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Human Leukocyte Antigens (HLA) and Mycobacterial Disease

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Introduction

The Immunogenetic Approach to Mycobacterial Disease

In most individuals extensive exposure to mycobacteria does not result in disease [21]. Many factors are involved in this apparent successful parasitism of mycobacteria. For instance, most mycobacterial species rarely or never cause disease. In this chapter we will only discuss genetic host factors that play a role in the outcome of an infection with potentially pathogenic mycobacteria. To this purpose we will confine ourselves to the two most important pathogenic mycobacteria, namely *Mycobacterium tuberculosis (M. tuberculosis)* and *Mycobacterium leprae (M. leprae)*. Of these two mycobacterial species *M. leprae* is by far the less virulent and toxic and, therefore, makes host factors particularly apparent.

Certainly in leprosy, but probably also tuberculosis, the immune response of the host to the bacillus is responsible for the disease symptoms rather than direct damage by the mycobacterium [22]. Virtually everybody has an intact immune system, but only few sufficiently exposed to and infected with *M. tuberculosis* or *M. leprae* develop a clinically relevant disease. Particularly during the last two decades it has become clear that subtle differences in immune reactivity exist between individuals and that these differences are genetically controlled and biologically relevant [32]. The study of genetically controlled differences in immune reactivity and their relevance for disease susceptibility historically belongs to a discipline called immunogenetics.

Immunogenetics was born as the AB0 bloodgroups and is thus 88 years old [30]. Because several blood group antigens also appeared to be transplantation antigens, immunology became not only a tool but also a subject of study for immunogeneticists. This led to the discovery of the major histocompatibility complex (MHC)-linked immune response (Ir-) genes [4, 33] and the role of their products in the regulation of the immune response [3, 51], which is the main subject of this chapter.

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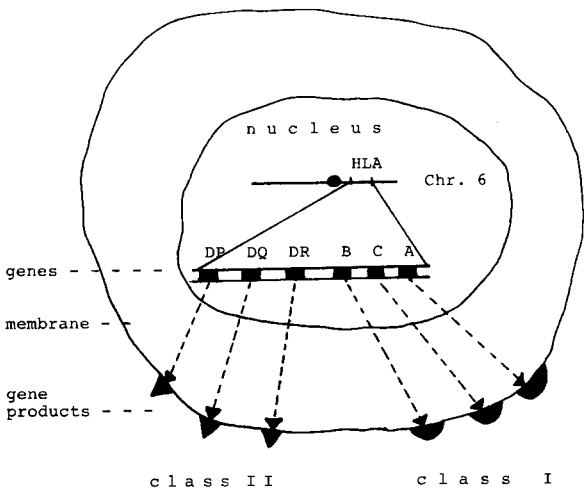
Ir-genes are polymorphic genes that code for differences in immune reactivity between individuals [4]. An important aspect of Ir-genes is that they may be studied in healthy individuals, thus providing a possibility to differentiate between cause and effect of disease. The goal of the immunogenetic approach to disease is to unravel the following chain of events (Fig. 1): (1) polymorphic Ir-genes code for: (2) qualitative or quantitative differences in expression of Ir-gene products, which (3) result in inter-individual differences in immune reactivity to certain antigens, which in turn (4) lead to differences in susceptibility to or severity of a certain disease. This immunogenetic approach to disease may be applied to specific and non-specific immune reactivity. However, in this chapter we will mainly focus on antigen-specific immune reactivity, because we will almost exclusively discuss the MHC-linked Ir-genes which code for antigen-specific differences in immune reactivity [4].

