HLA antigens and inflammatory bowel disease in a sample of Iraqi patients

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مستضدات « هلا » والمرض الالتهابي المعوي في عينة من المرضى العراقيـــين

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الخلاصة: دَرَسَ الباحثون الترابط بين مستضدات « هلا » وبين المرض الالتهابي المعوي لدى 65 مريضاً عراقيـاً (50 مصاباً بالتهاب القولون التقرحي و15 مصاباً بداء كرون) وقورنَتْ بـ67 من الـشواهد المناسبة. وقـد أظهـر المرضى في المنطقة I من الصنف « هلا » ارتفاعاً يُعتَـدُّ به إحصائياً في معدلات تكرار A9 وB41 مـع نقـص فـي A11. كما وجد الباحثون نتائج مشابهة عند النظر في الأنماط السريرية كل على حدة، باستثناء A11 الذي لم يكن الازدياد فيه يُعْتَدُّ به إحصائياً، أما في الناحية II من الصنف « هلا » فقد كان هناك زيادة يُعْتَدُّ بها إحصائياً في DR8 لدى العدد الكلي للمرضى، إلا أن هذا الترابط لم يكن مطّرداً في التهاب القولون التقرحي أو داء كـرون، وبـدلاً من ذلك فقد كان لدى داء كرون ترابط إيجابي مع DQ1، وقد أوضحت مقارنة الأنماط الـسريرية اختلافـاً يعتـد به إحصائياً في المستضد B16 مما يجعل B16 من الواسمات التفريقية لهذا المرض.

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ABSTRACT This study investigated the association between HLA antigens and inflammatory bowel disease in 65 Iraqi patients (50 ulcerative colitis, 15 Crohn disease) compared with 67 matched controls. At HLA class I region, the patients showed significantly increased frequencies of A9 and B41 and a decrease of A11. Similar results were found when the clinical types were considered separately, except for A11, which was not significant. At HLA class II region, DR8 was significantly increased in the total patients, but the association was not maintained for ulcerative colitis or Crohn disease patients; instead Crohn disease was positively associated with DQ1. Comparing the clinical types revealed a significant difference in the antigen B16, suggesting that B16 is a differentiating marker in the disease.

Antigènes HLA et maladies inflammatoires intestinales dans un échantillon de patients iraquiens

RÉSUMÉ Cette étude a porté sur l'association entre les antigènes HLA et les maladies inflammatoires intestinales chez 65 patients iraquiens (50 atteints de recto-colite hémorragique et 15 de la maladie de Crohn) par rapport à 67 témoins appariés. Dans la région HLA de classe I, les patients présentaient des fréquences significativement augmentées de l'antigène A9 et B41 et une diminution de l'antigène A11. Des résultats similaires ont été obtenus lorsque les types cliniques ont été considérés séparément, à l'exception de l'antigène A11 qui n'était pas significatif. Dans la région HLA de classe II, l'antigène DR8 avait augmenté de façon significative chez tous les sujets, mais chez les sujets atteints de recto-colite hémorragique ou de maladie de Crohn, il n'était pas associé à la maladie ; en fait, la maladie de Crohn était positivement associée au DQ1. La comparaison des types cliniques a permis d'observer une différence significative au niveau de l'antigène B16, ce qui semble indiquer que cet antigène est un marqueur de différenciation.

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Introduction

Inflammatory bowel disease (IBD) is a group of disorders defined by the presence of chronic gastrointestinal inflammation not due to a specific disease-producing organism. Two clinical forms of the disease exist—ulcerative colitis and Crohn disease—which have a number of different clinical and pathological features [*1*].

The aetiology of IBD is not well understood, although epidemiological as well as other clinical- and laboratory-based evidence suggests that the disease may be multifactorial, and genetic, immunological and environmental factors are all involved [*2*]. But the nature of these factors and how they interact to produce disease is a matter of speculation.

Evidence from family and twin studies suggests that genetics plays an important role in predisposing an individual to develop ulcerative colitis and Crohn disease [*3,4*]. Further evidence of a genetic predisposition comes from studies of the association between the human leukocyte antigen (HLA) system and IBD. However, an association has only been shown in homogeneous populations. In Japanese patients, HLA-B5 has been recorded as associated with ulcerative colitis, while in Caucasian patients the disease has shown an association with 2 HLA class I antigens (B27 and B44). Crohn disease patients behave in a similar manner, and the association has been reported with HLA-DR4 in Japanese patients and HLA-B44 in Caucasian patients (reviewed by [*1*]). Later studies, based on a molecular typing of HLA class II region, have emphasized the importance of HLA-DR and -DQ polymorphisms in the aetiology of IBD, but different associations have been reported in different populations [*5–10*].

