Different HLA associated gene combinations contribute to susceptibility for coeliac disease and dermatitis herpetiformis

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SUMMARY Forty two white patients of British or Irish descent with coeliac disease and 28 with dermatitis herpetiformis were typed for class I HLA-A, B, and C, and class II DR and DQ antigens. In coeliac disease there was a significant increase in the frequencies of A1, B8, DR3, DR7, and DQw2 compared with controls but no increase of DR2. In dermatitis herpetiformis there were similarly increased frequencies of A1, B8, DR3, and DQw2. In contrast with coeliac disease, however, the frequency of DR7 (18%) was no different from the control group but there was an increased frequency of DR2.

Coeliac disease and dermatitis herpetiformis are gluten dependent disorders. Both have a gluten sensitive enteropathy but the distinguishing feature between the two is a characteristic skin rash in dermatitis herpetiformis. In both conditions the lesions heal with a gluten free diet and recur on reintroduction of gluten.^{1 2} Previous reports of HLA associations have shown that patients with coeliac disease and dermatitis herpetiformis have similar significant increases in the incidence of both HLA-B8³⁻⁶ and HLA-DR3⁷⁻¹⁵ in different population groups. These data have suggested that disease susceptibility gene(s) for both conditions are closely linked to HLA-B8-DR3. Because one group of patients only develops an enteropathy and the other skin lesions with mild enteropathy, however, there may be different genes playing a role in the manifestation of the disease in the two groups.

In addition to the HLA-DR series a second serologically defined series of HLA class II antigens, the DQ series, was officially recognised at the 9th Histocompatibility Workshop¹⁶ after which 12 DR and three DQ antigens were established. Strong linkage disequilibrium exists between alleles of the DR and DQ series. In European caucasians DR3 is in strong linkage disequilibrium with DQw2 and

DR7 is also in strong linkage disequilibrium with DQw2, but less so with DQw3.¹⁷ A significantly increased frequency of both HLA-DR3 and DR7 in patients with coeliac disease in different caucasoid population groups is now well documented.^{9 10 12 14 15} It has been postulated that in coeliac disease¹⁸ and in dermatitis herpetiformis¹⁹ the primary association is with DQw2 and that DR3 and DR7 are raised as a consequence of their linkage disequilibrium with DQw2. Hitherto only DR3 and not DR7 has been found to be significantly increased in patients with dermatitis herpetiformis.

In this study, we have compared the incidence of the HLA class I A, B, C and class II DR and DQ antigens in white patients of British or Irish extraction with dermatitis herpetiformis and coeliac disease and have now shown significant differences in the frequencies of DR histocompatibility antigens in the two disorders.

Methods

PATIENTS

Forty two children (27 girls and 15 boys) with coeliac disease attending the Queen Elizabeth Hospital for Children and 28 patients with dermatitis herpetiformis (19 boys and nine girls) attending the Dermatology Department of St Mary's Hospital were investigated. The approximate age of onset of disease in each patient is given in Tables 1 and 2.

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Table 1 HLA Phenotypes of patients with coeliac disease

