Frequency of HLA Antigens in a Sample of Iraqi Brucellosis Patients

Ali H. Ad'hiah* ***Aaiad H. A. Al-Rikaby*****
Ammna N. Jasim****

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Abstract:

Fifty one patients with serologically confirmed brucellosis and 70 healthy controls were phenotyped for HLA-A, -B, -DR and -DQ antigens by using standard microlympho-cytotoxicity method, and lymphocytes defined by their CD markers (CD3, CD4, CD8 and CD19). The results revealed a significant (Pc = 0.001) increased frequency of HLA-DR8 (41.18 *vs*. 10.0%) in the patients . A significant increased percentage of CD8+ lymphocytes was also increased in the patients (25.15 *vs*. 22.0%; P = 0.006), while CD3+ lymphocytes were significantly decreased (75.1 *vs*. 79.4%; P = 0.02).

Key words: Brucellosis, HLA, CD markers.

Introduction:

Brucellosis is the most common zoonotic disease with worldwide distribution, and more than 500000 new cases are reported annually. It is caused by intracellular pathogens of the genus *Brucella* that have their natural reservoir in domestic and wild animals. The disease is transmitted to humans by consumption of contaminated dairy products or by occupational contact with infected animals [1]. The genus *Brucella* consist of seven species according to antigenic variation and primary host: *B. melitensis* (sheep and goats), *B. suis* (hogs), *B. abortus* (cattle), *B. ovis* (sheep), *B. canis* (dogs), *B. neotomae* (wood rats) and *B. maris* (marine mammals) [2].

Brucella infection occurs through inhalation or ingestion of organisms via the nasal, oral, or pharyngeal cavities, and following penetration of the mucosal epithelium, the bacteria are transported, either free or within phagocytic cells, to the regional lymph nodes [3]. The spread and multiplication of *Brucella* in lymph nodes, spleen, liver, bone marrow, mammary glands, and sex organs occurs via macrophages. In general, *B. melitensis*, *B. abortus* and *B. suis* can infect humans and the pathological manifestations of brucellosis in humans are meningitis, endocarditis, spondylitis, and arthritis [4].

Cellular immune responses are a critical part of the host defense against intracellular bacterial infections, but the response against *Brucellae* spp. involves the whole principles of the immune system from innate to adaptive immunity. In brucellosis, the different arms of the immune system, namely antigen-presenting cells, natural killer (NK) cells, CD4+ and CD8+ T cells and B cells, act together to provide a coordinated response [5]. The primary mechanism of control involves cell-mediated immunity rather than antibodies, although some immunity against re-infection is provided by serum immunoglobulins

*Tropical-Biological Research Unit, College of Science, University of Baghdad.
**Department of Biology, College of Education Ibin Al-Haitham, University of Baghdad.
***Department of Biology, College of Science for Women, University of Baghdad.

[6]. However, the immunological recognition of non-self antigens; viral, bacterial or parasitic, is dependent on self antigens, which are collectively known as human leukocyte antigens (HLA). These antigens are genetically controlled, and their expression shows an extensive polymorphism, and certain HLA alleles have shown positive associations with different diseases; including autoimmune, viral, bacterial and parasitic diseases [7], and human brucellosis is one of these diseases that has been studied in relation to HLA antigens [8,9,10].

Accordingly the present investigation aimed to investigate the association between HLA-class I (A and B) and class II (DR and DQ) antigens and brucellosis in a sample of Iraqi patients. The profile of lymphocytes (CD3+, CD4+, CD8+ and CD19+ cells) were also investigated.

Materials and Methods:

Blood samples (10 ml) were collected from 51 brucellosis patients (25 males and 26 females), who were administrated to the Al-Karama Teaching Hospital, and their age range at the time of diagnosis was 17-50 years. The diagnosis was made by the consultant medical staff, and based on a clinical examination and laboratory investigations, which included Rose-Bengal test and indirect immunofluorescence antibody test (IFAT). A control sample of 70 apparently healthy subjects (blood and kidney donors) was also included, and they were age, sex and ethnicity (Arab Muslims) matched.

The blood was drawn in a hepranized tube and lymphocytes were isolated by means of a density gradient centrifugation using lymphoprep as a separating medium. These lymphocytes were further separated into T- and B-cells using the nylon-wool method. The T lymphocytes were employed in the phenotyping of HLA-class I antigens (A and B), while B lymphocytes were used in the phenotyping of HLA class II antigens (DR and DQ). The microlymphocytotoxicity test was applied to determine these phenotypes using commercially available antisera (Biotest, Germany) that recognized 8 A, 14 B, 8 DR and 2 DQ antigens [11].

The total lymphocytes were also characterized in terms of their CD profiles (CD3+, CD4+, CD8+ and CD19+ cells), which were investigated using a commercially available monoclonal antibodies against each CD marker (Serotec, France). The method of detection was direct immunofluorescence as suggested by the kit's manufacturer.

