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Allelic distribution of human leucocyte antigen in historical and recently diagnosed tuberculosis patients in Southern Italy

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SUMMARY

This study addresses the analysis of the human leucocyte antigen (HLA) allele distribution in 54 historical and in 68 recently diagnosed tuberculosis (TB) patients. The historical cohort was characterized by the presence of large fibrocavernous lesions effectively treated with therapeutic pneumothorax during the period 1950–55. Patients and healthy controls enrolled in the study were from the Campania region of southern Italy. No significant association between HLA alleles and TB in the population of recently diagnosed TB patients was observed. On the contrary, among the historical TB patients there was a strong association with an increased frequency of the HLA-DR4 allele alone and/or in the presence of the HLA-B14 allele ($P = 0{\cdot}000004$; $P_c = 0{\cdot}0008$), as well as with a decreased frequency of the HLA-A2$^+$,-B14$^-$,DR4$^-$ allele association ($P = 0{\cdot}00005$; $P_c = 0{\cdot}01$). In order to exclude any interference from age-related factors, these results were confirmed by comparing the historical cohort of TB patients with an age-matched healthy control population of the same ethnic origin ($P = 0{\cdot}00004$; $P_c = 0{\cdot}008$; and $P = 0{\cdot}0001$; and $P_c = 0{\cdot}02$, respectively).

INTRODUCTION

Mycobacterium tuberculosis infects a large percentage of the world population, but only a small number of those infected actually present with clinical disease.

Substantial evidence exists that host genes are important in determining the outcome of mycobacteria as well as other intracellular pathogen infections.[1] In this context, interferon-γ receptor deficiencies,[2] abnormalities in mannose binding lectin (MBL) levels[3] and in vitamin D supplementation[1,3] as well as polymorphism in the so-called natural-resistance-associated-macrophage-protein-1 (NRAMP1)[4] were found to be associated with tuberculosis (TB).

One important element of resistance and susceptibility to infections is the major histocompatibility complex (MHC), known as human leucocyte antigens (HLA) in humans. MHC consists of a set of polymorphic genes encoding both class I and class II cell surface glycoproteins.[5] The main biological function of MHC molecules is to bind antigenic peptides and present them for T-cell scrutiny.[6] Moreover, MHC polymorphism tends to concentrate in hypervariable regions[7] corresponding to MHC binding pockets engaging specific anchor residues of their peptide ligands.

HLA genes have been extensively studied for the association with susceptibility or resistance to mycobacteria.[8] In many populations the susceptibility to leprosy and tuberculosis was associated with the class II antigen HLA-DR2, but data are still conflicting (reviewed in 1). In this context, the critical relevance of the availability of homogeneous groups of patients to be analysed for HLA allele association has already been established.[9,10] In order to address this issue we analysed the HLA phenotype/genotype in a cohort of historical TB patients characterized by the presence of large fibrocavernous lesions that had been effectively treated with therapeutic pneumothorax during the period 1950–55. All the patients and the healthy controls enrolled in the study were from the Campania region of southern Italy.

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MATERIALS AND METHODS

Patients and healthy control populations

A group of 54 unrelated patients (38 males and 16 females, aged 18–25 years at the time of their TB diagnosis), were enrolled in the study. All were coming from the Campania region of southern Italy and their TB diagnosis was performed during the period 1950–55 at the 'V. Monaldi' Hospital of Naples, a National Reference Centre for TB treatment and control since 1930. TB diagnosis was always confirmed by microbiological as well as X-ray evaluations. The analysis of their clinical reports revealed, at the time of their first hospital admittance, the presence of extensive TB fibrocavernous lesions treated with effective therapeutic pneumothorax.

A total of 68 unrelated patients (53 males and 15 females; aged 21–35 year), all TB diagnosed in the period June 2000–June 2002 at the 'Cattedra di Malattie dell'Apparato Respiratorio' of the University of Naples 'Federico II', c/o the 'V. Monaldi' Hospital, and all coming from the Campania region of southern Italy, were enrolled in the study. Patients were classified according to clinical, imaging and microbiological data obtained at their initial evaluation, that was always performed within 2 months from the clinical onset of the disease. Clinical evaluation was performed by using radiographic, scintigraphic and computed tomography imaging; when needed, biopsy and histopathological analysis of all suspicious extrapulmonary lesions were performed. Patients were not affected with significant associated diseases.

One thousand and eight-nine unrelated individuals, healthy blood donors at the Blood Transfusion Centre of the University of Naples 'Federico II', all from the Campania region of southern Italy, were entered in the study and considered as healthy controls. The parents and the grandparents (both maternal and paternal) of each volunteer had been born in Campania.

