HLA-linked control of the course of *M. leprae* infections

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Introduction

The only simple discovery in leprosy has probably been made more
than a century ago. It is clear from the disease spectrum that the
modulation of the host response to the lazy Mycobacterium has to be
a complicated matter. That genetic host factors play a role was al-
ready realized before Hansen (1). Probably the best and certainly
easily presentable evidence for an important role of genetic host
factors comes from twin studies conducted by Chakravartti and Vogel
only about 10 years ago (2). Table 1 shows that monozygotic twins
show a much higher concordancy not only for leprosy, but also for

Table 1. Genetic host-factors in leprosy: twin studies.

	both leprosy	
	type =	type ≠
monozygotic twins (n = 62)	32 (52%)	5 (8%)
dizygotic twins (n = 40)	6 (15%)	2 (5%)
(from: Chakravartti and Vogel 1973)		

leprosy type.
     There are two important reasons why a study of the HLA system
in relation to the disease spectrum in leprosy a priori seemed
worth while. In the first place, the HLA system shows more than
enough variation to be a good candidate for an important and there-
fore probably complicated host factor. In the second place, pro-
ducts of the HLA system have been shown to be involved in the im-
mune response and in particular in the interaction between T cells
and macrophages (3) supposed to be of crucial importance in the
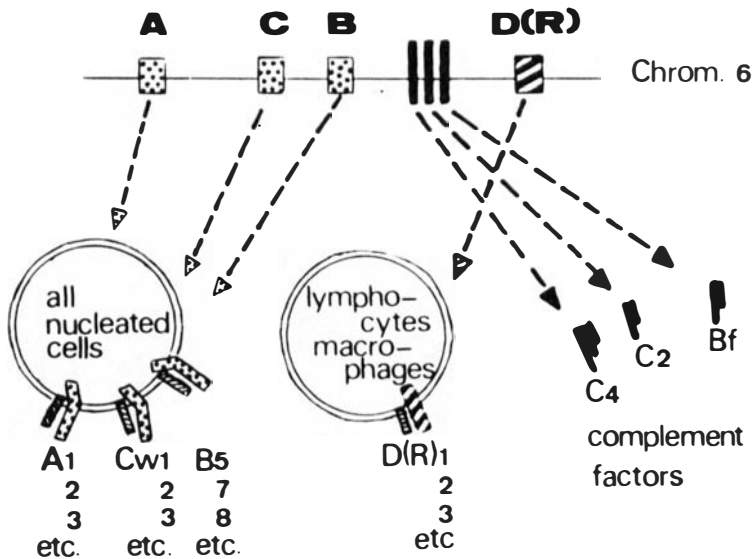host defense against Mycobacterium leprae (4).

The HLA system (3)

Figure 1 illustrates some important aspects of the HLA system and
its gene products. At least 7 loci have been defined, where the
genes are located coding for 3 types of molecules. The first type
is present on most if not all nucleated cells and is coded for by
genes situated at three loci: HLA-A, -B, and -C. Every determinant
is identified by a number (e.g. A1). If this definition is not yet
optimal, the number is preceded by the prefix "w" (e.g. Cw1). A
second type of determinants is also situated on the cell membrane,
but has a restricted tissue distribution. This type II determinant
is seen preferentially on lymphocytes (mainly B lymphocytes, but
also activated T lymphocytes) and macrophages. Originally these
determinants were defined by cell culture methods (mixed lymphocyte
cultures), but more recently this is also possible with serological
techniques. Although there are probably more loci containing genes
coding for these determinants, thus far only one locus has been well
defined: HLA-D, or -DR, if we mean the serologically defined deter-
minants. Finally a number of factors of the serum complement sys-
tem are coded by genes located in HLA: C2 and C4 of the classical
and factor B (Bf) of the alternative pathway.
    Both type I molecules (HLA-A, -B, and -C) and type II molecules
(HLA-D, -DR) are very polymorphic: about 20 different HLA-A deter-
minants (or alleles), 40 HLA-B, 8 HLA-C, and 10 HLA-D(R) determi-
nants are known. The complement factors show a much lesser degree
of polymorphism. Every individual has 2 copies of chromosome 6 and
therefore 2 HLA-A, -B etc. determinants, which because of this high
polymorphism will often be different. The complexity of the HLA

system is illustrated by a comparison with the ABO system: in the ABO system there is one locus with 3 alleles and 6 different geno-types, whereas the HLA system has at least 7 loci with more than 90 determinants and more than 40×10^6 possible combinations.

