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Histocompatibility antigens in inflammatory bowel disease[1]

Their clinical significance and their association with arthropathy with special reference to HLA-B27 (W27)

ELIAS G. MALLAS, PAULINE MACKINTOSH, P. ASQUITH, AND W. T. COOKE

From the Nutritional and Intestinal Unit, The General Hospital, and the Regional Transfusion Centre, Birmingham

SUMMARY Histocompatibility (HLA) antigen phenotypes have been studied in 100 patients with ulcerative colitis, 100 with Crohn's disease, and 283 normal controls. In addition the incidence of ankylosing spondylitis, sacroiliitis, and 'enteropathic' peripheral arthropathy was determined in the patients with inflammatory bowel disease (IBD). There was no significant difference in antigen frequency between patients and controls. However, the incidence of HLA-B27 was increased in the patients complicated by ankylosing spondylitis and/or sacroiliitis in both ulcerative colitis and Crohn's disease. In contrast, none of the 29 IBD patients with 'enteropathic' peripheral arthropathy had B27 antigen. Furthermore, ankylosing spondylitis was found more frequently in ulcerative colitis bearing HLA-B27 compared with non-B27 patients ($P < 0.01$). The same was found in Crohn's disease, although this difference was not statistically significant. In addition, 12 of 14 ulcerative colitis patients and five out of six Crohn's patients with HLA-B27 had total colitis, compared with the frequency of total colitis in non-B27 patients ($P < 0.024$ and < 0.03 respectively). The data suggest that B27 histocompatibility antigen could be a pathogenetic discriminator between the arthropathies in IBD and may be of prognostic significance with respect to extension and severity of the disease.

There has been increasing interest in the relationship between the HLA histocompatibility (tissue type, transplantation) antigens and disease (Dausset *et al.*, 1974). HLA-B27 is significantly associated with ankylosing spondylitis (Brewerton *et al.*, 1973; Schlosstein *et al.*, 1973), psoriatic rheumatism (Svejgaard *et al.*, 1973), Reiters disease (Brewerton *et al.*, 1973; Woodrow, 1973), and Yersinia arthritis (Aho *et al.*, 1973).

Evidence for the involvement of genetic factors in inflammatory bowel disease (IBD) is provided by the increased familial incidence found in both ulcerative colitis and Crohn's disease (Sherlock *et al.*, 1963; Singer *et al.*, 1973). Also the involvement of immunological mechanisms in the causation of these diseases has been emphasized (Kraft and Kirsner, 1971). Since HLA antigens are not only genetic markers but are probably in linkage disequilibrium with immune response or immune response associated antigens (McDevitt and Bodmer, 1972), determination of the histocompatibility antigens in inflammatory bowel disease could provide important information.

An association between IBD and arthropathy is well recognized. It has been suggested that the arthropathy seen in IBD consists of two distinct types, one, a peripheral (enteropathic) arthropathy which is seronegative and usually correlates with disease activity, and the other ankylosing spondylitis and sacroiliitis which seems to be independent of the bowel disease (Acheson, 1960; Ansell and Wigley, 1964; Bowen and Kirsner, 1965; Haslock and Wright, 1973; Palumbo *et al.*, 1973).

The HLA antigens in IBD and the association between the antigen B27 and ankylosing spondylitis complicating these disorders have been reported by a number of workers (Gleeson *et al.*, 1972; Asquith *et al.*, 1974; Brewerton *et al.*, 1974; Jacoby and

[1]The WHO Committee's new recommendation for HLA nomenclature has been used in this study.

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Jayson, 1974; Lewkonia *et al.*, 1974; Morris *et al.*, 1974; Nagant *et al.*, 1974; Russell *et al.*, 1975). Nevertheless, a relationship between HLA-B27 and either ulcerative colitis or Crohn's disease has not been convincingly demonstrated. Asquith *et al.* (1974) found that HLA-A3 in ulcerative colitis and HLA-A9 in Crohn's disease were significantly reduced compared with controls, but this has not been the experience of others (Gleeson *et al.*, 1972; Jacoby and Jayson, 1974; Russell *et al.*, 1975). Hence, a series of patients with IBD have been studied with special reference to HLA-B27 and the HLA phenotype of the patients complicated with arthropathy.