The human leukocyte antigen (HLA) system is the MHC of man [12]. As shown in Fig. 2, it contains two different sets of very polymorphic genes, namely class I and class II genes. These two sets of genes are the human MHC-linked Ir-genes. Several class I-like genes have recently been discovered, which are situated telomeric from the class I genes. The function of the products of these class I-like genes is unknown and they will remain outside the scope of this chapter. Between the class I and class II genes several genes coding for factors of the complement system (C2, C4 and factor B) are situated. These genes are neither structurally nor functionally related to the HLA class I and II genes and, therefore, we do not consider them to belong to the HLA system. This does not mean that these genes may not be relevant for differential susceptibility to or course of mycobacterial infections. However in this review we will focus on the HLA class (I and) II Ir-genes. There are three functional class I genes (A, B and C) and at least three sets of class II genes coding for class II products (DP, DQ and DR). Except for DP, these genes are all situated so close to each other (recombination frequency less than 2%) that they are usually inherited together or as a so-called haplotype.

The HLA system is by far the most polymorphic genetic system known in man. This implies that most individuals will have a unique set of HLA class I and II alleles, unless they are genetically closely related. Apart from the fact that this extreme polymorphism provides us with a powerful tool for genetic studies, it is also probably essential for the function of the system, as will be discussed later. The theoretically virtually infinite number of combinations is restricted to some degree because of so-called linkage disequilibria: certain combinations of alleles of different loci occur more (or less) often than predicted from their respective gene frequencies. These linkage disequilibria may be (or have been) functionally important, as will also be discussed later. Moreover, they have practical relevance for the demonstration of disease susceptibility genes because products of genes linked to a particular disease susceptibiliy gene may serve as genetic markers.

Fig. 1. The immunogenetic approach to mycobacterial disease. (Ir-=Immune response)

1. Ir-gene	:	A or non-A
2. Ir-gene product	:	a or non-a
3. Ir to antigen X	:	inappropriate or appropriate
4. result	:	disease or no disease



tissue distribution:	antigen presenting cells (APC)	all nucleated cells
molecular weight:	32 + 38 kD	46 + 12 kD
domain structure:	2 + 2	3 + 1
function:	antigen presentation to helper T cells	antigen presentation to cytotoxic T cells
polymorphism:	++	++
Ir-genes:	+	+
transplantation antigens:	+	+

Fig. 2. The human leukocyte antigen (HLA) system

In this chapter we will first review the epidemiologal studies demonstrating the HLA class II-linked control and the association with certain HLA class II types of the course of mycobacterial infections. Next we will discuss what is presently known on HLA class II Ir-genes, their products and their regulation of the immune response. Finally, we will review the studies indicating HLA class II Ir-gene control of the immune response against mycobacterial antigens and its implications for the pathogenesis of leprosy and tuberculosis, as well as for the development of an effective vaccine. Because most information is available for leprosy, that disease will be discussed in greater detail. However, we think that the main conclusions drawn for leprosy also apply to tuberculosis and other mycobacterial diseases.

Epidemiology

Figure 3 illustrates the changes in point of view on the genetics of leprosy that have taken place during the 19th and 20th century. In the middle of the 19th century

leprosy was thought to be a hereditary disease by several experts, including P. C. Danielssen, the father-in-law of G. Armauer Hansen [9]. However, Hansen argued after his discovery of the bacillus in 1874 that "a bacillary disease cannot be inherited" [25]. This simple concept has been popular for nearly a century. However, in the middle of the 20th century the idea that genetic host factors migth also be important in infectious diseases gained popularity [47]. For leprosy this culminated in 1973 in a twin study by Chakravartti and Vogel, which strongly suggested that both the risk of developing leprosy and the type of the disease are genetically controlled [8]. Although this and other studies arguing for genetic control of susceptibility to, or course of, leprosy may be rightly criticised in several ways [18], they paved the way for the immunogenetic studies to be discussed below, which definitely showed that at least the type of leprosy developing in a susceptible host is controlled by HLA class II Ir-genes [15, 18, 35]. It took some time before this "semi-hereditary" concept was generally accepted. This was partly due to a presumed incompatibility with leprosy control strategies (vaccination) and the fatalistic imago of the word heredity in this context. There was also scientific opposition of which the top right corner of fig. 3 is a humorous documentation. This copy of Hansen's paper was given to the first author of this chapter (RdV) "on behalf of Hansen" by Dr. Lorentz M. Irgens, an epidemiologist from Bergen (where Hansen also worked and lived), who had just published a monograph on the epidemiology of leprosy in which he provided arguments against an important role of genetic host factors [26]. Today (molecular) genetics has become a field of research where virtually nothing is impossible, and recently a joint IMMLEP/IMMTUB Immunology of leprosy and tuberculosis components of World Health Organization meeting was organized in Geneva to discuss its possible impact on the prevention and treatment of mycobacterial disease.