The present study was designed to investigate the association between HLA polymorphisms (class I and II antigens) and IBD in a sample of Iraqi patients. Disease heterogeneity was also considered and therefore 2 clinical entities of the disease (Crohn disease and ulcerative colitis) were studied.

Methods

Patients

A total of 65 Iraqi patients with IBD were investigated during the period May–December 2004. The patients were referred to the clinics of Al Kadhamyiah Teaching Hospital and the Gastrointestinal Tract and Liver Disease Centre in Baghdad, Iraq for diagnosis and evaluation. The disease was clinically diagnosed and the patients were selected based on criteria for inclusion and exclusion decided by the consultant medical staff at the 2 clinics. The diagnosis was based on clinical evaluation using colonoscopy and a histopathological examination of a biopsy sample. According to the consultants' viewpoint, patients were clinically subdivided into ulcerative colitis (50 patients) and Crohn disease (15 patients). None of the patients was on medication at the time of investigation. A further 67 individuals were also investigated, selected from healthy blood donors at the hospitals, and were considered as a control sample. They were matched with the IBD patients for age, sex and ethnic background (Arab Muslims) (Table 1).

HLA phenotyping

From each participant, 10 mL of venous blood was obtained and processed in less than 2 hours. Lymphocytes were collected by means of density-gradient centrifugation, and then separated into T- and B-cells by the nylon-wool method. Phenotyping of the cells for HLA class I (A and B) and

Table 1 **Inflammatory bowel disease patients and controls by sex and age range**

Variable	Inflammatory bowel disease patients						Controls (n = 67)	
	Total		Ulcerative colitis		Crohn disease			
	(n = 65)		(n = 50)		(n = 15)			
	No.	%	No.	%	No.	%	No.	%
Sex								
Male	29	44.6	25	50.0	4	26.7	30	44.8
Female	36	55.4	25	50.0	11	73.3	37	55.2
Age (years)								
Range	13–65		13–35		19–65		18–65	

n = *total number of patients.*

class II (DR and DQ) antigens was done by the microlymphocytotoxicity method [*11*]. The laboratory assessments were carried out at the histocompatibility laboratory, Al Karama Hospital, Baghdad.

Statistical analysis

Significant variations of HLA alleles between patients and controls were assessed by 2-sided Fisher exact probability, and the *P*-value obtained was corrected for the number of antigens tested at each locus. The correction factors were 11, 18, 9 and 3 for HLA-A, -B, -DR and -DQ loci, respectively. The results are presented in terms of numbers, percentage frequencies, odds ratio (OR), etiological fraction (EF) and preventive fraction (PF). The latter 2 estimations were calculated when the OR values were > 1 (positive association) and < 1 (negative association) respectively. The 95% confidence intervals (CI) of the OR are also given. The mathematical calculations of these estimations were carried out using the computer programme *PEPI*, version 4.04X.

Results

Total patients

The HLA antigens showing significant variations between total IBD patients and controls are shown in Table 2.

At the HLA-A locus, 4 antigens (A9, A11, A30 and A33) showed significant deviations when comparisons were made between the total patient group and the control group. The antigen A9 was found in a significantly higher proportion of patients than controls (52.3% versus 17.9%) (OR = 4.72, 95% CI: 2.02–11.36; P < 0.001. The EF value was 0.40. Moreover, this positive association remained significant after correction for the number of antigens tested (n = 11). In contrast, 3 antigens showed negative associations with IBD and significantly fewer patients had these antigens compared with controls: A11 (3.1% versus 22.4%) (P = 0.002), A30 (6.2% versus 20.9%) (P = 0.02) and A33 (4.6% versus 16.4%) (P = 0.05). After correcting the probabilities, there was only 1 significant negative association, that between A11 and IBD.

Table 2 **Antigens of human leukocyte antigen (HLA) class I and II regions showing significant difference between inflammatory bowel disease patients and controls**

HLA antigens	Inflammatory bowel disease (*n* = 65)		Controls (*n* = 67)		Odds ratio	EF or PF	*P*-value[a]	95% CI
	No.	%	No.	%				
A9	34	52.3	12	17.9	4.72	0.40	< 0.001	2.02–11.36
A11	2	3.1	15	22.4	0.11	0.20	0.002[b]	0.01–0.51
A30	4	6.2	14	20.9	0.25	0.16	0.02	0.06–0.86
A33	3	4.6	11	16.4	0.25	0.12	0.05	0.04–1.01
B41	43	66.2	4	6.0	30.78	0.64	< 0.001	9.35–127.42
B51	11	16.9	25	37.3	0.34	0.25	0.01	0.14–0.83
DR4	22	33.8	9	13.4	3.30	0.24	0.01	1.29–8.91
DR5	11	16.9	2	3.0	6.62	0.14	0.01	1.34–63.23
DR7	5	7.7	16	23.9	0.27	0.16	0.02	0.07–0.83
DR8	20	30.8	6	9.0	4.52	0.24	0.003	1.57–14.73
DQ2	16	24.6	7	10.4	2.80	0.16	0.05	0.99–8.65