Name	Age of onset (yrs)	Class I			Class II		
		A	B	Cw	DR	DQw	DR52/53
LDa	6	2 29	45 57	6	3 7	2 3	2 3
KTo	2	2 30	13 14	6	3 7		2 3
DMi	<1	1 29	8 44	7	3 7	2	2 3
AvR	5	1 2	8 41	7 7	3 7	2 3	2 3
RWi	<1	2 23	8 44	4 7	3 7	2	2 3
JCu	2	2 32	8 14	8 7	3 7	2	2 3
EoC	<1	1 28	8 44	7	3 7	2	2 3
LBa	na	2 11	8 7		3 7	2	2 3
SSh	9	1 29	8 44	7	3 7	* 3	2 3
SBr	9	1 32	8 44		3 7	*	2 3
GGo	<1	1 24	8 13	6	3 7	*	2 3
DSe	<1	1 3	8 44	7	3 7	*	2 3
LWe	2	1 29	8 44	7	3 7	*	2 3
PNc	<1	1 2	8 44	7	3 7	*	2 3
YBe	<1	1	8 44	7	3	2	2
JMa	<1	1 30	8 18	5 7	3	2	2
CCr	<1	1	8 14	7	3	2	2
NPu	4	1 2	8	7	3	2	2
NMu	<1	1	8		3	2	2
SMa	2	2	8 44	5 7	3	2	2
FNc	<1	1 3	8 27	1 7	3 1	2 1	2
TSt	6	24 25	8 27	2 7	3 2	2 1	2
JWe	na	1 32	8 35	4	3 2	*	2
SB1	9	1 3	8 7	7	3 2	2 1	2
PWi	6	1 3	8 44	5 7	3 2	2 1	2
RBr	10	2 29	8 57	6 7	3 2	Nt	2
JSc	<1	3 24	8	7	3 4	2 3	2 3
CA1	na	1 28	8 62	3 7	3 4	2 3	2 3
RWh	<1	1 3	8 27	1 7	3 4	2 3	2 3
LTa	<1	1 28	8 60	3 7	3 4	2 3	2 3
SFr	3	1 2	8 44	5	3 5	2 3	2
JGr	<1	2 11	39 61	2 7	3 5	2 3	2
PMa	<1	2	62 18	3 7	3 5	2 3	2
DPa	2	11 24	18 55	3 5	3 6	2 1	2
JLi	5	1 3	8 7	7	3 6	2 1	2
LWi	8	1 2	8 51	7	3 6	2 1	2
CMc	<1	2 3	8 14	7	3 6	2 1	2
LHa	<1	1	8 44	7	7	2	3
PHo	na	2 3	7 44	5 7	7	2	3
GHa	<1	2	44 72	2 7	7 4	2 3	3
WMc	<1	2 24	18 62	3	7 5	2 3	2 3
FKc	<1	2 25	49 57	6 7	7 5	2 3	2 3

Nt=not tested, *=not tested for DQw2, <=less than, na=not available.

The minimal criteria for the diagnosis of coeliac disease were based on biopsies showing subtotal villous atrophy of the jejunal mucosa on a gluten containing diet followed by a clinical remission on a gluten free diet. The diagnosis of dermatitis herpetiformis was based on the clinical features of the rash and the presence of granular deposits of IgA in the upper dermis of uninvolved skin. No patient with linear IgA deposits along the basement membrane was included in this study. The control group consisted of 161 unrelated healthy British white volunteers who were typed in the Department of Immunology, The London Hospital Medical College.

TISSUE TYPING

The class I, HLA-A, B, C and class II, HLA-DR and DQ antigens were identified by a modified NIH technique²⁰ and double immunofluorescence method²¹ respectively. It is not possible to define DQw2 in DR3/7 heterozygous individuals by conventional serology as the DQw2 sera in use have either DR7 or DR3 antibodies as well. The presence of DQw2 was determined in eight coeliac disease patients who were DR3 and DR7 by (i) using an anti-DQw2, DR7 antiserum previously absorbed with a DQw3-DR7 EBV transformed homozygous cell line, and (ii) assessment of the inhibition of the cytotoxic reaction between a DQw2-DR3 cell line

Table 2 HLA Phenotypes of Patients with dermatitis herpetiformis

Name	Age of onset (yrs)	Class I			Class II		
		A	B	Cw	DR	DQw	DR52/53
JM	50	1 24	8 7	7	2 3	1 2	2
GR	27	1 3	8 7	7	2 3	1 *	2
UH	18	3 26	8 7	7	2 3	1 2	2
GW	69	2 3	7	7	2 3	1 *	2
RO	27	1	8 7	7	2 3	1 2	2
CG	47	1 3	8 7	7	2 3	1 2	2
TF	5	2 29	8 7	7	2 3	1 2	2
JM	50	1 24	8 7	Nt	2 3	1 2	2
EB	17	1 11	8 55	3 7	2 3	1 2	2
MH	20	1 2	8 60	3 7	2 3	1 2	2
MG	33	1 2	8 35	4 7	2 3	1 2	2
JJ	43	1 2	8 44	5 7	2 3	1 *	2
MJ	43	1 11	8 62	1 7	2 3	1 2	2
ML	24	2	8 44	5 7	2 3	1 2	2
SB	46	2 3	8 44	Nt	2 3	* 1	2
FB	3	1 3	18	5	2 3	1 2	2
MG	33	1 2	8	7	3	2 3	2
TB	25	1 68	8 51	5 7	3	2	2
PD	12	1	8	7	3	2	2
HS	33	3	8 18	7	3 7	*	2 3
JS	25	1 24	8 39	7	3 7	*	2 3
EE	31	3 24	18 7	5 7	3 7	*	2 3
DH	41	1 29	8 44	5 7	3 7	*	2 3
LMM	12	1 11	35 14	4	3 1	1 2	2
KP	35	1 3	8 44	5	3 6	1 2	2
JL	27	1 11	27 18	2 5	3 8	2 3	2
GG	17	1 3	51 35	4	4 1	1 3	3
IHB	67	68 11	44 18	7	4 7	Nt	3

