HLA and cell-mediated immunity in HB_sAg negative chronic active hepatitis¹

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SUMMARY In 20 patients with severe HB_sAg negative chronic active hepatitis a significant association has been found between the HLA determinants A1, B8, and Dw3 and cell-mediated immunity directed against human liver specific lipoprotein *in vitro*. This observation induces further speculation that genetic immunoregulatory action might be involved in the pathogenetic mechanisms of chronic active hepatitis *in vivo*.

It has been well established that there is a high prevalence of the HLA determinants A1, B8, and Dw3 in patients with HB_sAg negative chronic active hepatitis (CAH) compared with healthy controls (Mackay and Morris, 1972; Galbraith *et al.*, 1974; Page *et al.*, 1975). The loci of the major histocompatibility complex (in man known as HLA) are located on the chromosome close to genes that regulate certain immune responses or Ir-genes (Bach and van Rood, 1976a; Marx, 1976). Altered cell-mediated immunity, governed by genetic factors, may be important to the susceptibility for chronic active hepatitis (Mackay and Morris, 1972; Page *et al.*, 1975) and it has been suggested that these associations of CAH with HLA determinants might be significant to the course of the disease (Eddleston *et al.*, 1976), to its response to immunosuppressive treatment (Vogten *et al.*, 1976), and to prognosis (Opelz *et al.*, 1977).

In addition, it has recently been shown that peripheral blood mononuclear cells (PMC) of patients with chronic active hepatitis may be cytotoxic *in vitro* for autologous hepatocytes (Geubel *et al.*, 1976) and for avian erythrocytes coated with liver specific protein (LSP) purified from human liver (Vogten *et al.*, 1978) and it has been suggested that such immune mechanisms may be involved in the

pathogenesis of the disease (Cochrane *et al.*, 1976).

In the course of extensive studies of the cytotoxicity of peripheral mononuclear cells for LSP-coated target cells *in vitro* we decided to evaluate whether any relationship existed between the HLA-determinants A1, B8, and Dw3 and the degree of cell-mediated cytotoxicity in patients with chronic active hepatitis and the purpose of this report is to present our findings.

Methods

PATIENTS

We studied 20 consecutive, Caucasian, HB_sAg negative patients (six male, 14 female, aged 19-56 years) with severe chronic active hepatitis diagnosed by previously established clinical, biochemical, and morphological criteria (Soloway *et al.*, 1972). Histological confirmation of the diagnosis in these patients was based on a liver biopsy showing chronic active hepatitis (with or without cirrhosis), subacute hepatitis with multilobular necrosis, or subacute hepatitis with bridging. All patients were initially randomly assigned to standard treatment with prednisone alone or in combination with azathioprine (Schalm *et al.*, 1976). Some clinical data of the individual patients and the time of cytotoxicity testing in relation to duration of disease, medication, and disease activity are summarised in Table 1.

TECHNIQUES

HLA typing of 18 alleles from locus A and 16 alleles from locus B was performed with 120 well-defined antisera using microcytotoxicity techniques (Mittal *et al.*, 1968) and the typing of the HLA-D locus was done by a semimicro mixed leucocyte

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Table 1 Clinical data of 20 patients with HB_sAg negative chronic active hepatitis

Patient no.	Initial liver histology*	LE/ANA/ASMA	Duration of disease before therapy (months)	At time of cytotoxicity testing		
				Duration of therapy (months)	Treatment†	AST-level (U/l)
1	SHMN	+/+/+/-	3	6	P	31
2	SHB	-/-/+	4	15	P	21
3	CAH	-/-/+	4	24	P	31
4	CAH	-/+/-	6	12	—	35
5	SHB	-/+ +/+	2	36	P	25
6	SHMN	-/-/+	9	12	P	38
7	CAH	+/+/+	15	55	P/A	39
8	CAH	-/+ +/+	60	9	P/A	61
9	SHMN	-/-/+	16	36	—	19
10	SHB	-/-/+	8	9	P/A	101
11	CAH + cirrh	-/+ +/+	72	12	P	40
12	SHMN	-/-/+	15	6	—	28
13	SHMN	-/-/+ +	6	12	—	47
14	CAH	+/+ +/+ +	4	3	P	36
15	CAH	-/-/+ +	12	40	P	21
16	SHMN	-/+ +/+	8	36	P	157
17	SHB	-/+/-	14	16	P/A	27
18	SHB	+/+ +/+ +	4	3	P/A	537
19	SHMN	-/+ +/+	8	16	P	19
20	CAH	+/+/-	24	36	—	21

*SHMN: subacute hepatitis with multilobular necrosis. SHB: subacute hepatitis with bridging. CAH (+cirrh): chronic active hepatitis (with cirrhosis).