[a]*Fisher exact test.*
[b]*Corrected* P-*value significant.*
n = *total number of patients; EF = etiological fraction, PF = preventive fraction, CI = confidence interval.*

At HLA-B locus, the B41 antigen was significantly increased in the patients compared with controls (66.1% versus 6.0%) (OR = 30.78, 95% CI: 9.35–127.42; *P* < 0.001; EF = 0.64) while the antigen B51 was significantly decreased in the patients (16.9% versus 37.3%) (*P* = 0.01). Correcting the probabilities of the 2 associations gave 1 significant positive association, between B41 and IBD.

At HLA class II region (DR and DQ loci), 5 antigens (DR4, DR5, DR7, DR8 and DQ2) showed different frequencies in patients and controls. Increased frequencies were observed in patients for DR4 (33.8% versus 13.4%) (OR = 3.30, 95% CI: 1.29–8.91), DR5 (16.9% versus 3.0%) (OR = 6.62, 95% CI: 1.34–63.23), DR8 (30.8% versus 9.0%) (OR = 4.52, 95% CI: 1.569–14.73) and DQ2 (24.6% versus 10.4%) (OR = 2.80, 95% CI: 0.99–8.65). The EF values were 0.24, 0.14, 0.24 and 0.16 re-spectively. However, 1 positive association remained significant after correction, and this was with DR8. In contrast, DR7 was significantly decreased in the patients (7.7% versus 23.9%), but the negative association also failed to retain statistical significance after correction.

Ulcerative colitis patients
HLA antigens showing significant variations between ulcerative colitis patients and controls are summarized in Table 3.

At HLA-A locus, 2 antigens (A3 and A9) showed increased frequencies in the patients compared with controls, and 2 other antigens (A11 and A30) showed decreased frequencies. The antigen A3 (present in 26% of patients versus 8.9% of controls) showed a positive association that was significant before correction (*P* = 0.03). The antigen A9 was also positively associated with

Table 3 **Antigens of human leukocyte antigen (HLA) class I and II regions showing significant difference between ulcerative colitis patients and controls**

HLA antigens	Ulcerative colitis (*n* = 50)		Controls (*n* = 67)		Odds ratio	EF or PF	*P*-value[a]	95% CI
	No.	%	No.	%				
A3	13	26.0	6	8.9	3.57	0.19	0.03	1.13–12.36
A9	22	44.0	12	17.9	3.60	0.32	0.004	1.45–9.15
A11	2	4.0	15	22.4	0.14	0.20	0.008	0.02–0.68
A30	2	4.0	14	20.9	0.16	0.18	0.01	0.02–0.75
B41	36	72.0	4	6.0	40.50	0.70	< 0.001	11.40–173.87
DR4	18	36.0	9	13.4	3.63	0.26	0.008	1.35–10.18
DR5	8	16.0	2	3.0	6.19	0.13	0.03	1.14–61.69
DR7	3	6.0	16	23.9	0.20	0.19	0.02	0.04–0.79
DR8	14	28.0	6	9.0	3.95	0.21	0.01	1.27–13.55
DQ1	20	40.0	12	17.9	2.94	0.26	0.02	1.17–7.54
DQ2	13	26.0	7	10.4	3.01	0.17	0.05	1.00–9.69

[a]*Fisher exact test.*
n = *total number of patients; EF = etiological fraction, PF = preventive fraction, CI = confidence interval.*

ulcerative colitis (44% versus 17.9%) (OR = 3.60, 95% CI: 1.45–9.15) (EF = 0.32). The probability was significant before (*P* = 0.004) and after (*Pc* = 0.044) correction. In contrast, the antigens A11 and A30 showed negative associations with ulcerative colitis, and each of the 2 antigens were found in nearly 4% of patients, compared with 22.4% and 20.9% of controls. Although these 2 associations were significant (*P* = 0.008 and 0.01 respectively), the corrected probabilities failed to attain significance.