Method

One hundred patients with ulcerative colitis and 100 with Crohn's disease were randomly selected from the follow-up clinic of the Nutritional and Intestinal Unit. By chance 11% from the previous series of Asquith *et al.* (1974) without reference to their typing have been included and retyped with the extended range of HLA antisera. The diagnosis was made using accepted criteria (Schachter and Kirsner, 1975). The control group consisted of 283 normal panel members and local kidney donors.

A full history and examination were carried out with special attention to arthropathy. Those with arthritic symptoms were *x*-rayed (spine, sacroiliac joints, and symptomatic peripheral joints) together with the sacroiliac joints and spines of all HLA-B27 positive patients and the films read independently. Criteria for the radiological diagnosis of sacroiliitis were irregularity of sacroiliac joint margins, periarticular sclerosis, loss of joint space, and fusion of the joints (Murray and Jacobson, 1971). Patients with ankylosing spondylitis had classical clinical and radiological features of the disease (Kellgren, 1962). A diagnosis of the peripheral, 'enteropathic' arthropathy was made on examination or with a typical history, on the basis of a seronegative polyarthritis without any deformity, but usually related with bowel disease activity. In all, venous blood was tested for rheumatoid factor by Latex fixation test and routine haematological and biochemical determinations were performed. All the patients were negative for rheumatoid factor. Twenty-four different HLA antigens were determined in each, using a modification of the microlymphocytoxicity test of Terasaki and McClelland (Terasaki and McClelland,

Table 1 *Frequency of HLA antigens in patients with IBD and controls*

Ulcerative colitis (100)				*Normal controls (283)*	*Crohn's disease (100)*		
PHEN	*NA*	*CHISQ*	*χ*	*NC*	*NA*	*CHISQ*	*χ*
A1	43	0·845	1·181	103	30	0·870	0·824
A2	51	0·100	1·053	137	62	2·626	1·280
A3	21	1·000	0·781	76	23	0·424	0·856
A9	20	1·622	1·415	40	14	0·000	0·990
A10	6	0·733	0·679	25	4	2·280	0·452
A11	8	1·619	0·611	37	4	5·583	0·305
A28	6	0·486	1·414	12	5	0·096	1·179
AW 19	12	0·883	0·738	46	14	0·239	0·861
SUM	167	7·291	0·738	476	156	12·222	0·861
B5	16	1·087	1·372	33	10	0·181	0·857
B7	23	0·583	0·834	78	29	0·054	1·052
B8	31	0·634	1·185	74	24	0·133	0·917
B12	29	0·973	0·812	101	31	0·471	0·868
B13	9	4·450	2·547	10	7	2·000	1·981
BW35	12	0·007	1·029	33	7	1·536	0·600
BW40	12	0·313	1·212	28	10	0·000	1·010
B14	2	4·895	0·277	25	7	0·297	0·792
BW15	10	0·062	0·912	31	18	2·867	1·643
BW16	1	0·096	0·943	4	3	1·017	2·201
BW17	3	6·214	0·287	34	11	0·064	0·915
B18	4	0·542	0·665	17	1	3·941	0·242
BW21	1	0·277	0·771	5	1	0·277	0·771
BW22	1	1·049	0·499	8	6	2·035	2·122
B27	14	4·552	2·085	19	6	0·057	0·893
BW37	5	2·958	2·830	5	2	0·021	1·286
SUM	173	28·701		505	173	14·959	

Generalisation of Woolf's method (Edwards, 1974).
NA: number of responses for affected individuals.
NC: number of responses of control individuals.
χ: relative liability.

1964) and assessed for either an increase or decrease in frequency in each of the 24 antigens tested. P values were obtained using the chi-squared test applying the Yates' correction and multiplied by 24, the number of antigens tested (Grumet *et al.*, 1971). In addition, the chi-squared test was studied for evidence of a generalised variation in liability in HLA status (Edwards, 1974; Svejgaard *et al.*, 1975).

Results

The frequency of the 24 HLA antigens in ulcerative colitis, Crohn's disease, and control subjects is shown in Table 1. No significant difference at the 5% level was found for HLA-A antigens in either disease group or for HLA-B antigens in Crohn's disease, compared with controls, but there was a significant difference in locus HLA-B between ulcerative colitis and normal subjects ($\chi^2_{15} = 28{\cdot}7$ $P < 0{\cdot}05$). This significance still remains even if those cases with ankylosing spondylitis are excluded. When allowance was made for the numbers of tests, there was no difference at a statistically significant level in individual phenotypes. However, at the same locus there was a substantial increase in risk for phenotypes B13, B27, BW37, and an apparent decrease for B14 and BW17 The relative liability data in both groups of patients is graphically represented in the Figure.

