Ulcerative colitis and HLA phenotype

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SUMMARY The distribution of HLA A, B, C, DR antigens was investigated in a British population with ulcerative colitis. Fifty six patients were typed for HLA, A, B, C and 46 additionally for DR. No association was found between the HLA phenotype and the presence or absence of ulcerative colitis. Serum from 52 patients was tested for the presence of the anticolon antibody. There was no relation between the presence of the antibody and the HLA phenotype. Finally, no correlation was found between the HLA phenotypes, the age of onset of the disease, the extent and the clinical course.

Ulcerative colitis is not a classical genetic disorder. The involvement of genetic factors is suggested by the familial aggregation¹ and the immune abnormalities found in patients² and their relatives.³

The search for an association between the disease and human histocompatibility antigens (HLA) has produced conflicting results.⁴⁻⁷ These differences have been explained on the basis of different genetic backgrounds of the patients studied.

The association of HLA-AW24 in patients with an early onset of their disease reported in a Jewish population has lead to the suggestion that further attempts should be made to search for an association with age of onset, extent and severity of the disease.⁶

Asakura *et al*, on the basis of an increase of DR 2 in a Japanese population have suggested that immune responsiveness coded within HLA might play an important role in the pathogenesis of ulcerative colitis.⁷

The aim here was to study the distribution of HLA A, B, C, DR antigens in a group of British patients suffering from ulcerative colitis and to investigate whether an association existed between HLA phenotype and age of onset, extent of disease, the clinical course and the presence of circulating antibodies to colonic epithelium.

Methods

PATIENTS

Fifty six patients attending the ulcerative colitis

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clinic at the John Radcliffe Hospital were studied. All patients were typed for HLA, A, B, C, antigens and 46 were additionally typed for DR antigens. There were 24 women and 32 men ranging in age from 16-76 years. The patients were divided into three groups according to the extent of the involvement: nine had proctitis, 25 had left-sided disease and 22 had total colitis - seven out of nine with proctitis, 20 out of 25 with left sided, 18 out of 26 with total colitis were studied for DR. Patients were divided into those with chronic intermittent disease (40), chronic continuous disease (8), or a single attack only (8). The diagnosis of ulcerative colitis was made on clinical sigmoidoscopic and radiological criteria. The control group consisted of 167 ethnically matched healthy individuals.

ANTICOLON ANTIBODIES

Serum from 52 patients were tested for the presence of antibodies to colonic epithelium. A 1 cm block of human colon was snap frozen within two hours of surgical resection and stored at -70°C until use. The following sera were obtained from Dako Laboratories: antihuman gammaglobulin of rabbit origin conjugated with fluorescein isothiocyanate and rabbit antihuman immunoglobulins conjugated with peroxidase.

Cryostat sections (6µ) at -20°C were fixed at 95% ethanol for 10 minutes at 4°C. The colonic sections were covered with a drop of undiluted test serum and placed in a moist chamber for 30 minutes. The sections were then washed with phosphate buffered saline (PBS) for 10 minutes and then used for immunofluorescence and immune peroxidase staining.

IMMUNOFLUORESCENCE

One drop of FITC conjugated antisera diluted 1:10 with PBS was added to the sections, incubated for 30 minutes, washed for a further 10 minutes and then mounted in glycerol and PBS.

IMMUNOPEROXIDASE TECHNIQUE

After application of serum for 30 minutes, two drops of 1:5 diluted normal swine serum was added for 10 minutes and the peroxidase labelled rabbit anti-human immunoglobulins (diluted 1:20 with PBS, and further diluted 1:20 swine serum to give a final dilution of 1:40) were added for 30 minutes. After a further wash in PBS the sections were developed in DAB (3,3-Diaminobenzadine tetrahydrochloride) (BHD Chemicals Ltd) and mounted in DPX (Raymond A Lamb, London).

The fluorescence sections were examined by ultraviolet fluorescence microscopy (Leitz, Ortholux).

HLA TYPING

Lymphocytes were isolated from defibrinated peripheral venous blood using a Ficoll-hypaque gradient. HLA antigens were detected by the standard lymphocyte cytotoxicity technique.^{8,9} T and B lymphocytes were separated by rosetting the T cells with sheep erythrocytes. These cells were used for HLA-A, B, C typing and the B lymphocytes were used for HLA-DR typing. The standard HLA-A, B, C antigens were typed for in all patients, and HLA-DR1-W10 were typed for in 46 patients.

STATISTICAL ANALYSIS

A comparison of HLA antigen frequencies between the patients and control groups was analysed using the χ^2 test. For the small number of samples Yates correction was used. The p value was corrected for the number of antigens tested.

