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HLA-DR3 associated genetic control of response to multiple skin tests with new tuberculins

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SUMMARY

Multiple skin testing with mycobacterial antigenic preparations reveals distinct reaction patterns, which might be relevant to the development of mycobacterial disease in man. Previous work has shown that HLA-DR associated factors correlate with the position of a leprosy patient in the immunopathological spectrum of leprosy. This study was undertaken to see whether these skin test patterns in healthy persons do show any association with HLA-DR types. Out of a group of 74 healthy Caucasoid individuals HLA-DR3 was observed to be absent from the 16 individuals who did not respond to any of the mycobacterial antigens tested. This is a striking difference from the distribution of HLA-DR3 both among the 17 individuals who responded to all mycobacterial antigens tested ($P=0{\cdot}005$) and the 41 individuals who responded to some but not all antigens ($P=0{\cdot}015$). These data show that an HLA-DR3 associated genetic factor controls, albeit indirectly, skin test responsiveness to mycobacterial antigens. It may be significant that this same HLA-DR determinant is implicated in deciding the type of disease to be developed by a leprosy patient.

INTRODUCTION

One of the characteristic features of mycobacterioses in man is their immunological spectrum (Turk & Bryceson, 1971). The host factors, which are thought to determine the relative position of an individual in this disease spectrum may be either acquired or intrinsic.

Acquired factors include the development of immune mechanisms to environmental mycobacteria. Such mechanisms may have a significant effect on subsequent susceptibility to, or protection from, challenge with pathogenic mycobacteria (Palmer & Long, 1966) and on the efficacy of BCG vaccination (Stanford, Shield & Rook, 1981b; Rook, Bahr & Stanford, 1981). In the present study we seek to determine whether HLA associated genetic factors might play a role in the determination of the pattern of reactivity against environmental mycobacteria.

The development of the New Tuberculins, (Paul, Stanford & Carswell, 1975) with their improved specificity over earlier preparations has made it possible to assess to some extent, the parts played by individual mycobacterial species in the mosaic of naturally occurring sensitization. The data obtained by testing human populations, (largely secondary school children) in numerous countries with sets of four of these reagents has led to the inevitable observation that some people respond to all four reagents (category 1), some to none of them (category 2) and the majority to between one and three reagents (category 3). Calculation of the numbers of persons that might be

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expected to behave in these different ways soon showed that there was a discrepancy in the observations in that there were always more individuals responding to all or none of the reagents than might have been expected. This suggested that there must be a group of individuals more likely to respond to mycobacteria than other people and a second group less likely to do so. The mechanisms underlying these were thought to be effective recognition of common mycobacterial antigens and some form of regulation of response to them respectively. Whether or not these were themselves intrinsic or acquired phenomena was not known, but categorisation of people according to their responses to four simultaneously administered New Tuberculins seemed to be potentially meaningful (Stanford, 1981). This categorization is most readily seen where contact with environmental mycobacteria is very common and the observation that the proportions in the different categories are different along the leprosy spectrum suggests that there might be some relationship between categorization and susceptibility to particular forms of the disease (Stanford *et al*, 1981a).

The involvement of HLA encoded genetic factors in leprosy has been shown most convincingly by family studies (De Vries *et al*, 1976; Fine *et al.*, 1979; De Vries *et al.*, 1980; Van Eden *et al.*, 1980). In particular HLA-DR determinants have been found to associated with certain types of the disease. The HLA-D region is the human analogue of the H-2I region in mice, which was originally defined to contain the so called Ir-genes, (immune response genes).

In multiple case families from India, HLA-DR2 has been shown to be associated with tuberculoid leprosy (De Vries *et al.*, 1980; Van Eden *et al.*, 1980). In a recent study of patients from a mixed African–Caucasoid population originating from Surinam, HLA-DR3 was found to be frequently present in polar tuberculoid leprosy patients and very uncommon in lepromatous patients, indicating that in this population a DR3 associated factor controls the type of the disease that develops after infection with *Mycobacterium leprae* (Van Eden *et al.*, 1982).

Thus both the individual pattern of tuberculin reactivity and the individual HLA-DR determinants might be expected to direct to some extent the individual liability to develop disease at certain parts of the leprosy spectrum. This study has been carried out to investigate whether there is an inter-relationship between tuberculin skin test reactivities and the presence of certain HLA-DR determinants in healthy individuals.

