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Interaction of HLA and Gm in autoimmune chronic active hepatitis

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SUMMARY

An immunogenetic study of autoimmune chronic active hepatitis (CAH) showed the relative risk (RR) for this disease was 11·6 for patients who were HLA-B8, 11·7 for patients who were DR3 and 2·3 for patients who were Gma+x+. Moreover, the Gm haplotype Gma+x+ was present in 18 of 40 (45%) patients with HLA-B8, but in none of 10 patients negative for HLA-B8, whereas in 180 healthy controls Gma+x+ was evenly distributed among those positive (24%) and negative (18%) for HLA-B8. The RR was lowest in patients lacking HLA-B8 but positive for Gma+x+. Relative to this low-risk group, the risk was increased 39 times in subjects with both HLA-B8 and Gma+x+, 15 times in subjects with HLA-B8 who were not Gma+x+ and twice in subjects who were neither HLA-B8 nor Gma+x+. Statistical analysis indicated that the three-factor effect (disease risk affected by non-additive effects of HLA-B8 and Gma+x+) was significant ($P < 0.01$), as were the main effects of HLA-B8 ($P < 0.001$) and Gma+x+ ($P < 0.02$). Thus in the presence of HLA-B8, genes linked to Gma+x+, an immunoglobulin C_H allotype, may contribute to the development of autoimmune chronic active hepatitis; in the absence of HLA-B8 these same genes appear to be inactive. This may indicate interactions between MHC gene products and V_H gene products in the presentation and recognition of autoantigen(s) in autoimmune hepatitis.

INTRODUCTION

Evidence from studies in inbred strains of mice points to a substantial degree of genetic control of immune responses through regulatory genes in the major histocompatibility complex (MHC) (Paul & Benacerraf, 1977) and structural genes, presumably germline V genes linked to immunoglobulin genes (Eichmann, 1972; Pawlak & Nisonoff, 1973; Sher & Cohn, 1972). Experimental evidence for analogous genetic control of immune responsiveness in man is more difficult to obtain, but some supportive data are available from studies of particular human leucocyte antigens (HLA) (Sasazuki *et al.*, 1978; Whittingham *et al.*, 1980), and from studies of Gm (Whittingham *et al.*, 1980), the C_H region allotype locus which is presumed to be in linkage disequilibrium with immunoglobulin V_H genes. Moreover, there are associations of immunologically-mediated diseases with HLA, notably HLA-B27 and HLA-B8 and DR3 (Svejgaard & Ryder, 1977), although different mechanisms may determine these HLA associations. In autoimmune chronic active hepatitis (CAH) the reported

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frequencies of HLA-B8 and D3/DR3 are generally high, conferring a relative risk of developing this disease ranging from 3–15 (Freudenberg *et al.*, 1977; Galbraith *et al.*, 1974; Lindberg *et al.*, 1975; Mackay & Morris, 1972; Mackay & Tait, 1980; Morris *et al.*, 1977; Opelz *et al.*, 1977; Page *et al.*, 1975), and there is considerable linkage disequilibrium between HLA-B8 and DR3 (Mackay & Tait, 1980).

In this study we have shown that genes associated with HLA-B8-DR3 and Gma+x+haplotypes may interact to cause autoimmune CAH.

PATIENTS AND METHODS

Patients. The study included 41 female and 11 male patients with autoimmune CAH. All were Caucasians, and all but one were from families of Northern European origin. The diagnoses of CAH were made according to recently established clinical and histological criteria (Leevy, Popper & Sherlock, 1976); patients selected for this study were in the group defined as 'autoimmune' on the basis of the presence of characteristic autoantibody markers (Morris *et al.*, 1977). Forty-nine of the 52 (94%) patients had antibody to human polymorphonuclear leucocyte nuclei, 32 (62%) had antibody to rat liver cell nuclei, 42 (81%) had antibody to smooth muscle, and two (4%) had antibody to mitochondria when sera were tested at a dilution of 1 in 5 by immunofluorescence (Whittingham, 1972). In all cases the test using radioimmunoassay for hepatitis B surface antigen (HBsAg) was negative.

Controls. Controls comprised 299 randomly selected healthy Australians whose origins were similar to those of the patients. One hundred and eighty controls were tested for Gm allotypes and HLA-A and -B locus antigens and 119 for HLA-D locus antigens.

Gm allotyping. Gm phenotypes were established by testing the sera of the 52 patients and 180 controls for the allotypic markers Glm (f,z,a,x) and G3m(b0,b1,b3,b5,c3, s,t,g) by the passive haemagglutination inhibition procedure described by Schanfield (1978). Two patients who gave uninterpretable typing results because of the presence of antibodies were excluded, leaving 50 for analysis.