An additional control group of 44 unrelated healthy people, age-matched with the historical TB population (all over 70 years old), and coming from the Campania region of southern Italy were also entered in the study and considered as homogeneous controls for the historical patient group.

None of the controls referred the occurrence of clinical TB. All individuals, patients as well as healthy controls, gave their informed consent before being enrolled in the study.

Typing techniques

Serological typing for HLA-A, -B, and -C loci, as well as for HLA-DR and -DQ loci was performed by standard microcytotoxicity assay.[11] HLA class II molecular analysis was carried out by using polymerase chain reaction with sequence specific primers (PCR–SSP), as described.[12] Commercial kits were purchased from Dynal A.S. Oslo, Norway and used according to the manufacturer's instructions.

Statistical analysis

The statistical analysis and the P calculation were performed by using Student's t-test and Fisher's two-tailed exact text, as indicated. Results were considered significant when a P-value $<0{\cdot}05$ was obtained. The corrected P-values (P_c) were calculated by multiplying the P-value by the number of the tested alleles at each locus, i.e. 24 alleles at the A locus, 51 alleles at the B locus and 23 alleles at the DR locus. In order to address the hypothesis that any of the HLA alleles detected might be associated with TB, each allele was treated similarly. Therefore, we used as a correction factor the total number of alleles typed (always 98) regardless of the locus/loci analysed in the comparison. In addition, a further correction of 2 was applied to test the hypothesis that each given allele could be associated to TB with either an increased or a decreased frequency. This method was considered to be an acceptable approximation of the so-called 'Bonferroni' correction for multiple comparisons.

RESULTS

Analysis of HLA allele frequency in a historical cohort of TB patients and in a group of recently diagnosed patients

In order to address the study of HLA allele frequency in homogeneous groups of TB patients, we analysed the HLA phenotype/genotype in a historical cohort of individuals whose TB diagnosis was performed during the period 1950–55. Interestingly, these patients were characterized by the presence of large fibrocavernous lesions effectively treated with therapeutical pneumothorax. In addition, a group of 68 human immunodeficiency virus (HIV) negative, recently diagnosed TB patients was analysed.

Relevant differences in the frequencies observed, are reported in Table 1. As shown, no significant differences in HLA allele frequency were found in the TB patients after the P correction. Interestingly, in the group of historical TB patients an increase of the HLA-DR4 and -B14 allele and a decrease of the -A2 allele was observed, compared with both the healthy controls and the age-matched healthy population. In addition, none of the recently diagnosed TB patients showing fibrocavernous TB ($N = 8$) revealed -DR4 and/or -B14 alleles in their HLA phenotype (not shown). In this context, the possibility must be considered that chance effects caused by sampling error in a small population could account for this result.

To better analyse the DR4 antigen association observed in the historical TB cohort, molecular subtyping of the $DR4^+$ patients was performed, using the PCR–SSP technique. The heterogeneous distribution of the -DR4 alleles in -$DR4^+$ patient population shown in Table 2 indicates a lack of significant association with single -DR4 allele specificities in the historical TB patients. Analogous -DR4 allele distribution was observed in the cohort of -$DR4^+$ recently diagnosed TB patients.

A significant increase in the HLA-B14 and/or HLA-DR4 alleles as well as a decrease of the HLA-$A2^+$,-$B14^-$,$DR4^-$ antigen association characterize the historical group but not the recently diagnosed individuals

Our previous data indicate an increase in the -DR4 and in the -B14 allele frequency as well as a decreased frequency of the HLA-A2 antigen in our historical cohort of TB patients. Moreover, the molecular subtyping of the -$A2^+$-individuals revealed the presence mainly of the A*0201 allele, both in the patients as well as in the control groups.

Table 1. Comparative analysis of HLA allele frequency in historical and recently diagnosed TB patients, versus healthy controls in the Campania region of southern Italy