Before discussing the epidemiology of HLA and mycobacterial disease, it seems appropriate to briefly review some general and methodological aspects of HLA and disease studies. Following the discovery in 1964 by Lilly et al. [31] that resistance to virally induced leukaemia is controlled by MHC-linked genes,

Heredity of Leprosy.

By

Dr. G. Armauer Hansen.

Zambaco Pacha has published a treatise: „L'hérédité de la lèpre", in which he thinks undoubtedly to have proved the heredity of leprosy. He seems to think that the contagionists do not acknowledge anything to be hereditary. This is at any rate not the case with me, but I say that a bacillary disease can not be inherited. Zambaco says, page 7: „L'héritage et

Fig. 3. Leprosy and heredity: an historical perspective reproduced from Arch Dermatol Syphilol 110: 225 (1911)

susceptibility to a large number of mainly immunopathological diseases was shown to be associated with certain HLA types. There are two important reasons why this area of research has been so productive. In the first place, the HLA system is an unmatched genetic marker system for population and family studies, due to its exceptional polymorphism. Secondly, as discussed in the previous section, the HLA class I and class II products have a central role in the regulation of the immune response and are the products of immune response genes. It should be stressed at this point that it is extremely unlikely that the HLA system was meant to confer disease susceptibility. There is evidence that at least one important function of the HLA system is to confer resistance to infectious diseases and the present form of the HLA system might have been shaped to a great extent by the vast selective pressures of virulent infectious agents [11, 14, 40]. These virulent pathogens might have selected haplotypes associated with a relatively aggressive immune response. These haplotypes, however, become harmful in the presence of less virulent pathogens or other subtle antigenic changes by leading to auto-destruction. Such haplotypes will not quickly disappear if the disadvantage is expressed as morbidity later in life rather than decreased Darwinian fitness, as is the case for nearly all diseases associated with HLA. The HLA and disease associations may, thus, be the result of a response which was molded to confer immunity, but sometimes may lead to immunopathology. Until now the major contribution from the study of HLA and disease associations has been in the elucidation of genetic aspects of the diseases in question and for some diseases the mode of inheritance has been clarified. Furthermore, these studies have contributed a great deal to a better insight into the pathogenesis of several diseases by leading to the subdivision of apparently genetically heterogeneous entities or by demonstrating a common genetic factor for other diseases. Finally, we are now beginning to gain insight into the possible mechanisms of a few HLA and disease associations.

HLA and disease studies may be divided from a methodological point of view into population and family studies. Both types of studies have their merits and disadvantages. The majority of HLA and disease studies have been population studies, in which the HLA antigen frequencies in a sample of unrelated patients are compared with those in a matched control population. The numbers of patients and controls who are positive and negative for each HLA-antigen are entered in a 2×2 table. A significantly increased or decreased frequency of a given HLA-antigen among the patients indicates an association between that particular antigen and disease. Most of the pitfalls encountered in carrying out and statistically analyzing population studies have been reviewed by Svejgaard et al. [48]. Apart from chance, heterogeneity, or technical (typing) artifacts, such an association may be a direct result of the presence of that particular antigen (causation), or to linkage disequilibrium between the HLA allele coding for the associated antigen and a linked susceptibility (or resistance) gene. With respect to the latter, it is important to realize that linkage disequilibria vary between populations, which means that the associations may also vary between populations. The strength of an association is usually expressed as the relative risk (RR), which is the cross product calculated from the 2×2 table, as initially described by Woolf and modified by Haldane [48].