HLA typing. Fifty patients and 180 controls were typed for HLA-A and -B locus antigens, and 39 of the 50 patients and 119 controls were typed for HLA-DR locus antigens. Lymphocytes were separated from heparinized blood by centrifugation through Ficoll–Isopaque and tested for HLA-A and -B locus antigens by a standard microlymphocytotoxicity procedure (Mittal *et al.*, 1968; Terasaki & McClelland, 1964). B lymphocytes were separated from the lymphocyte preparation by a modification of procedures described by Nelson *et al.* (1977) and Grier *et al.* (1977), and typed for 7 DR locus antigens (DR1–7) by the standard microlymphocytotoxicity assay but using longer incubation times (Mackay & Tait, 1978).

Statistical analysis. The association of HLA and Gm antigens with autoimmune CAH was analysed by fitting log-linear models to multidimensional contingency tables as described by Bishop, Fienberg & Holland (1975); the computational algorithm described by Haberman (1972) was incorporated in a locally written FORTRAN program running on the CYBER-73 computer system at the University of Melbourne.

For most analyses, subjects were grouped according to three variables: disease (patients or controls), Gm (using the five exclusive and exhaustive phenotypes a−x−b+, a+x−b−, a+x−b+, a+x+b−, a+x+b+) and HLA (presence or absence of each antigen). For analyses involving a particular HLA antigen, the significance of the 'three-factor interaction' (disease risk affected by non-additive effects of HLA and Gm) and of each of the 'two-factor effects' (disease risk affected by HLA or by Gm) was assessed by examining the appropriate G^2 likelihood ratio statistic (asymptomatically distributed as χ^2). Using the hierarchy principle (Bishop *et al.*, 1975), the overall G^2 was broken down into separate contributions in a way analogous to the hierarchical decomposition of variance in ANOVA or in stepwise multiple regression.

For some analyses, Gm phenotypes were dichotomized according to the presence or absence of the Gma+x+ haplotype.

RESULTS

Among the patients with autoimmune CAH females predominated 5:1. All patients and controls who were G1m(a) typed as Gm(z,a,g) and all G3m(b) subjects typed as Gm(f,b0,b1,b3,b5), so that subjects could be grouped according to five common phenotypes b+, a+x−, a+x−b+, a+x+b− and a+x+b+.

Forty of the 50 patients (80%) were positive for HLA-B8 compared with 46 of 180 (26%) of healthy controls ($G^2 = 41{\cdot}03$, $P < 0{\cdot}001$, RR = 11·6), and 33 of 39 patients typed (85%) were positive for DR3 compared with 38 of 119 (32%) healthy controls (RR = 11·7). Thirty-one of the 33 patients (94%) who were DR3 were also HLA-B8.

Examination of the distribution of Gm phenotypes in patients and controls (Table 1) showed there was an excess of patients with the Gm phenotypes a+x+b+ (observed = 16, expected = 8·04), and the heterogeneity over all Gm phenotypes was unlikely to be due to chance ($G^2 = 16{\cdot}02$, d.f. = 4, $P < 0{\cdot}005$). Analysis of the relationship of Gm phenotype to disease according to the presence or absence of HLA-B8 (Table 2) showed that the association of disease with Gma+x+b+ was confined to those patients who were positive for HLA-B8 (observed = 16, expected = 5·22). There was also a slight excess of patients with Gma+x+b− and HLA-B8 (observed = 2, expected = 1·09). Overall, the relative risk for the Gma+x+ haplotype was 2·3 with 95% confidence limits of 1·82–2·97.

Further analysis of the data (Table 2) indicated that the three-factor interaction (disease risk affected by non-additive effects of HLA-B8 and Gm) was suggestive but did not achieve statistical significance ($G_4^2 = 6{\cdot}05$, $P > 0{\cdot}05$). The effect of HLA-B8 on disease, taking into consideration the

Table 1. Observed (and expected)* numbers of patients and controls grouped according to their Gm-phenotype

	Gm phenotype					
Group	b+	a+x−	a+x−b+	a+x+b−	a+x+b+	Total
Patients	20 (23·48)*	0 (2·61)	12 (12·39)	2 (3·48)	16 (8·04)	50
Controls	88 (84·52)	12 (9·39)	45 (44·61)	14 (12·52)	21 (28·96)	180
Total	108	12	57	16	37	230

* Expected numbers based on the null hypothesis of independence; $G^2 = 16{\cdot}02$, d.f. = 4 ($P < 0{\cdot}005$).