At HLA-B locus, the antigen B41 showed a significant (*P* < 0.001) increased frequency in patients versus controls (72.0% versus 6.0%), and the positive association was highly significant after correction (OR = 40.50, 95% CI: 11.40–173.87; EF = 0.70).

At HLA class II region (DR and DQ loci), several deviations in antigen frequencies were observed in the patients when comparisons were made with control subjects. Increased frequencies of antigens DR4 (36.0% versus 13.4%), DR5 (16.0% versus 3.0%), DR8 (28.0% versus 9.0%), DQ1 (40.0% versus 17.9%) and DQ2 (26.0% versus 10.4%) were observed in patients. A negative association was also observed between DR7 and ulcerative colitis (6.0% versus 23.9%). However, none of these deviations maintained a significant level after correction.

Crohn disease

HLA antigens showing significant variations between Crohn disease patients and controls are summarized in Table 4.

At HLA-A locus, the antigen A9 was found in 60% of patients compared with only 17.9% of controls. The deviation was significant before (*P* = 0.004) and after (*Pc* = 0.044) correction (OR = 6.88, 95% CI: 1.75–27.67; EF = 0.51).

Table 4 **Antigens of human leukocyte antigen (HLA) class I and II regions showing significant difference between Crohn disease patients and controls**

HLA antigens	Crohn disease ($n = 15$)		Controls ($n = 67$)		Odds ratio	EF or PF	P-value[a]	95% CI
	No.	%	No.	%				
A9	9	60.0	12	17.9	6.88	0.51	0.004	1.75–27.67
B16	5	33.3	3	4.5	10.67	0.30	0.009	1.69–76.11
B41	7	46.7	4	6.0	13.78	0.43	< 0.001	2.66–75.77
B51	1	6.7	25	37.3	0.12	0.33	0.03	0.003–0.898
DR8	6	40.0	6	9.0	6.78	0.34	0.01	1.42–31.08
DQ1	9	60.0	12	17.9	6.88	0.51	0.004	1.75–27.67

[a]Fisher exact test.

n = *total number of patients; EF = etiological fraction, PF = preventive fraction, CI = confidence interval.*

At HLA-B locus, 3 antigens (B16, B41 and B51) exhibited variations between patients and controls. Increased frequencies of B16 (33.3% versus 4.5%) and B41 (46.7% versus 6.0%) were observed in the patients (OR = 10.67, 95% CI: 1.69–76.11 and OR = 13.78, 95% CI: 2.66–75.77 respectively) (EF = 0.30 and 0.43 respectively). The probabilities of such positive associations were significant before correction ($P = 0.009$ and $P < 0.001$ respectively), but after correction, only the association between B41 and Crohn disease remained significant. The antigen B51, in contrast, showed a significantly decreased frequency in patients (6.7% versus 37.3%) ($P = 0.03$; PF = 0.33), but the difference lost significance after correction.

At HLA class II region, increased frequencies of DR8 (40.0% versus 9.0%) and DQ1 (60.0% versus 17.9%) were observed in the patients ($P = 0.004$). Such variations were significant before correction, with the exception of DQ1, which maintained a significant corrected P-value (OR = 6.88; 95% CI: 1.75–27.67).

Immunogenetic heterogeneity of IBD

The immunogenetic heterogeneity of IBD was assessed by comparing the antigen frequencies of HLA-A, -B, -DR and -DQ loci between ulcerative colitis and Crohn disease patients. Several antigens (A2, A3, A9, A30, B16, B41, B51, DR4, DR6, DR7, DR8, DR10, DQ1 and DQ2) showed different distributions in the 2 groups of patients, but none of these differences maintained a significant level. The antigen B16 was an exception. Out of 50 ulcerative colitis patients, only 1 (2.0%) expressed B16, while 5 (33.3%) out of 15 Crohn disease patients expressed this antigen ($P = 0.002$). This was significant even after correction for the number of antigens tested at the HLA-B locus ($Pc = 0.036$).

Discussion

The present study demonstrated that immunogenetic predisposition may be considered an important requirement for the development of IBD, as several markers of

human major histocompatibility complex in our study showed different distributions in patients and controls.