The distance between the different HLA loci is very small: only in about one out of 100 meioses a cross-over occurs. The informa-tion for the different HLA determinants on one chromosome, or haplo-type, is therefore nearly always inherited as such. Every indivi-dual has 2 HLA haplotypes, which can lead to 4 different combina-tions, if he or she marries and gets children. One in 4 children will therefore be identical for HLA. Unrelated individuals have of course a much smaller chance to be HLA identical if we think of the enormous number of possible combinations. It appears however that some combinations occur much more frequently than expected on the basis of the respective gene frequencies. This phenomenon is call-ed linkage disequilibrium and is illustrated in figure 2.

Figure 2. Linkage disequilibrium (A1-B8-DRw3); 75 DRw3 positive haplotypes on HLA-A versus HLA-B. (from Lamm et al. 1977)

It is quite possible that individuals carrying these preferential combinations have had a better chance to survive infectious diseases in the past (4). It should be noted that there is a great variation both of gene frequencies and linkage disequilibria between differ-ent populations.

In the present context, we will confine ourselves to mention only one functional aspect of the products of HLA, which is probab-

ly the most pertinent with respect to leprosy. The type II mole-
cules, that is HLA-D(R), are crucial in the interaction between T
cells and macrophages (3). This is illustrated in <u>figure 3</u> for the
response to PPD.

Figure 3. PPD responses of allogeneic T lymphocyte/macrophage com-
binations expressed as relative antigen-specific stimulation (RAgS).
The ranges and medians are given. Note that for an adequate res-
ponse, T lymphocyte and macrophage donors must share at least one
HLA-D antigen. (from Bergholtz and Thorsby 1977)

**Number of HLA-D antigens shared
between T and M Φ donor**
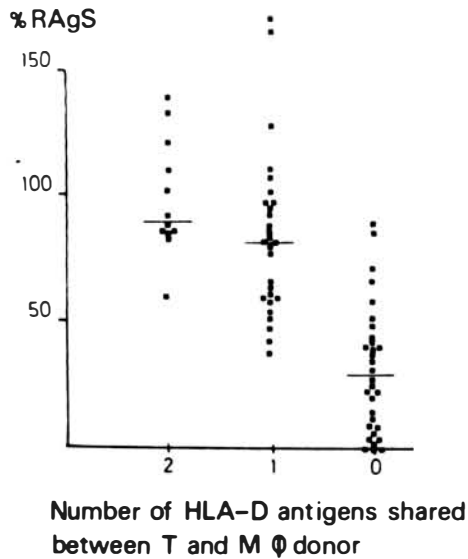
Some general methodological comments on HLA and disease studies

When looking for a possible relationship between HLA and a disease,
two different approaches have been used: a population study or a
family study. In a population study we look for an association be-
tween a particular HLA type and a disease, which may be due to
either causation (that is the associated antigen itself is respons-
able for the higher or lower susceptibility) or linkage disequili-
brium of a susceptibility gene with the associated HLA marker. For
the basically simple protocol, the statistics and pitfalls of these
studies, the interested reader is referred to Svejgaard et al. (6).
    Family studies have two big advantages compared with population
studies: 1)
lation heterogeneity and 2) they do not rely on linkage disequili-
brium. Of course a third important aspect of family studies is,
that they may provide extra genetic information, such as the inheri-
tance, penetrance and frequency of disease susceptibility genes.
Apart from the fact that appropriate families are often difficult
to collect, a methodological problem is that familial cases of a

given disease may not be representative of the patient population. The statistics of these family studies basically concern the question: do affected siblings more often than expected share HLA haplotypes?

HLA-A, -B, -C and leprosy: population studies

We know of 16 publications addressing the question of a possible association between HLA-A, -B, and/or -C antigens and leprosy or leprosy-type (7-22). Before attempting to draw general conclusions from these studies a few comments seem useful. In the first place, the leprosy classification varies from study to study. Furthermore these studies often concern small patient samples or small samples from certain subgroups. Sometimes there have been clear technical problems in HLA typing (8), and in general the investigators did not use the same typing reagents. All these factors make comparisons and interpretation a difficult task. Perhaps the most important methodological criticism is, that often not enough care seems to have been taken in matching of the control group. Probably just the healthy donors or staffmembers of the HLA typing center often do not represent a well matched control group. This is elegantly shown for instance by Smith et al. (11).