Table 2. Observed (and expected)* numbers of patients and controls grouped according to Gm phenotype and HLA-B8 status

		Gm phenotype					
Group	HLA-B8	b+	a+x−	a+x−b+	a+x+b−	a+x+b+	Total
Patients	+	14 (8·26)*	0 (0·43)	8 (3·70)	2 (1·09)	16 (5·22)	40
	−	6 (15·22)	0 (2·17)	4 (8·70)	0 (2·39)	0 (2·83)	10
Controls	+	24 (29·74)	2 (1·57)	9 (13·30)	3 (3·91)	8 (18·78)	46
	−	64 (54·78)	10 (7·83)	36 (31·30)	11 (8·61)	13 (10·17)	134
		108	12	57	16	37	230

* Expected numbers based on model (Bishop *et al.*, 1975) where the terms for three-way interaction, and for two-factor effects (B8 × disease and Gm × disease) are missing.

Table 3. Effect of Gma+x+ and HLA-B8

	Gma+x+ present		Gma+x+ absent	
Group	B8+	B8−	B8+	B8−
Patients	18	0	22	10
Controls	11	24	35	110
Risk*	>39 (4·4–340)†	1 —	>15 (1·8–120)	>2 (0·7–7)

* Relative to low-risk group.

† Approximate 95% confidence limits for risk, relative to low-risk group.

Table 4. Association between HLA-B8 and DR3 in patients with CAH type A

		Gma+x+ present		Gma+x+absent	
		B8+	B8−	B8+	B−
DR3	+	14	0	15	2
DR3	−	1	0	0	5

three-factor effect, was highly significant ($G_1^2 = 41{\cdot}03$, $P < 0{\cdot}001$), and the effect of Gm on disease, taking into consideration both the three-factor effect and the HLA-B8 effect, was also significant ($G_4^2 = 16{\cdot}00$, $P < 0{\cdot}005$), as is expected from the additive property of the G^2 likelihood-ratio statistic.

The data were re-analysed with Gm phenotypes dichotomized according to the presence or absence of the Gma+x+ haplotype to examine possible non-additive effects of Gm and HLA-B8 (Table 3). Eighteen of the 50 patients (36%) had Gma+x+ and this haplotype was detected in 18 of 40 (45%) of HLA-B8-positive patients compared with none of 10 without HLA-B8. In controls the frequency of Gma+x+ was 24% in those with HLA-B8 and 18% in those without HLA-B8. Analysis showed that the three-way term was significant ($G_1^2 = 6{\cdot}81$, d.f. $= 1$, $P < 0{\cdot}01$), indicating that the effect of Gma+x+ on disease was influenced by HLA-B8 (and vice versa). This reflects the finding that Gma+x+ was only associated with disease when HLA-B8 was also present. The main effects of HLA-B8 ($G_1^2 = 44{\cdot}82$, $P < 0{\cdot}001$) and of Gma+x+ ($G_1^2 = 5{\cdot}54$, $P < 0{\cdot}02$) were little altered.

The risk of disease was greatest in patients with Gma+x+ who were also positive for HLA-B8, and least in those who were Gma+x+ and negative for HLA-B8 (Table 3). Although the concept of relative risk is not simply defined in tables such as these, estimates of risk were made for the other phenotypes relative to the risk in Gma+x+ patients negative for HLA-B8. Since the numbers were small, these estimates are unstable; nevertheless, the magnitude of the effect associated with the interaction between HLA-B8 and Gma+x+ (RR > 39) was such that it is unlikely to be completely attenuated in a larger series. Since HLA-DR3 was closely associated with HLA-B8 (Table 4), it could also be presumed that the risk of autoimmune CAH was high in patients who were Gma+x+ and positive for DR3. However, as Gm phenotypes were not measured in DR-typed controls, this could not be shown directly.

Of the other HLA antigens, A1 was positively associated with disease ($G_1^2 = 26{\cdot}53$, $P < 0{\cdot}001$, RR = 6·30) reflecting its linkage disequilibrium with HLA-B8. HLA-B12, by contrast, was negatively associated with disease; only four of the 50 (8%) patients were positive for HLA-B12 compared with 44 of 180 (24%) controls. Although the decrease in HLA-B12 was considerable

($G_1^2 = 8{\cdot}16$, $P < 0{\cdot}01$, RR = 0·27), this must be at least partly a consequence of the increase in HLA-B8. This effect of HLA-B12 on disease was independent of the effect of Gm phenotype.