At HLA class I region, remarkable deviations were observed for the antigens A9, A11 and B41. Both A9 and B41 showed increased frequencies in the patients. The antigen A9 was observed in around 50% of the patients, while B41 antigen was much higher (about two-thirds of patients). These 2 deviations had OR values of 4.72 and 30.78 respectively, and maintained EF values of 0.40 and 0.64 respectively. A review of other studies of the association of HLA with IBD in other world populations revealed associations with other HLA class I antigens: B5 in the Japanese and B27 and B44 in Caucasians [1]. Such a discrepancy can be explained by racial differences, especially if we consider that HLA antigens show different frequencies in different populations including Iraqis [11,12]. This can be confirmed by looking at the estimated EF values for an antigen, which can range from 0 (no association) to 1 (maximum association). A value of 1 means that the antigen is fully responsible for the development of the disease, and if the value is between 0 and 1 the marker is partially involved in disease development and other factors (i.e. environmental pathogens) are operative [13]. The EF values for A9 (0.40) and B41 (0.64) in our study support this statement, and suggest that 60% and 36% contributions of other factors in association with A9 and B41 respectively are required in the development of IBD. The nature of these factors is unknown and further studies are certainly required to tackle this subject.

A further inspection of HLA class I antigens in the clinical types of IBD (ulcerative colitis and Crohn disease) revealed a similar picture, and both A9 and B41 antigens were positively associated with the 2 clinical entities of IBD. In this sense, these 2 antigens may confer an immunogenetic predisposition to IBD in general, irrespective of its clinical types, although the strength of the 2 associations was different. The B41 antigen may be considered more important than A9 in ulcerative colitis (EF values were 0.70 and 0.19 respectively), while the opposite was observed in Crohn disease (EF values were 0.43 and 0.51 respectively).

At HLA class II region, further antigens (DR4, DR5 and DR8) had positive associations with IBD, but only DR8 showed statistical significance after correction of probability. Although these associations were not as strong as that of the HLA class I region, the polymorphism of the HLA class II loci has gained much more interest in HLA–disease association studies because both α and β chains, which are coded by structural genes on chromosome 6, are highly polymorphic, especially at the HLA-DQ and -DP subregions [14]. Accordingly, it has been suggested that susceptibility to IBD is partially genetically determined, and the HLA class II genes are candidates for a role in genetic susceptibility to IBD, because their products play a central role in the immune response [15]. The present results strongly support such a theme; however, multiple studies have reported associations between HL-DR and -DQ phenotypes and IBD, both ulcerative colitis or Crohn disease, although much of the data are still controversial [5–10]. These studies have demonstrated that DR1, DR4, DR5 and DR7 are positively associated with Crohn disease, while DR2, DR3 and DR8 are negatively associated. For ulcerative colitis, positive associations have been found with DR2, DR6 and DR12, and recently DR13 has been added to the list in Iranian patients [8], and negative associations with DR2, DR6 and DR7 have been observed. Moreover, ulcerative colitis in Chinese patients has recently been shown to

have no significant association with the polymorphism of HLA-DRB1 gene [10]. For HLA-DQ locus, the studies have reported further associations, and ulcerative colitis has been positively associated with DQ2 and negatively associated with DQ3, while in patients with Crohn disease increased frequencies of DQ3 and DQ4 and decreased frequencies of DQ1 and DQ6 have been reported. None of these findings were confirmed in the present study, and in contrast, DR8 was positively associated with IBD, both in the total patient group and when subdivided by clinical type. However, some of these associations (positive and negative) were observed in our IBD patients (DR4, DR5, DR7 and DQ2), ulcerative colitis patients (DR4, DR5, DR7, DQ1 and DQ2) and Crohn disease patients (DQ1), but the significance was lost when the probability was corrected, with the exception of DR8 (total IBD patients) and DQ1 (Crohn disease patients). Therefore, the discrepancy can be ascribed to either racial differences (different associations in different populations), low sample size (affecting the level of significance) or environmental impacts (different causative pathogens).

Comparing the 2 clinical forms of IBD in terms of HLA antigen frequencies revealed that the antigen B16 was significantly different in ulcerative colitis and Crohn disease. Although such an observation has not been recorded before, it may help to answer crucial questions regarding the genetics of IBD, and how the 2 clinical forms are related to each other. Family studies carried out in ulcerative colitis and Crohn disease families have demonstrated that the 2 clinical forms do not always segregate independently within families [16]. Accordingly, it has been suggested that there are 3 genetic forms of IBD, one leading to ulcerative colitis alone, one to Crohn disease alone, and a third leading to both ulcerative colitis and Crohn disease. Fine mapping of the HLA region in IBD families may support this, and explain some the discrepancies in HLA–IBD association studies [9]. Evidence for a linkage of both ulcerative colitis and Crohn disease around the HLA region on the short arm of chromosome 6 has been presented [5]. Moreover, these studies have described further predisposing loci on different chromosomes [16], which highlights the heterogeneity of the disease. We can consider B16 as a differentiating marker between the 2 clinical types of IBD, although further confirmation is required before reaching a definitive conclusion.

Acknowledgements

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