The data of HLA were presented as observed numbers and percentage frequencies, and antigens showing significant variation (Fisher's exact probability; P) were further presented as odd ratio (OR), etiological fraction (EF) and preventive fraction (PF). The probability was corrected for the number of antigen tested at each locus [11]. The statistical analysis of these presentations was carried out using the computer programme PEPI version 4. The data of CD profile were given as means ± standard errors (S.E.), and significant differences between means were assessed by the least significant difference (LSD) using the computer programme SPSS version 15.

Results:

HLA antigens showing significant variations between brucellosis patients and controls are given in table 1. Seven antigens showed a significant increased frequency; A23 (11.76 *vs*. 1.43%), B44 (25.49 *vs*. 8.57%), DR1 (39.22 *vs*. 18.57%), DR2 (33.33 *vs*. 18.57%), DR8 (41.18 *vs*. 10.0%), DQ1 (33.33 *vs*. 15.71%) and DQ2 (37.25 *vs*.

20.0%), while three antigens showed a significant decreased frequency; A11 (5.89 *vs*. 18.57%), A32 (0.0 *vs*. 11.43%) and B35 (7.84 *vs*. 20.0) in the patients as compared to controls. Correcting the P values of these variations revealed that only the increased frequency of DR8 maintained a significant corrected level (Pc = 0.001), moreover, such variation was associated with an OD value of 6.30 and EF value of 0.34.

The CD profile of lymphocytes revealed that CD3+ cells showed a significant decreased percentage (75.1 *vs*. 79.4%; P = 0.02), while CD8+ cells showed a significant increased percentage (25.15 *vs*. 22.0%; P = 0.006) in the patients as compared to controls. The CD4+ lymphocytes were also decreased in the patients (38.95 *vs*. 39.55%), but the difference did not attained a significant level (P > 0.05). Whereas the CD4/CD8 ratio maintained a significant decrease (1.58 *vs*. 1.82; P = 0.003) in the patients. The CD19+ cells showed a non-significant increase in the patients (19.35 *vs*. 18.70%) (Table 2).

Discussion:

Infectious diseases are a major selective pressure, and the genes involved in the immune response are the most numerous and diverse in the human genome, indicating the evolutionary advantages of a varied immunological response to a wide range of infectious pathogens. This is most obvious at the HLA loci, the prototypical candidate genetic region for infectious disease susceptibility [12]. The present results demonstrated that brucellosis was associated with abnormalities in the percentage of CD3+, which are pan T-lymphocytes, and CD8+, which are T-cytotoxic. The latter cells are involved in cell-mediated immunity, which is the responsible immunity against *Brucella* infection, although humoral immunity might have a role [5,6]. However, both immunities are MHC-restricted, and the HLA antigens may have an effect as they are the recognition molecules [13]. Comparing the patients and controls revealed that HLA-DR8 was significantly increased in the patients, and such deviation was associated with an OR value of 6.30 and EF value of 0.34. Such positive association may highlight the importance of such antigen as a predisposing immunogenetic marker conferring about 34% predisposition. Additionally, CD8+ lymphocytes were also significantly increased in the patients. Therefore, part of the genetic susceptibility to brucellosis may be attributed to polymorphisms in the HLA region genes. These genes encode for the cell-surface human HLA class I (HLA-A, -B, and -C) and class II (HLA-DR, -DQ, and -DP) molecules, which play an important role in the regulation of the immune system. Most nucleated cells express HLA class I genes, whereas expression of HLA class II genes is restricted to specialized antigen-presenting cells. HLA class I and class II molecules present antigenic peptides to CD8+ and CD4+ T cells, respectively [7]. The variability observed among these molecules, HLA-DR8 antigen in the present investigation, is located in the peptide-binding region, which is important in determining the antigen repertoire that is displayed to T cells by each HLA molecule. The interaction between the HLA-peptide complex and the T cell receptor is an essential and specific step in T cell activation [14]. In this regard, it has been demonstrated by using immunofluorescence that lipopolysaccharide (LPS), a major amphiphilic molecule located at the outer membrane of *Brucella*, is able to accumulate in an intracellular

compartment enriched in MHC class II molecules, and by the use of immunoprecipitation, it was illustrated that *Brucella abortus* LPS is associated with MHC class II molecules in a haplotype-independent manner. Taken together, these results raised the possibility that *B. abortus* LPS may play a role in T-cell activation, which is dependent on the MHC class II molecule [15].

Regarding HLA polymorphisms and the risk of brucellosis, discordant results have been observed among different populations or even in the same population. For instance, an earlier study in Spanish patients recorded a significant decreased frequency of HLA-Cw2 [16], while a later study reported a significant increased frequency of HLA-B39, especially in patients who had osteoarticular complications [8], but both investigations showed no association with HLA-B27. These three antigens showed no significant variations between the present brucellosis patients and controls. The latter antigen (HLA-B27) shared most of the controversy in relation to brucellosis especially in patients who had osteoarticular complications and arthritis, and the studies either reported a significant increase [9,10], or no association was recorded [16], including Iraqi patients [17].