DISCUSSION

In this study we have shown that autoimmune CAH is not only associated with the HLA phenotypes HLA-B8 and HLA-DR3, but also with the immunoglobulin allotype Gma+x+. It is of particular interest that Gma+x+ was detected with a high frequency in patients who were positive for HLA-B8, whereas Gma+x+ was not detected in any patient negative for HLA-B8. Compared with the low-risk group – that is, patients who were HLA-B8 negative and Gma+x+ positive – the relative risk was 39 times greater in subjects with both Gma+x+ and HLA-B8, 15 times greater in subjects with HLA-B8 but lacking Gma+x+, and twice in subjects with neither HLA-B8 nor Gma+x+. These findings suggest that in the presence of HLA-B8, genes linked to the Gma+x+ haplotype are important contributory causes of autoimmune CAH; in contrast, in the absence of HLA-B8 these genes appear to be inactive. The non-additive (interactive) effects of Gma+x+ and HLA-B8 imply that gene interactions of a complex kind are involved in the determination of susceptibility to autoimmune CAH. Moreover, Gma+x+ interacted with the HLA-B8-DR3 haplotype which, in previous studies, was found to be strongly associated with autoimmune CAH (Mackay & Tait, 1980). In control subjects HLA and Gm phenotypes were independent of each other, making it unlikely that the findings represent an artefact arising from the claimed localization of the Gm locus (Smith & Hirschhorn, 1978) and the HLA complex to the same chromosome (No. 6) (see Note added in proof). Furthermore, although there are major gradients in the frequencies of HLA-B8 and Gma+x+ through European populations (Dausset & Colombani, 1973; Johnson, Kohn & Steinberg, 1977), the present findings could not be accounted for in terms of differences in the immigrant origins of patients and control subjects.

There is some precedent for the concept that the MHC and immunoglobulin allotype-linked genes can interact to cause CAH and other autoimmune diseases. In animals, the immune response to certain antigens is determined by genes segregating within the MHC (Paul & Benacerraf, 1977), and by genes associated with immunoglobulin allotypes (Eichmann, 1972; Pawlak & Nisonoff, 1973; Sher & Cohn, 1972); at the cellular level, the immune response depends upon the association of antigen with cell membrane structures coded by MHC genes, and the recognition of antigen by lymphocyte receptors coded for by immunoglobulin V genes linked to immunoglobulin C region genes. In man, we have shown that the immune response to a bacterial antigen flagellin is determined by interactive effects of Gm- and HLA-linked genes (Whittingham *et al.*, 1980), and there is evidence that susceptibility to autoimmune thyrotoxicosis is also influenced by Gm- and HLA-linked genes (Farid *et al.*, 1977).

If predisposition to autoimmunity does depend upon the interaction of immune response genes linked to the HLA-B and DR loci with immunoglobulin V region genes linked to the Gm allotype locus, the specificity of the interaction of HLA-B8 with Gma+x+ may reflect the specificity of recognition of separate determinants on the antigen molecules which are presumed to initiate the autoimmune process. Thus, when expressed on the surface of antigen-presenting cells, products of genes linked to the HLA-B8-DR3 haplotype could associate with certain determinants of initiating antigen(s) in such a way as to facilitate an appropriate recognition of other antigenic determinants by products of immunoglobulin V region genes linked to genes specifying the Gma+x+ haplotype. The recent finding of kappa (Seidman *et al.*, 1978; Lenhard-Schuller, Hohn & Brack, 1978) and heavy chain V region repertoires of 100–200 genes in the germline of the mouse (Kemp, Cory, & Adams, 1979) adds weight to the concept of a genetically determined antibody repertoire and favours the concept of structural gene-linked immune response capacity.

Autoimmune CAH is mostly reported in female Caucasians of Northern European origin; this distinctive feature sets it apart from the other major type of chronic active hepatitis, namely that associated with persistence of the hepatitis B virus in the liver, which is most prevalent in males from Southern European, Asian and African countries, with a low population frequency of HLA-B8 (Dausset & Colombani, 1973).

It is plausible that any hypothetical environmental agents causing autoimmune CAH would require genetic help from the host, and that the nature of the help and the precise alleles involved would depend upon the nature of the original stimulus. Whether there is a 'single event' which sets the disease process of autoimmune CAH on course is uncertain; the genetic determinants described in this study could act by predisposing to a particular type of initial injury by an environmental agent, or to a continuing inflammation after various types of initial injury. The occurrence of autoimmune CAH in persons with neither HLA-B8 nor Gma+x+ implies either that there is at least one other sub-type of disease caused by a different set of interacting genetic and environmental causes and/or that the susceptibility alleles are only in partial linkage disequilibrium with HLA-B8 and Gma+x+.

Further work is needed to test our observations in autoimmune CAH, other organ-specific and non organ-specific autoimmune diseases, in coeliac disease and other HLA-B8-associated non-autoimmune diseases, and to explain the specificity of each disease in terms of the interactive effects of environmental agents and genes at other loci determining susceptibility. For example, the excess of autoimmune diseases among females (Mackay & Burnet, 1963), which occurs even in some autoimmune diseases which are weakly associated with HLA-B8, suggests an important, yet undefined, role for the X chromosome in CAH and other autoimmune diseases. It is uncertain whether the effect of the X chromosome is direct, or indirect and mediated through hormonal influences on immune responsiveness.

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Note added in proof

There is recent evidence that the Gm locus is on chromosome 14 (Croce *et al.*, *Proc. Natl. Acad. Sci. USA*, **76,** 3416).