Results

There was no difference in the distribution of HLA-A, B, C, DR antigens in the patients with ulcerative colitis compared with controls (Tables 1 and 2). Data for HLA-C are not shown. The phenotype frequencies of patients with ulcerative colitis were correlated with the presence of anticolon antibodies (data shown only for DR in Table 2), the age of presentation, the extent of the disease, and the clinical course. A higher frequency of DR 5 was found in those patients with anticolon antibodies, compared with the controls ($p < 0.05$). This increase, however, was not significant after correction for the number of antigens tested. B44 and DR

Table 1 Frequency of HLA A-B antigens in patients with ulcerative colitis and controls

HLA A locus	Patients (56) %	Controls (169) %	HLA B locus	Patients (56) %	Controls (169) %
A1	30	28	B5	3.5	1
A2	51	45	B7	28.5	31
A3	21	30	B8	21.4	21
A9	5.8	1	B13	1.75	2
A11	12.5	14	B14	8.9	8
A23	3.5	5	BW15	17	9
A24	12.5	17	BW16	3.5	2
A26	11.6	8	BW17	3.5	7
A28	3.5	6	BW18	5.3	5
A29	11	8	BW22	0	4
AW30	1.7	2	BW27	7	8
AW31	7	7	BW35	16	18
AW32	5	8	BW37	5.3	3
AW33	7	5	B38	1.7	2
			B40	10.6	17
			B41	1.7	1
			BW44	35.7	22
			BW45	1.75	3
			BW49	5.3	4.5
			B51	5.3	4.9

Table 2 Human lymphocyte antigen phenotype frequencies (PF) in patients with ulcerative colitis and correlation with age of onset and anticolon antibodies

Locus	Ulcerative colitis 46	Controls 169	Age of onset		Anticolon antibody	
			<30 20	>30 26	Positive (17)	Negative (28)
	PF%	PF%	PF%	PF%	PF%	PF%
DR 1	23.5	18	25	23	11	21.5
DR 2	23.5	31	30	19.2	11	28.5
DR 3	21.7	22	25	19.2	23	17.5
DR 4	21.7	29	25	19.2	23	21.5
DR 5	21.7	12	10	26.9	35*	7
DR 6	19.5	27	30	26.9	23	32.1
DR 7	28.2	21	10	19.2	29	17
DR 8	15.2	3	5	3	—	5
DR 9	4	2	—	—	—	—

* $\chi^2 = 5.2$ $p < 0.05$ NS corrected for the number of antigens

1 were increased in patients with left sided disease (Table 3), but this again was not statistically significant after correction for the number of antigens. No phenotype correlated with the clinical course.

Discussion

This study shows no significant association between HLA-A, B, C, DR antigens in British patients with ulcerative colitis. Furthermore, no association was

Table 3 Human lymphocyte antigen phenotype frequencies in patients with ulcerative colitis and controls

	BW 44 %	DR 1 %	DR 5 %
Controls	22	18	12
UC total	35.7	23	19.5
Age <30	41.6	25	2
Age >30	28	23	26.9
Rectum	25	10	10
Left sided	52*	40†	15
Total	27	11	16
Anticolon antibody positive	33	11	35‡
Negative	32	21	7

* $\chi^2 = 8.80$ $p < 0.01$ not significant when corrected for the number of antigens.

† $\chi^2 = 4.21$ $p < 0.05$ not significant when corrected for the number of antigens.

‡ $\chi^2 = 5.20$ $p < 0.05$ not significant when corrected for the number of antigens.

found between early onset, extent of the disease, clinical course and the HLA antigens.

The relationship between the presence of anticolon antibody and HLA phenotype was studied. A previous report has shown an increase of anticolon antibodies in the relatives of patients with UC.³ No close association, however, was found although DR 5 appeared to be more frequent in patients possessing anticolon antibodies but this was not significant when corrected for the number of antigens tested.

This negative study is in agreement with other European studies^{10 11} which have shown no correlation between HLA antigens and ulcerative colitis. Positive associations obtained by other authors^{6 7 12} may be explained by either a different genetic background or by a failure to allow for the number of antigens tested in the statistical analysis.

The major problem with all studies has been the small number of patients studied. It may be necessary to type several hundred patients before definitive results can be obtained; an exercise which is not encouraged by the negative studies so far published

and even the associations that have been found are weak.

Because there is a familial incidence of ulcerative colitis, future studies should be based on families where more than one sibling is affected either with ulcerative colitis or with Crohn's disease.

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