Table 2 shows the incidence of ankylosing spondylitis, sacroiliitis, and peripheral arthropathy in the current series and also the HLA-B27 incidence in those patients. Five patients (5%) had ulcerative colitis and ankylosing spondylitis and four out of the five were HLA-B27 positive, six (6%) had ulcerative colitis and sacroiliitis, three of whom were HLA-B27 positive. In Crohn's disease, there were two (2%) with ankylosing spondylitis of which one had HLA-B27 and 11 (11%) with sacroiliitis, four being HLA-B27 positive.

Twelve per cent of patients with ulcerative colitis and 17% of those with Crohn's disease were found to have seronegative (enteropathic) peripheral arthropathy. In Crohn's disease, 11 out of the 17 patients with peripheral arthropathy had the HLA-A2, B12 combination as compared with eight with the same combination out of 83 non-arthropathy patients ($P < 0{\cdot}01$). None of the 12 patients with ulcerative colitis and peripheral arthropathy had the HLA-A2-B12 combination and no patient with peripheral arthropathy had HLA-B27.

The HLA-B27 frequency in ulcerative colitis (14%) was considerably greater, though not significantly so, than in Crohn's disease (6%) or in normal controls (6·7%). In ulcerative colitis four out of 14 patients with B27 had ankylosing spondylitis compared with one in 86 B27 negative patients ($P < 0{\cdot}01$): in Crohn's disease one of the six B27 positive patients had ankylosing spondylitis compared with one in 94 B27 negative patients ($P < 0{\cdot}24$).

Twelve patients of the 14 with ulcerative colitis and B27, had total colonic involvement, compared with 30 of 86 without B27 ($P < 0{\cdot}024$). In Crohn's

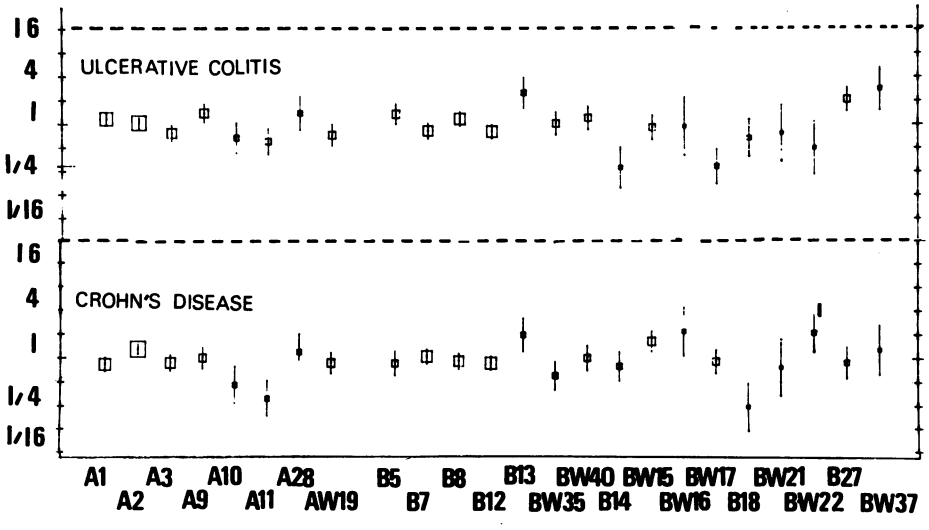

Figure *Relative liability (X) (logarithmic scale) in patients with Crohn's disease and ulcerative colitis. The vertical lines show standard error. The size of the squares is proportional to the numbers of the individuals in each group.*

Table 2 *Frequency of HLA-B27, in IBD patients with ankylosing spondylitis, sacroiliitis, and peripheral arthropathy*

	Ulcerative colitis (100)				*Crohn's disease (100)*			
	Patients		*B27 Positive patients*		*Patients*		*B27 Positive patients*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Ankylosing spondylitis	5	5	4	80	2	2	1	50
Sacroiliitis	6	6	3	50	11	11	4	36
Peripheral arthropathy	12	12	0	0	17	17	0	0

Table 3 *Frequency of HLA-B27 in ankylosing spondylitis and sacroiliitis complicating IBD as reported in literature*