HLA Alleles	Recently diagnosed TB patients % ($N = 68$)	Historical TB patients % ($N = 54$)	Controls % ($N = 1089$)	Healthy age-matched controls* % ($N = 44$)
HLA-A2	52·94 RR = 1·49 †P = NS‡	29·62 RR = 0·6 P = 0·06 §P_c = NS	42·24	52·27 RR = 0·38 P = 0·03 P_c = NS
HLA-A3	10·29 RR = 0·39 P = 0·008 P_c = NS	16·66 P = NS	22·68	18·18 P = NS
HLA-B14	8·82 RR = 1·9 P = NS	16·66 RR = 3·9 P = 0·001 P_c = NS	4·77	NF¶ P = 0·003 P_c = NS
HLA-B50	2·94 RR = 0·79 P = NS	11·11 RR = 3·2 P = 0·01 P_c = NS	3·67	9·09 P = NS
HLA-DR4	8·82 RR = 0·6 P = NS	29·62 RR = 2·7 P = 0·001 P_c = NS	15·36	20·45 RR = 1·6 P = NS
HLA-DR7	10·29 RR = 0·37 P = 0·005 P_c = NS	29·62 P = NS	23·59	27·27 P = NS
HLA-DR8	11·76 RR = 3·2 P = 0·007 P_c = NS	5·55 P = NS	3·94	6·82 P = NS

*Statistical analysis was performed by using Fisher's two tailed exact test.
†Not significant.
‡Corrected P-value (see Materials and methods for details).
§To be compared with the historical TB patients.
¶Not found.

In an effort to analyse the possible relationship between the HLA-A2 allele and TB resistance/susceptibility, we measured the frequency distribution of the HLA-A2$^+$, -B14$^-$, -DR4$^-$ antigen association in our group of historical TB patients. As shown in Table 3 a significant increase of -DR4 and/or -B14 alleles ($P = 0{\cdot}000004$; $P_c = 0{\cdot}0008$) and a decrease of the HLA-A2 ±, B14$^-$ -DR4$^-$ antigen association ($P = 0{\cdot}00005$; $P_c = 0{\cdot}01$) was revealed in the historical TB patient group.

Interestingly, these differences were confirmed by the comparison with the age-matched healthy control population

Table 2. DR4 allele distribution in historical, recently diagnosed TB patients and controls

Allele	Frequency in DR4$^+$ recently diagnosed TB patients % ($N = 6$)	Frequency in DR4$^+$ historical TB patients % ($N = 16$)	Frequency in DR4$^+$ controls % ($N = 80$)
DRB1*0401	50	6·25	29·4
DRB1*0402	16·66	6·25	17·6
DRB1*0403	16·66	37·5	17·6
DRB1*0405	NF*	31·25	11·2
DRB1*0406	NF	6·25	<1
DRB1*0407	16·66	6·25	5·8
DRB1*0409	NF	6·25	<1

*Not found.

Table 3. Analysis of HLA antigen association in historical and recently diagnosed TB patients versus controls

HLA phenotype	Recently diagnosed TB patients % ($N = 68$)	Historical TB patients % ($N = 54$)	Controls % ($N = 1089$)	Healthy age-matched controls* % ($N = 44$)
HLA-A2$^+$,-DR4$^-$	50	16·66	34·89	43·18
	RR = 1·86	RR = 0·37		RR = 0·26
	†P = 0·009	P = 0·003		P = 0·003
	‡P_c = NS	§P_c = NS		P_c = NS
HLA-A2$^+$,-B14$^-$	51·47	20·37	39·11	52·27
	RR = 1·6	RR = 0·39		RR = 0·23
	P = 0·03	P = 0·003		P = 0·0009
	P_c = NS	P_c = NS		P_c = NS
HLA-A2$^+$,-DR4$^-$,B14$^-$	48·52	9·25	33·33	43·18
	RR = 1·88	RR = 0·2		RR = 0·13
	P = 0·008	P = 0·00005		P = 0·0001
	P_c = NS	P_c = 0·01		P_c = 0·02
HLA-A2$^-$,-DR4$^+$,B14$^+$	NF¶	5·55	1·28	NF
		RR = 1·88		P = NS
	P = NS	P = 0·008		
		P_c = NS		
HLA-DR4$^+$ and/or -B14$^+$	17·64	61·11	20·75	20·45
	RR = 0·8	RR = 6		RR = 6·1
	P = NS	P = 0·000004		P = 0·00004
		P_c = 0·0008		P_c = 0·008

*Statistical analysis was performed by using Fisher's two tailed exact test.
†Corrected P-value (see Materials and methods for details).
‡Not significant.
§To be compared with the historical group of TB patients.
¶Not found.

($P = 0·00004$; $P_c = 0·008$; and $P = 0·001$; $P_c = 0·02$, respectively),

These data indicate that a significant increase of -DR4 and/or –B14 alleles, as well as a decrease of the HLA-A2$^+$, -B14$^-$, -DR4$^-$ antigen association, can be observed in the historical cohort of TB patients characterized by the presence of large fibrocavernous lesions susceptible to therapeutic pneumothorax treatment. The comparative analysis versus the age-matched healthy control population excluded the interference of survival at old-age related factors. No significant HLA allele associations were found in the recently diagnosed TB patient group of the same ethnic origin.