†P: prednisone. P/A: prednisone + azathioprine. —: no treatment (remission).

culture method (Opelz *et al.*, 1977). Cell-mediated cytotoxicity was tested using peripheral mononuclear cells from the peripheral blood as the effector cells for ⁵¹Cr labelled pigeon erythrocytes coated with human liver specific protein as the targets, as reported elsewhere (Vogten *et al.*, 1978). Using the same method, cytotoxicity also was tested in a group of 60 HB_sAg-negative healthy volunteers (34 male, 26 female, aged 19-54 years). All experiments were done at least in duplicate and arithmetical means ($\pm$ SEM) are given. Student's *t* test was applied for the calculation of statistical significance.

Results

The results of individual experiments are shown in Table 2. In summary, the degree of cytotoxicity shown by peripheral mononuclear cells from 10 HLA-A1 positive patients was significantly higher than in 10 who were HLA-A1 negative ($P < 0.005$). In addition, the B8-positive patients with chronic active hepatitis were significantly more cytotoxic ($P < 0.01$) than a comparable group of B8-negative patients, as were the PMC of Dw3-positive patients when compared with those from Dw3-negative patients ($P < 0.05$). No significant correlations were found between the HLA determinants A1, B8, and Dw3 and age, sex, liver histology, and duration of treatment and follow-up period. All patients who possessed all three HLA determinants showed a high degree of cytotoxicity ($14 \pm 3\%$), whereas those who

Table 2 Relationship between HLA and cell-mediated cytotoxicity in 20 patients with HB_sAg negative chronic active hepatitis

Patient* no.	HLA-A1	HLA-B8	HLA-Dw3	%specific	⁵¹ Cr-release
				Mean	Range
1	+	+	+	5	0.3-15.7
2	+	-	+	2	1.1- 3.4
3	-	-	-	2	0.9- 2.2
4	-	-	-	5	4.2- 5.9
5	+	+	+	7	2.9-10.1
6	+	-	-	5	4.2- 5.6
7	+	+	+	18	18.1-18.6
8	-	-	-	2	1.4- 2.0
9	+	-	+	14	13.5-15.2
10	-	-	+	0	0.2- 0.5
11	-	-	+	6	4.0- 7.3
12	-	-	-	-1	-0.1- -0.7
13	+	+	+	26	24.5-27.0
14	+	-	+	17	17.1-17.7
15	-	-	+	4	2.7- 4.8
16	+	+	+	9	7.4-11.2
17	-	-	?	9	3.7-13.7
18	+	+	+	16	14.1-18.2
19	-	-	+	1	0.7- 1.2
20	-	-	+	5	3.9- 5.5

*The patient numbers refer to the same patients as Table 1.

lacked all three determinants were essentially negative ($2 \pm 1\%$). In the group of 60 healthy volunteers no significant cytotoxicity was found ($1.1 \pm 0.2\%$).

Discussion

The HLA determinants can be used to divide patients with chronic active hepatitis into one

subgroup bearing one or more of the HLA determinants A1, B8, or Dw3 and another consisting of patients lacking these determinants. Previously, we have found an association between HLA-Dw3 and a poor response to treatment, and postulated a genetic influence on both susceptibility to chronic active hepatitis and response to treatment with immunosuppressive drugs (Opelz *et al.*, 1977). Our present data show that *in vitro* cytotoxicity of peripheral mononuclear cells against LSP-coated target cells was primarily found in the first of these subgroups of such patients.

As this cytotoxicity measures immune reactivity directed against liver lipoprotein(s) (Vogten *et al.*, 1978) we may conclude that, in our patients with chronic active hepatitis, the presence of certain HLA determinants was apparently associated with increased cytotoxicity of peripheral mononuclear cells for target cells coated with liver specific protein, although whether this association would be sustained in a much larger series of patients is at present unknown. However, it has been suggested by some authors (Meyer zum Büschenfelde and Hopf, 1974) that autoimmune reactions to liver specific protein may be important in the production of chronic liver cell injury and are related to piecemeal necrosis in the liver biopsy (Cochrane *et al.*, 1978).

To explain the high incidence of HLA determinants A1, B8, and Dw3 in series of patients with chronic active hepatitis several possible mechanisms have been proposed (Bach and van Rood, 1976b; Marx, 1976) including cross-reactivity of these HLA-antigens with antigenic components of the infecting virus and suggestions that HLA-antigens may act as receptors for the virus. However, because many patients with chronic active hepatitis (especially those associated with HBsAg in the serum) lack these HLA determinants we find these hypotheses unattractive. Our finding of an association between certain HLA determinants and cell-mediated cytotoxicity *in vitro* in patients with HBsAg negative chronic active hepatitis lends support to the hypothesis that a genetic linkage disequilibrium exists between the genes of the major histocompatibility complex and the immunoregulatory genes controlling autoimmunity against liver specific protein. However, whether such a mechanism contributes to the pathogenesis of chronic active hepatitis *in vivo*, or to its course, remains to be established.

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