References	*No. of patients*	*Ankylosing spondylitis*			*Sacroiliitis*		
		No.	*%*	*%B27 positive*	*No.*	*%*	*%B27 positive*
Crohn's disease							
Jacoby *et al.* (1974)	74	0	0	0	3	4	0
Nagant *et al.* (1974)	117	5	4·2	27	6	5	—
Russell *et al.* (1975)	77	11	14	90	—	—	—
Morris *et al.* (1974)	18	5	27	75*	—	—	—
Present series	100	2	2	50	11	11	36
Ulcerative colitis							
Brewerton *et al.* (1974)†	28	28		72	—	—	—
Morris *et al.* (1974)	11	2	18	75*	—	—	—
Present series	100	5	5	80	6	6	50

*In this series no separation was made between ulcerative colitis and Crohn's disease.
†Selected series.

disease, five out of six patients with B27 had total colonic involvement compared with 17 out of 94 patients without B27 ($P < 0{\cdot}03$). Ten out of these 17 patients with inflammatory bowel disease have had panproctocolectomy.

Discussion

No significant difference in the incidence of any of the 24 antigens was found between ulcerative colitis, Crohn's disease, or the panel of control subjects as has been noted by others (Gleeson *et al.*, 1972; Jacoby *et al.*, 1974; Russell *et al.*, 1975). Asquith *et al.* (1974) found HLA-A3 to be rarer in ulcerative colitis: in this series HLA-A3 was slightly reduced. They also found HLA-A9 rarer in Crohn's disease; in this series, the percentage of A9 in both patients and controls was 14%.

In addition, the total chi-squared was studied for evidence of a generalised variation in liability in HLA status (Table 1). (Edwards, 1974; Svejgaard *et al.*, 1975). No significant difference at 5% level was found for HLA-A antigen in either disease or for HLA-B antigen in Crohn's disease. There was a significant association in ulcerative colitis but as this was out of four tests and as two of the major contributors to the total χ^2 was due to low, rather than high, liability (B14 and BW17), it seems likely that this was fortuitous. However, only by the combination of data from several series of similar size will it be possible to distinguish between sampling effects and disease association with reasonable confidence

In the normal population, 6·7% had B27; of these about 5% are likely to have ankylosing spondylitis (Brewerton *et al.*, 1973). This disease occurs in approximately 1 in 2000 of the normal population and 88-96% of such cases have HLA-B27 (Schlosstein *et al.*, 1973). This occurrence in the normal population should be compared with ulcerative colitis, in which the incidence of B27 was 14% of which 28·7% had ankylosing spondylitis. Five per cent of the ulcerative colitis patients had ankylosing spondylitis, 80% of which were B27. In the Crohn's disease group the incidence of B27 was almost the same as the normal controls (6%), but a higher percentage (16·5%) had ankylosing spondylitis. The incidence of this disease was 2%, 50% being B27. These data certainly support the suggestion put forward by Morris *et al.* (1974) that patients with inflammatory bowel disease with HLA-B27 are more likely to develop ankylosing spondylitis than others who do not possess this antigen. But whether this is more likely than a suggestion that ankylosing spondylitis predisposes to inflammatory bowel disease cannot be deduced from the data presented (Table 3).

In the present series the majority of patients with Crohn's disease and B27 had total colonic involvement and this was a statistically greater incidence than those B27 negative patients. A similar significantly increased incidence of total colonic involvement was present in ulcerative colitis. Jacoby *et al.*

(1974) noted three out of six patients with Crohn's disease and B27 had colonic involvement, compared with 16 out of 68 B27 negative, though in that instance the findings were not statistically significant. Nevertheless, it would appear the antigen B27 may have some influence on the severity and location of the inflammatory bowel disease.

It was also noteworthy, as had been reported by Morris *et al.* (1974) that none of the 29 patients with inflammatory bowel disease and peripheral arthropathy had the B27 antigen, suggesting that this type of arthropathy is distinct from the complication of ankylosing spondylitis. Whether the A2, B12 combination present in 11 of the 17 patients with Crohn's disease with enteropathic arthropathy and its absence in all 12 patients with ulcerative colitis is of similar significance must remain to be confirmed in view of the small number of patients involved.

In conclusion, HLA-B27 may well have some prognostic and clinical significance with respect to the extent and severity of inflammatory bowel disease, while it may also be of some value in differentiating between the arthropathies complicating it.

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