DISCUSSION

This study indicates the presence of a strong increase in the HLA-DR4 allele alone and/or in the presence of B14 ($P = 0·000004$; $P_c = 0·0008$) in a group of historical TB patients characterized by the occurrence of large fibrocavernous lung lesions susceptible to pneumothorax treatment. Interestingly, a significant decrease in the frequency of the allele association HLA-A2$^+$,-B14$^-$,-DR4$^-$ ($P = 0·00005$; $P_c = 0·01$) in the same cohort of historical TB patients was also observed. In order to exclude the possible interference of age-related factors, these data were confirmed by comparison of this cohort with age-matched healthy controls of the same ethnic origin.

A number of studies have revealed the association of TB with the HLA-DR2 specificity, but the literature is still conflicting (reviewed in 1). The presence of an increased frequency of the B14 antigen in tuberculosis has already been described in a number of studies performed essentially in Asian populations.[13] In addition, B14-restricted CD8$^+$ T-cell clones were recently isolated by using a limiting dilution approach in the presence of *M. tuberculosis* infected targets.[14] Data available indicate the significant association of DR4 with a high response to micobacterial-specific antigens in patients affected with rheumatoid arthritis.[15] Studies on the association of the A2 allele with TB are still controversial. Data prevalently obtained in Asian populations indicate an increased frequency of this antigen in TB, but a correlation with a sputum-negative disease has also been described.[16]

Extensive studies of the HLA allele distribution show that populations of the same ethnic origin, likely to be exposed to the same environmental factors and sharing a common microbiological background, also share homogeneous HLA allele frequency.[17] In this context, it seems possible that the profile of HLA allele frequency distribution in different populations could be defined by the ability of different alleles to provide varying degrees of protection against infectious pathogens. The theory of pathogen-driven MHC diversity requires that individuals of different MHC types should differ in their susceptibility to at least some major infectious pathogens.

The study of the HLA phenotype in survivors of typhoid and yellow fever epidemics in Surinam,[18] as compared with the original MHC asset of that population, indicates that HLA antigens may have influenced the outcome of these diseases. In addition, the convincing association between MHC polymorphism and a fatal infection, observed in Marek's chicken

disease[19] strongly supports the hypothesis of a pathogen-driven MHC diversity.

The HLA-Bw53 class I allele and DRB1*1302, DQB1*0501, class II haplotype, common in West Africans but rare in other racial groups, are associated with protection from severe malaria.[9] In this population the presence of an effective Bw53 restricted cytotoxic response against *Plasmodium*-derived peptides was also described.[10] These data suggest that the effectiveness of the immune responses against endemic infectious agents could contribute to the definition of the HLA allele frequency distribution. Moreover, the critical need for homogeneous criteria in selecting the clinical groups to be analysed for HLA association has been also established.

Both the epidemiology and the clinical outcome of TB have been substantially modified by environmental changes occurring in the last 50 years, with a mortality rate drop from 27·2%, observed in 1950[20] to 2·8%, as reported in 1999.[21] Therapeutic pneumothorax was the treatment of choice in the prechemotherapy era (1950–55) for TB fibrocavernous lesions, representing the most frequent clinical form of pulmonary TB at that time.[22] Moreover, the occurrence of pulmonary TB recovery by pneumothorax treatment must be considered as a relatively rare event in the period 1950–55, involving not more than 26·9% of the people treated.[23] Therefore, the TB historical cohort might represent a group of patients characterized by the occurrence of a favourable prognosis, whose relationship with a specific genetic background could be hypothesized. In this context, our results suggest the involvement of HLA in this genetic habitus.

No association with HLA alleles was revealed in the recently diagnosed TB patient population. This apparent discrepancy suggests the association of HLA with a favourable TB prognosis rather than with the simple occurrence of TB, at least in our population. Indeed, the historical TB patients represent a selected group of individuals affected with TB. In this respect, although they develop symptomatic disease while most exposed people do not, these patients were able to effectively react to TB lesions. The identification of such a group of patients should be quite difficult to observe in the recent diagnosed TB populations, likely caused by the interference of the current successful antimicrobial therapies. Therefore, the possibility that this and other interference factors could cause a sort of underestimation of the HLA relevance in the interplay between genetics and *M. tuberculosis* infection should be considered. The observation that HLA involvement in the control of infectious diseases has so far been identified in models showing a limited interference of therapeutic approaches,[9,10,18,19] is consistent with this hypothesis.

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