MATERIALS AND METHODS

Individuals tested. Seventy-four healthy Caucasoids, living in London in the period of study, were skin tested and typed for HLA-DR.

Skin test reagents. The reagents used were a range of new tuberculins, (Shield *et al.*, 1977) which are soluble, sonicate preparations of mycobacteria, (Paul *et al.*, 1975). The protein concentrations of these reagents are assayed spectrophotometrically and adjusted to 20 μg protein/ml in the cases of Vaccin and Non-chromogenicin, 10 μg/ml in the case of Leprosin A and 2 μg/ml for all other reagents.

Skin tests. For skin testing 0·1 ml of reagent was injected intradermally into the front surface of the forearm. Four reagents were tested at the same time, two on each arm. Reactions were read after 72 h by measuring the diameters of induration in mm. Reactions of 2 mm or more were considered positive.

Skin test categories. Categorization in this study has been based on results of 4–20 skin tests on different individuals. Category 1: positive to all reagents, (recognition of common antigenic determinants. Category 2: negative to normal doses of all reagents, (naive or regulated). Category 3: positive to at least one reagent and negative to at least one other reagent, (recognition of species specific antigens) (Stanford *et al.*, 1981a).

For the purposes of this study the numbers in categories 1 and 2 were deliberately increased by the inclusion of individuals known to belong to these categories.

HLA-DR typing. Typing for HLA-DR specificities was performed with 80 platelet absorbed sera in the two colour fluorescence test, as described previously (Van Leeuwen & Van Rood, 1980).

Statistics. The significance of differences in HLA antigen frequencies among individuals in different skin test categories were calculated using Woolf's method as modified by Haldane (1955).

RESULTS

Seventy-four individuals were skin tested and available for HLA typing. Seventeen were categorized as common antigen reactors (category 1), 16 were categorized as zero responders (category 2) and 41 were categorized as species specific reactors (category 3).

The HLA-DR antigen frequencies among the individuals of the different skin test categories are shown in Table 1. The HLA-DR frequencies among Caucasoids, as calculated from the combined results of the 1980 International Histocompatibility Workshop (Terasaki, 1980), are shown for comparison. Data on HLA-DRw6 has been excluded from the latter, since no consensus has been achieved so far over its definition.

HLA-DR3 was observed to be absent from the zero-responders (category 2), whereas the DR3 frequency was observed to be 0·41 amongst common antigen reactors and 0·29 amongst species specific reactors. No significant differences in frequencies between the various skin test categories were observed for the other HLA-DR specificities.

The numerical distribution of HLA-DR3 among the different skin test categories is shown in Table 2. Seven out of 17 common antigen reactors carried DR3, whereas none of 16 zero responders carried this specificity ($P=0·005$). The difference in frequency of HLA-DR3 among zero responders and among species specific reactors is less significant ($P=0·015$) and the difference between the common antigen reactors and species specific reactors is not significant.

Table 1. HLA-DR antigen frequencies among individuals belonging to different skin test categories and among European Caucasoids

	Category 1 ($n=17$)	Category 2 ($n=16$)	Category 3 ($n=41$)	Caucasoids*
DR1	0·12	0·19	0·12	0·14
2	0·24	0·25	0·34	0·32
3	0·41	0·00†	0·29	0·26
4	0·41	0·44	0·22	0·26
5	0·24	0·38	0·29	0·26
w6	0·18	0·13	0·20	‡
7	0·24	0·50	0·42	0·39
w8	0·00	0·00	0·02	0·01
w9	0·00	0·00	0·05	0·03
w10	0·06	0·06	0·00	0·03

* As calculated from the combined results of the 1980 International Histocompatibility Workshop (Terasaki, 1980).

† Significantly decreased as compared to category 1 ($P=0·005$) and as compared to category 3 ($P=0·015$).

‡ See Results section.

Table 2. HLA-DR3 and skin test categories

	DR3			
	+ve	−ve	χ^2	*P* value
Category 1	7	10	7·834	0·005
Category 2	0	16		
Category 3	12	29	5·952	0·015

DISCUSSION

The data from the present study suggest that the presence of different categories of individuals according to mycobacterial skin test reactivities within a certain population, living in more or less the same environment, may be attributable, at least partly, to the presence of a polymorphic HLA-DR encoded or linked genetic trait in the population. Skin test reactivities may reflect the individual ability to express tuberculin type hypersensitivity to antigens present in the injected reagent. These delayed type hypersensitivity responses have been shown to depend on interactions between immunologically competent cells, presumably macrophages and T cells. In these cellular interactions major histocompatibility, class II, determinants have been shown to be involved, (Zinkernagel *et al.*, 1977; Strassman, Eshbar & Mozes, 1980; Bianchi *et al.*,1981; Kaufmann & Hahn, 1982).