The main advantages of family studies are: (a) they exclude artifacts due to population heterogeneity; (b) they may reveal linkage in the absence of an association at the population level, because they do not rely on linkage disequilibrium; and (c) they may provide extra genetic information. Apart from the fact that appropriate families are often difficult to collect, a potential danger of family studies is that familial cases of a particular disease may not be representative of the patient population. Two main problems are encountered in performing linkage analyses of HLA and disease family studies. The first applies to most diseases which are not inherited in a simple Mendelian fashion, while the second is particularly related to diseases which show an association with an HLA phenotype in the population. Both preclude standard linkage analyses. A more refined linkage program combined with a complex segregation analysis (POINTER) has been developed by Morton and Lalouel [28, 34] to cope with these problems but it seems far from the final answer. Simpler methods have been developed for detecting linkage without estimation of the recombination frequency. These methods basically test for deviations of HLA-haplotype sharing as an indication for co-segregation of HLA and disease. Several methods are available for analyzing HLA-haplotype sharing among affected sibs pairs [10, 50]. The observed frequencies of affected sib pairs sharing two, one, or zero HLA haplotypes are compared with those expected, with excess sharing indicating linkage. The deviations observed may be used to estimate the mode of inheritance, frequency and penetrance of a hypothetical disease susceptibility gene. Other methods have the advantage that sibships containing more than two affected sibs may also be analyzed, that information of sibships of various sizes may be combined, that unaffected sibs may also be analyzed, and that no a priori assumption on the mode of inheritance of disease susceptibility genes is required [13, 23, 54].

Table 1 summarizes the results of family studies indicating that both susceptibility to polar tuberculoid leprosy and susceptibility to BL/LL leprosy are controlled by HLA-linked genes [13, 52]. HLA-linked genes do not confer susceptibility to leprosy per se, because the segregation of HLA haplotypes to healthy siblings occurs randomly. Furthermore, if genes conferring susceptibility to leprosy per se were linked to HLA, one would expect those HLA haplotypes shared between leprosy patients in a given sibships to occur less frequently among the healthy siblings of that sibship. This was not the case in three independent studies per-

Table 1. Children suffering from TT or BL/LL leprosy share human leukocyte antigen (HLA) haplotypes more frequently than expected

Children	Observed*	Expected*	P
TT leprosy	188	139.6	5×10^{-6}
BL/LL leprosy	89	64.5	0.0008
Healthy	128	125.8	n. s.

* The figures represent, respectively, the observed difference between the number of affected siblings carrying one and those carrying the other parental haplotype and the expected difference assuming random segregation. The method allows pooling data from a large number of multicase families [13]. Data from references given in [52]. TT: polar tuberculoid; BL/LL: lepromatous
n.s.: not significant

formed respectively in India, Venezuela and China [52]. Another argument against linkage of susceptibility to infection with HLA was that the presence of antibodies directed against *M. leprae*-specific phenolic glycolipids did not co-segregate with HLA in families (Buchanan, Ottenhoff and De Vries, unpublished observations). Thus genetic factors not linked to HLA may control susceptibility to infection [46], but the type of leprosy that develops in – genetically or otherwise – susceptible individuals is at least partly controlled by HLA-linked genes.

The mode of inheritance of the HLA-linked gene(s) controlling leprosy type is not quite clear yet. Most studies are compatible with susceptibility to (polar) tuberculoid leprosy being controlled by a recessive, and susceptibility to lepromatous leprosy by a dominant, HLA-linked gene [19, 44, 52]. However, at least one study is difficult to fit in such an hypothesis [16]. This discrepancy may partly be explained by the fact that susceptibility to leprosy type is in all probability a multifactorial condition, which complicates the analysis of just one contributing factor (in this case an HLA-linked gene). On the other hand, there is evidence that lepromatous leprosy, for example, may be a heterogeneous condition; suppressor T cells being demonstrable only in a subgroup [35]. So differences, e. g., geographic ones, in the frequencies of such subgroups with a different pathogenesis might be another explanation for the observed discrepancies.