Nt=not tested, *=not tested for DQw2.

and a DR7, DQw2 antiserum.²² In the latter test, B cells from DR3/7 heterozygous patients were transformed with EBV virus into lymphoblastoid cell lines. Detergent soluble preparations of the class II antigen from these cell lines were used as inhibitors of the cytotoxic reaction between an anti-DQw2 (and DR7) serum and a DQw2 (but DR3) cell line. The presence of the DQw2 antigen in the preparation derived from DR3/7 heterozygous EBV transformed cells resulted in the inhibition of the cytotoxic reaction between the DQw2 positive cell and the DQw2 serum.

STATISTICAL METHODS

The data were analysed using computer software developed by Dr J. D'Amato, The Department of Immunohaematology, Leiden, The Netherlands. The significance of differences in the distribution of the HLA antigens in the patients and controls were tested by Haldane's modification²³ of Woolf's method.²⁴

Results

The HLA-A, B, C, DR and DQ phenotypes of 42

coeliac disease patients and 28 dermatitis herpetiformis patients are shown in Tables 1 and 2. The frequencies of the DR and DQ antigens in the 42 coeliac disease patients and 28 dermatitis herpetiformis patients of British or Irish descent are compared with 161 controls of similar ethnic origin (Table 3).

DISTRIBUTION OF DR3, DR7, DR2, AND DQw2 IN COELIAC DISEASE PATIENTS (Table 3)

DR3 and DQw2 occurred in 93% and 100% of coeliac disease patients compared with 31% ($p<0.00001$) and 41% ($p<0.002$) respectively in the controls. The frequency of DR7 in the coeliac disease group was also significantly increased when compared with the 27% in the control group ($p<0.025$). As shown in Table 1 all coeliac disease patients of British or Irish descent were DR3 and/or DR7.

DISTRIBUTION OF DR3, DR7, DR2 AND DQw2 IN DERMATITIS HERPETIFORMIS PATIENTS (Table 3)

DR3 and DQw2 occurred in 88% and 95% of dermatitis herpetiformis patients compared with 31% ($p<0.000001$) and 41% ($p<0.002$) respectively

Table 3 Per cent HLA-DR phenotype frequencies in coeliac disease and dermatitis herpetiformis patients compared with healthy controls

Antigen	Control (n=161)	CD (n=42)	DH (n=28)
DR1	16	2	7
DR2	31	12	57 [‡]
DR3	31	93*	88*
DR4	36	12	7
DRw11 (DR5)	14	10	0
DRw6	19	10	4
DR7	27	43§	18
DRw8	7	0	4
DRw9	1	0	0
DRw10	1	0	0
DQw1	59	20	69
DQw2	41	100 * [‡]	95¶ [‡]
DQw3	44	32	11

*p<0.000001, †p<0.002, ‡p<0.01, §p<0.025, ||n=34, ¶n=19.
CD vs DH
DR2 p<0.0003
DR7 p<0.01

of controls and DR7 was present in 18% of dermatitis herpetiformis patients which was not significantly lower than the 27% found in the control group. The 57% frequency of DR2 in dermatitis herpetiformis patients was significantly higher than the 31% found in the control group (p<0.01). All DR2 positive patients were DR3 positive as well (Table 1) and the two patients who were not DR3 or DR2 were both DR4. When the DR antigen frequencies are compared in the two conditions DR3 is not significantly different whereas DR7 is increased in coeliac disease (p<0.01) and DR2 in dermatitis herpetiformis (p<0.0003).

DISTRIBUTION OF CLASS I ANTIGENS IN COELIAC DISEASE AND DERMATITIS HERPETIFORMIS PATIENTS

HLA-A1, -B8 and -Cw7 occurred in 60%, 79%, and 76% of coeliac disease patients compared with 32% (p<0.002), 26% (p<0.0001) and 37% (p<0.00006) of the control group respectively (Table 4).

These antigens were also present at significantly increased frequencies in the dermatitis herpetiformis patient group compared with controls. A1 was present in 71% (p<0.0005), B8 in 75% (p<0.0005) and Cw7 in 75% (p<0.0005) (Table 4).