Thus, the outcome of the present study is compatible with the existence of differences in the capacity of the distinct HLA-DR determinants to serve as restricting elements in the cellular interactions occurring in reactions to mycobacterial antigens.

Although the present findings remain significant after correction for the number of DR antigens tested, the numbers of individuals per catagory are too small to allow correction for the total number of comparisons (30) made. So, in order to draw definite conclusions, these data should be confirmed. Nevertheless, it is remarkable that the statistically significant heterogeneity in the DR antigen frequency distributions among the different skin test categories appaers to be confined to HLA-DR3. The DR3 specificity was previously shown to occur in a relatively high frequency among polar tuberculoid leprosy patients, (TT) and in a very low frequency among lepromatous leprosy patients, (BL and LL) in a mixed African–Caucasoid population, originating from Surinam (Van Eden *et al.*, 1982). Although the latter work and the present study concern different populations, the similarity with regard to HLA-DR3 encourages speculation on the relationships between mycobacterial skin test reactivities and leprosy.

There are several possible explanations for the lack of skin test responses of category 2 individuals. Amongst children naivety, i.e. not yet having developed responses to mycobacteria, is the explanation in many cases. In our study group, however, this cannot be the explanation since all of those in category 2 had received BCG in the past and had been at least fleetingly Tuberculin positive.

Numerous other possible explanations exist and these include different thresholds for recognition of mycobacterial antigens at a variety of sites and immunoregulation resulting in suppession of responses.

Whatever the explanation, the HLA studies described, associate category 2 individuals with lepromatous leprosy patients by their common lack of the DR3 determinant. An obvious hypothesis based on this is that such people fail to respond adequately to invading leprosy bacilli allowing the bacilli to establish themselves in the tissues long enough to produce the immunosuppressive, or perhaps immunodiverting substances that may be the source of immunological sequelae of lepromatous disease. An extension of this hypothesis is that individuals carrying HLA-DR3 are protected from recognizing suppression inducing determinants and as a consequence are protected from developing the lepromatous type of leprosy. Such suppression inducing deteminants have been described in lyzozyme (Yowell *et al.*, 1979) and non-specific suppressor substances have been shown to be in mycobacteria (Ellner & Daniel, 1979; Wadee, Sher & Robson, 1980). In a recent paper Nye *et al.* (1983) have demonstrated that suppressive factors are amongst the species specific attributes of certain fast growing mycobacteria. The same authors showed that these substances work in such a way that they not only suppress the responses to other mycobacterial antigens injected with them but also block responses to these other antigens injected simultaneously at a distant site.

Despite the fact that our evidence suggests that category 2, HLA-DR3 lacking, individuals may develop lepromatous disease if they catch leprosy, there is no evidence whatsoever that such persons are more susceptible to infection. Similarly the evidence from the MRC trial of BCG in Great Britain showed that those individuals who did not remain Tuberculin positive after BCG

vaccination were, nevertheless, just as well protected from tuberculosis as were Tuberculin positive individuals (MRC, 1956, 1959, 1963).

Finally, we cannot exclude the possibility that HLA-DR protects from zero or low responsiveness to mycobacterial skin test reagents in a non-specific way. It has been suggested previously that HLA-DR3 carrying individuals may be considered to some extent to be the human counterpart of the Biozzi high responder mice (Legrand *et al.*, 1982). After BCG infection, high responder Biozzi mice were observed to be more capable of expressing tuberculin type hypersensitivity as measured by foot pad swelling than were low line mice (Lagrange, Hurtrel & Thickstun, 1979). So, the presence of HLA-DR3 might confer some degree of general DTH high responsiveness and thus protect from zero or low responsiveness to all kinds of antigens, including mycobacteria.

Further studies are needed to help determine the implications of HLA-DR heterogeneity for mycobacterioses in man. In particular we need to identify genetic factors associated with disease susceptibility if they exist. Furthermore, additional studies need to be carried out to investigate the presence of HLA-DR3 in leprosy patients from a pure Caucasoid population.

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