With respect to lymphocyte phenotypes, the most significant findings were decreased percentage of CD3+ cells and increased percentage of CD8+ cells, while CD4+ lymphocytes showed a non-significant decrease. The significance of CD4+ and/or CD8+ T cells in *Brucella* immunity has been controversial. Araya *et al*. [18] reported that both T-cell populations are important. However, Oliveira and Splitter [19] reported that major MHC class I-deficient mice, which have no CD8+ T cells, control the infection more slowly than do wild-type mice, while MHC class II deficient mice, defective in CD4+ T cells, control the infection at a similar rate to wild-type mice. These results suggest that CD8+ T cells play a critical role although the role of CD4+ T cells in brucellosis should not be ignored. Moreno-Lafont *et al*. [20] reported an increase in the percentage of CD8+ T-cells in the peripheral blood of patients chronically infected with *Brucella* spp. In addition, they found increased numbers of *Brucella* antigen-specific CD8+ T cells. Gazapo *et al*. [21] observed both a decreased percentage of CD4+ lymphocytes and an increase in CD8+, hence an inverted CD4+/CD8+ ratio that was also observed in the present study, at the time of diagnosis.

In conclusion, the HLA profile in brucellosis patients may be subjected to race variations, because HLA antigens show different frequencies in different populations [22], but in each population there may be a single antigen that is able to predispose the individual to develop brucellosis in interaction with the strain that cause the infection, and such interaction involves CD8+ T lymphocytes.

Table 1: HLA antigens showing significant variations between brucellosis patients and controls.

HLA Antigens	Patients(No. = 51)		Controls(No. = 70)		Odd Ratio	EF or PF	P	Pc
	No.	%	No.	%				
A11	3	5.89	13	18.57	0.27	0.14	0.04	N.S.
A23	6	11.76	1	1.43	9.20	0.11	0.02	N.S.
A32	ND	ND	8	11.43	-	-	0.01	N.S.
B35	4	7.84	14	20.00	0.34	0.11	0.05	N.S.
B44	13	25.49	6	8.57	3.64	0.18	0.01	N.S.
DR1	20	39.22	13	18.57	2.83	0.25	0.01	N.S.
DR2	17	33.33	13	18.57	2.19	0.18	0.05	N.S.
DR8	21	41.18	7	10.00	6.30	0.34	0.0001	0.001
DQ1	17	33.33	11	15.71	2.68	0.21	0.02	N.S.
DQ2	19	37.25	14	20.00	2.38	0.21	0.03	N.S.

EF: Etiological fraction; PF: Preventive fraction; P: Probability; Pc: Corrected P; ND: Not detected.

Table 2: Percentage of lymphocyte phenotypes in the peripheral blood of brucellosis patients and controls.

Lymphocytes	Mean ± S.E. (%)		Probability
	Patients (No.= 20)	Controls(No.= 20)	
CD3+	75.10 ± 1.42	79.40 ± 1.10	0.02
CD4+	38.95 ± 0.70	39.55 ± 0.59	Not significant
CD8+	25.15 ± 0.91	22.00 ± 0.60	0.006
CD19+	19.35 ± 0.68	18.70 ± 0.69	Not significant
CD4/CD8 ratio	1.58 ± 0.06	1.82 ± 0.05	0.003

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تكرار مستضدات خلايا الدم البيض البشرية لعينة عراقية من مرضى داء البروسيلا

علي حسين أدحية* ***أعياد حميد عاجل الركابي**** ***آمنة نصيف جاسم*****

*وحدة الابحاث البيولوجية للمناطق الحارة، كلية العلوم، جامعة بغداد.
**قسم علوم الحياة، كلية التربية ابن الهيثم، جامعة بغداد.
***قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد.

الخلاصة:

نمط مظهريا احدى وخمسون مريضا من مرضى داء البروسيلا المشخصين مصليا وسبعين من الأصحاء لمستضدات خلايا الدم البيض البشرية A و B و DR و DQ باستخدام الطريقة القياسية Microlymphocytotoxicity test، وفضلا عن توصيف الخلايا اللمفية في ضوء بعض الواسمات (CD3 و CD4 و CD8 و CD19). أوضحت النتائج زيادة معنوية(الاحتمالية المصححة = 0.001) بتكرار المستضد HLA-DR8 (41.18 مقابل 10.0%) في المرضى. كما ارتفعت في المرضى ايضا وبفرق معنوي النسبة المئوية للخلايا اللمفية الحاملة للواسم CD8 (25.15 مقابل 22.0%؛ الاحتمالية = 0.006)، في حين انخفضت معنويا الخلايا الحاملة للواسم CD3 (75.1 مقابل 79.4%؛ الاحتمالية = 0.02).