With these considerations in mind, we choose not to make an extensive analysis of all positive and negative data, but rather to see whether there might be a pattern in the positive results.

Table 2. **Leprosy** and HLA: associations with HLA-A and -B.

Country (ref.)	HLA antigen	Antigen freq. in patients vs controls	
		T	L
Ethiopia (7)	Bw21	↑(RR^x=?)	= (LL)
India (12)			
Thailand (16,18,20)	A9	↓(RR = 0.4)	=
Singapore (21)	B17	↑(RR = 3.2)	=
Spain (9)	B14	=	↑?(RR = 13)

^{x}RR = Relative risk = how many times more frequently the disease occurs amongst individuals carrying the antigen as compared to those without it. In this particular case the Bw21 antigen did not occur amongst healthy controls (and LL patients) so that **theoretically** the RR is infinite.

Table 2 shows the associations, which were either confirmed in other studies (A9 and B17), or significant after correction for the numbers of comparisons made and not disproved by later studies (which were simply not performed to our knowledge: Bw21 and B14). If we then try to draw some general conclusions, we could state the following:

The fact that only 7 out of the 16 studies are listed means that a lot of studies were negative or not confirmed.

With one exception (9) the associations are with tuberculoid leprosy. It is perhaps interesting that in Spain the ratio tuberculoid versus lepromatous leprosy is 1:2.5, whereas in the other studies this ratio is ⩾1:1. Another exception might be Japan with a T:L ratio of 1:3, where no significant differences in HLA antigen frequencies might be found between tuberculoid and lepromatous leprosy, but both differing from healthy controls (Isumi et al., personal communication). The Relative Risk (see note to Table 2), for the confirmed associations is in the order of 3, which means that it is certainly not an "all or none" phenomenon.

The fact that associations with different HLA antigens are found is most easily explained by assuming that the associations are nót due to causation but most probably due to linkage disequilibrium of the associated antigen with leprosy susceptibility genes. It was stated earlier that there is marked inter-population variation in linkage disequilibria. This logically led us to family studies.

Family studies

Because the detection of linkage in family studies does not rely on linkage disequilibrium, these studies seemed most appropriate to test whether the assumption, that the associations found were due to linkage disquilibrium of the associated HLA antigen and a leprosy susceptibility gene was correct. As far as we know 6 family studies were performed (23-28), of which 3 by the same group, namely ourselves (23,25,27). The positive results of these studies are shown in Table 3.

Table 3. Segregation of HLA haplotypes from healthy parents in siblings affected with tuberculoid leprosy.

Families studied	Observed ΣD	Expected Σd	$\Sigma\sigma_d^2$	χ^2	p value
Surinam	26	17	14	5.16	<0.025
India I	19	11.5	10.75	4.56	<0.025
India II	10	9	7.5	0.03	n.s.
India I + II	29	20.5	18.25	3.51	<0.05
Total	55	37.5	32.25	8.96	<0.0025

The conclusions from these 3 studies were a) that (a) gene(s) **conferring susceptibility to tuberculoid leprosy is linked to HLA**

and b) that the data best fitted with a recessive inheritance. This was confirmed in a large study by Fine et al. (26), that is only evidence for susceptibility to tuberculoid leprosy being linked to HLA and again suggestion for recessive inheritance. Not easily explainable by us is the observation in the latter study that the data were only significant in families, where both parents were healthy. A study of one 3 generation family in Brasil (28) is compatible with these data. A study by Stoner et al. (24) used a quite different protocol, but is a confirmation of our and Fine's negative results, namely that no evidence was found for linkage of susceptibility to lepromatous leprosy with HLA.

Associations with HLA-DR?

As stated earlier it was expected for functional reasons, that stronger associations than with HLA-A, -B, and -C might be found with HLA-DR (3). The first indication, that this might indeed be the case came from our family studies in India (25,27), where no evidence for an association of tuberculoid leprosy with a particular HLA-A, -B, or -C antigen was found, but a significant association with HLA-DR2. Moreover, a preferential segregation of HLA-DR2 from healthy parents to children affected with tuberculoid leprosy could be shown in the first study (25) and confirmed in the second family study (27). Table 4 shows the combined data of the two studies.

