pittman *et al.*, 1982) and noted a complete absence of DR5 in our diabetic population. Other workers (Solow *et al.*, 1979; Farid *et al.*, 1979) have not found a significantly reduced frequency of DR5 among Type I diabetics but a similar finding has been reported among identical twins with insulin-dependent diabetes (Johnston *et al.*, 1983).

The insulin-dependent diabetics with necrobiosis included in this study were predominantly female, had classical skin lesions and in 12 of 15 cases the diagnosis of diabetes was made prior to or at the same age as the diagnosis of necrobiosis. They were matched for age, sex and duration of diabetes with patients who did not have necrobiosis. In the comparison of HLA antigens between insulin-dependent diabetics with and without necrobiosis lipoidica, only HLA-A2 had a significantly different frequency in the two groups. Considering the large number of HLA antigens tested, this single difference is unlikely to indicate that these two groups with diabetes are distinct. The lack of major differences between the two groups may also account for the similar prevalence

TABLE 1. HLA phenotypes of diabetic patients with and without necrobiosis

Patient	Diabetics with necrobiosis	Patient	Diabetics without necrobiosis
1	A1, A11, B8, BW35, CW4, DR3	1	A3, AW24, BW44, B15, CW2, CW3, DR4
2	A1, A3, B8, B37, CW4, DR3, DR4	2	A1, A2, B8, BW39, DR3, DRW8
3	A3, A11, B7, B27, CW1, DRW8	3	A2, BW44, B15, CW3, DR4
4	AW24, A28, BW35, CW4, DR1	4	A2, AW30, B8, B15, CW3, DR3, DR4
5	A2, AW24, B51, B40, CW3, DR1	5	A2, B7, BW44, DR4
6	A1, A3, BW35, B40, CW3, CW4, DR3	6	A1, A2, B17, BW44, CW4, DR4
7	AW24, B7, B8, DR3, DR4	7	A2, BW44, BW51, DR4
8	AW24, AW34, B14, BW39, DR4	8	A2, A3, BW40, CW3, DR4
9	AW24, B7, BW42, DR4	9	A2, A3, B15, BW51, CW3, DR3, DR4
10	A11, AW30, BW39, B13, DR3, DR7	10	A1, B8, BW35, CW4, DR3, DR4
11	A2, B15, CW1, CW3, DR3, DR4	11	A2, B27, BW44, CW3, DR4
12	A1, A11, B8, BW22, CW3, DR1, DR3	12	A1, A2, B8, BW60, CW3, DR3, DR4
13	A2, A3, B8, BW39, DR3	13	AW25, BW44, BW61, CW2, DR2, DR4
14	A1, A3, B8, BW35, CW4, DR1	14	A1, A2, B8, BW44, DR3, DR4
15	AW24, B8, BW44, CW5, CW7, DR3, DR4	15	A2, A26, B14, BW60, CW3, DR4, DR7
		16	A2, B18, BW62, CW3, DR3, DR4
		17	A1, A2, B8, BW62, CW3, DR3, DR4
		18	A3, AW24, B8, B17, DR3, DR7
		19	A1, A30, B8, DR3, DRW9, DRW10
		20	A1, A2, B8, BW62, CW3, DR3, DR4
		21	A2, AW24, B7, BW35, CW4, DR3, DRW6
		22	A2, A3, B7, B27, CW1, DR2, DRW6

TABLE 2. HLA antigen frequencies in diabetic patients with and without necrobiosis lipoidica

HLA antigen	Patients with necrobiosis (n = 15)	Patients without necrobiosis (n = 22)	P*
A2	0.200	0.773	0.0008†
A11	0.267	0	0.020
DR1	0.267	0	0.020
DR4	0.400	0.773	0.025

*Fisher exact test. †Remains significant when corrected for number of antigens tested.

lence of diabetic complications among patients with and without necrobiosis.

Some skin disorders other than necrobiosis also have HLA associations. Generalized granuloma annulare, a skin disease sometimes observed among diabetics, has been associated with a high frequency of BW35 (Friedman-Birnbaum *et al.*, 1978) and psoriasis vulgaris has been associated with B17, CW6 and DR7 (Marcusson, Johanesson and Moller, 1981). In both instances the HLA antigen associations are different from the negative association with HLA-A2 observed in necrobiosis lipoidica diabetorum.

Eighty percent of patients with necrobiosis lipoidica have or will develop diabetes mellitus (Muller and Winklemann, 1966). However, necrobiosis is rare and hence the lack of previous studies addressing the HLA antigen associations of this condition. Even the present results must be interpreted with caution because of the small number of patients studied. Although we found that diabetic patients with necrobiosis have a decreased frequency of HLA-A2

compared to patients without necrobiosis, our findings do not provide convincing evidence that the tendency to develop necrobiosis is genetically determined. Therefore, it seems likely that metabolic and/or vascular factors are more important than genetic factors in the aetiology of necrobiosis diabetorum.

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