BIOPSY REPORTS ON DR2 POSITIVE COELIAC DISEASE PATIENTS

To determine whether coeliac disease patients who are DR2 had a less severe enteropathy, jejunal biopsies taken from four of the five patients after

Table 4 Per cent phenotype frequencies of relevant class I antigens in coeliac disease and dermatitis herpetiformis patients compared with controls.

Antigen	Controls (n=161)	Coeliac disease (n=42)	Dermatitis herpetiformis (n=28)
A1	32 ^a	60 ^d	71 ^e
B8	26 ^b	79 ^c	75 ^h
CW7	37 ^c	76 ^f	75 ⁱ

a vs d p<0.002
b vs e p<0.0001
c vs f p<0.00006
a vs g p<0.0005
b vs h p<0.0005
c vs i p<0.0005

presentation and before introduction of a gluten free diet were reviewed. In two cases, the dissecting microscope appearance showed the mucosa to be flat and the other two as only convoluted. The severity of coeliac disease, however, also depends on the length of small intestine affected and a single biopsy taken from the proximal intestine would not necessarily reflect the extent of pathology.

Discussion

In addition to confirming the previously reported associations between dermatitis herpetiformis and DR3, this study shows for the first time a significant increase in the frequency of DR2 in dermatitis herpetiformis patients when compared with the control group. A non-significant increased frequency of DR2 in Austrian patients (38%) compared with controls (25%) has been previously reported.¹³

The increase of DR2 in dermatitis herpetiformis compared with the control group is no longer significant when the p value is corrected – that is, multiplied by the number of antigens involved in the analysis, a standard procedure to exclude the possibility of a chance finding with so many variables. Because of the profound increase of DR3 in the same group of patients, however, there should be a proportionate compensatory decrease in the frequency of non-involved antigens, as indeed there is for DR1 and DR4. Further support for the belief that the increased frequency of DR2 is real in dermatitis herpetiformis is underlined by the fact that the increase in frequency of DR2 in dermatitis herpetiformis is significant when compared with coeliac disease after correcting the p value.

The increased frequencies in coeliac disease of A1, B8, DR3, and DR7 confirm the findings of several other groups. Although the high frequency

of DQw2 has been previously reported,¹⁸ the study was based only on its presence or absence. Our data on the frequencies of DQw1, DQw2 and DQw3 in dermatitis herpetiformis and coeliac disease patients and the control group show a similar very high increased frequency of DQw2 in coeliac disease as well as dermatitis herpetiformis. Two patients with coeliac disease, however, albeit from different ethnic origins, (unpublished data) and one with dermatitis herpetiformis, were not DQw2 and it is unlikely that DQw2 *per se* is the gene product directly involved in disease susceptibility. The very high frequency of DQw2 in both groups of patients may be explained on the basis of the high linkage disequilibrium which exists between DR3 and DQw2 in this population. It is interesting to note that all three patients who were not DR3 or DR7 were DR4 as were all the other patients in the literature who were neither DR3 nor DR7.

Scholz and Albert,¹⁵ after combining population and sib-pair data, suggested that two genes, one associated with DR3 and the other with DR7 code for susceptibility for coeliac disease. In our data DR3 as well as B8 and DQw2 on the one hand, and DR7 in association with B44 and DQw2 on the other are increased. It is interesting to note that DQw2 associated with DR3 can be biochemically (although not serologically) distinguished from the DQw2 product associated with DR7²⁵; thus different susceptibility genes may be associated with different DQw2 products. Similarly in dermatitis herpetiformis, two susceptibility genes may be involved, one associated with DQw2-DR3 and the other with DQw1-DR2.

Current investigations using HLA class II cDNA probes may disclose differences and/or similarities between the DR3 and DQw2 genes in healthy individuals and in patients with coeliac disease, dermatitis herpetiformis and other DR3 associated disorders.

The pathogenesis of both dermatitis herpetiformis and coeliac disease involve gluten intolerance and it is tempting to speculate that a disease susceptibility gene associated with DR3 is the same in both diseases and is responsible for the gluten intolerance. In coeliac disease a DR7 associated susceptibility gene may localise the pathology to the small intestine and a DR2 associated susceptibility gene in dermatitis herpetiformis may protect against the severe enteropathy found in coeliac disease, as suggested by Scholz and Albert,¹⁵ with the result that there is a milder enteropathy which may allow a gluten diet to be tolerated leading later to skin involvement. It could be suggested, however, that the DR2 susceptibility gene not only has a protective effect on the small intestine but also

predisposes to the development of the skin lesions found in dermatitis herpetiformis, thus accounting for the different clinical features found in coeliac disease and dermatitis herpetiformis.

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