Table 4. Inheritance of DR2 haplotypes by the children affected with tuberculoid (T) leprosy of each DR2 **heterozygous** parent (27).

| Families[x] | Parents | Children with T leprosy[o] | | χ^{2}[*] | p |
		DR2 inherited	DR2 not inherited		
India I	Healthy	8	3	2.27	0.013
	All not affected with T leprosy	11	3	4.57	0.03
India II	Healthy	12	1	9.31	0.002
	All not affected with T leprosy	12	5	2.88	0.09
India I+II	Healthy	20	4	10.67	0.001
	All not affected with T leprosy	23	8	7.26	0.007

[x]Data from India I is from (25) and from India II is from (27). India I + II represents data that were combined from the two studies.
[o]Numbers in these columns refer to number of DR segregations, not to numbers of children.
[*]Calculated for deviation from expected 1:1 ratio.

Of course we then performed a population study to test whether this association could also be shown in non-familial cases. To our disappointment no association with HLA-DR2 (nor any other HLA antigen) could be shown for tuberculoid leprosy patients without family members known to be affected with leprosy (van Eden et al., Tissue Antigens in press). Although not expected, this negative result in our mind is interesting for epidemiologists, because it suggests a different epidemiological situation for familial and "sporadic" tuberculoid leprosy.

Also unexpected was that in two absolutely different populations an association with HLA-DR2 was reported, namely in Mexican Indians (29) and in Japan (Isumi et al., personal communication). In Japan the association was not only with tuberculoid but also with lepromatous leprosy (see also the paragraph on HLA-A, -B, -C population studies). This might indicate, that HLA-DR2 itself might be responsible for the increased susceptibility to (tuberculoid) leprosy.

Table 5.   HLA-DR3 in healthy controls versus leprosy patients.(30)

	HLA-DR3 pos.	HLA-DR3 neg.	χ^{2}*	p value
Healthy controls	23	63		
TT patients	16	13	7.61	0.006
BT patients	4	13	0.03	n.s.
BL + LL patients	1	22	4.89	0.027

*Significance of difference when compared with healthy controls.

That this does not seem to be the case, at least not in every population, is shown by the interesting results of a recent study conducted among Surinam people shown in Table 5 and graphically illustrated in figure 4. This study most clearly suggests, that the association is not with susceptibility to leprosy, but rather with the response to the bacillus.

Figure 4.   Relative risks to develop different types of leprosy for
Surinam individuals carrying HLA-DR3 as compared to those lacking
this determinant (30).
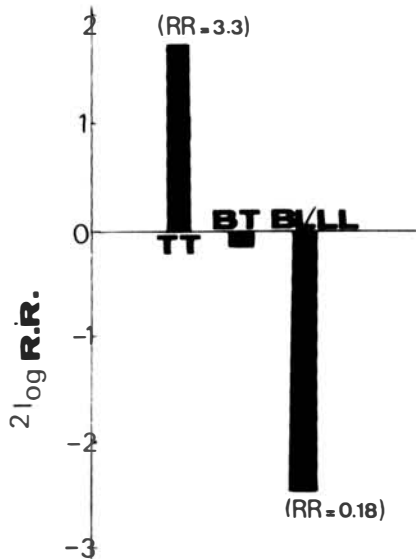
General conclusions

    The genetic factors controlling the course of M.leprae infec-
tions are not simple, not confined to HLA, and probably different
and/or differently modulated by environmental factors in different
populations.
    As far as HLA-linked factors are concerned: most evidence exists
regarding the development of tuberculoid leprosy. This means that
individuals prone to develop tuberculoid leprosy may be genetically
different from those, who stay healthy after exposure to the
bacillus. We realize that this is not in keeping with the ideas
many epidemiologists have on tuberculoid leprosy.
    It seems that the HLA-linked factor(s) do nòt confer suscepti-
bility to infection, but rather modulate the type of immune response
to M.leprae. This is similar to observations in mice for other in-
tracellular growing bacteria, such as Listeria and Leishmania,
where the susceptibility to infection is not, but the development
of specific immunity is linked to H-2, the equivalent of the HLA
system in the mouse (31,32).

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