Many studies have been performed in different parts of the world, searching for an association between particular HLA markers and leprosy or leprosy type. The early studies only looked for associations with HLA class I (A, B and C) antigens. Although some significant associations were reported, they were weak and/or not confirmed in other populations, and different associations were reported in different populations [15]. This suggested to us that these associations were not due to the associated HLA class I antigen itself, but to a closely linked susceptibility gene, which led to the family studies just discussed. In the meantime typing for HLA class II alleles had become possible and, as expected, stronger and more consistant associations were found with HLA-DR and other class II antigens [15, 52]. Notably HLA-DQ1 might be a universal marker for susceptibility to lepromatous leprosy [16]. For (polar) tuberculoid leprosy there seems to be more interpopulation heterogeneity [15, 52]. We will discuss in some detail one particular association observed in a mixed Caucasoid-negroid population in Surinam (South America), namely between DR3 and polar tuberculoid leprosy (see Table 2). One should realise that the risk of developing leprosy or a particular leprosy type in

Table 2. HLA-DR3 is associated with TT leprosy in a Surinam negroid population [35]

Subjects	HLA-DR3		χ^2	P	Relative risk
	Positive	Negative			
Healthy controls	49 (23%)	163 (77%)			
			9.93	0.002	3.30
TT patients	16 (50%)	16 (50%)			
			10.85	0.001	7.00
BL + LL patients	4 (11%)	31 (89%)			

individuals with a certain "high risk" HLA type is still very low (generally less than 1%).

Data from twin and family studies provide strong evidence for the involvement of genetic factors in the susceptibility to tuberculosis. Compared to leprosy much less attention has been paid to the relationship between tuberculosis and HLA. Some associations have been reported between HLA class I alleles and tuberculosis, but varying between populations [1, 24, 43]. One of these studies suggested that disease severity is associated with HLA type [1]. A similar observation was made in Chinese children, where tuberculous meningitis was associated more strongly with a particular HLA-antigen (Bw35) than pulmonary tuberculosis [27]. A family study in India showed significant co-segregation of pulmonary tuberculosis with HLA-DR2 [45]. In a recent large population study in Indonesia in collaboration with J. Ivanyi (London), we observed a significant association between sputum-positive tuberculosis and (also) DR2. In that study a strong association between DR2 and high antibody titer to an epitope on a 38-kDa protein of *M. tuberculosis* was observed, indicating that a DR2-associated Ir-gene may be the mechanism for this association (Bothamley et al., submitted for publication).

HLA Class II Ir-genes and Leprosy Type

The products of HLA class I genes (A, B and C) are glycoproteins which constitute the so-called heavy chain (molecular weight 44 kDa) of class I molecules. This heavy chain is a transmembrane protein produced by and present on the membrane of virtually all nucleated cells. Non-covalently attached to the heavy chain is β_2-microglobulin (β_2m), which has a molecular weight of 12 kDa and is coded by a non-polymorphic gene situated on chromosome 15. The extracellular part of the class I heavy chain consists of three immunoglobulin-like domains and β_2m is attached to the domain closest to the cell membrane. The polymorphism of the HLA class I genes is mainly expressed by the two outer domains of the heavy chain. Recently, the three-dimensional structure of an HLA class I molecule has been deduced from X-ray defraction studies of crystals [5]. The main surprise was that the two outer "domains" together form a groove, the bottom of which is formed by anti-parallel beta-stands and the sites by two alpha-helices. This groove is in all probability the antigen-binding site, whereas the two alpha-helices contain the T cell-recognition sites [6].

HLA class II molecules are also found on the cell membrane, but usually only on cells belonging to the immune system. They show a particularly strong expression on specialized-antigen-presenting cells, such as dendritic and Langerhans cells, and are also present on (most) B cells, macrophages (to a variable degree) and activated T cells. They consist of two non-covalently associated glycoprotein chains, which are called α and β. In this case both chains have two domains, both penetrate the cell wall and both are coded by HLA genes. Thus the three class II molecules officially recognized today [51], namely DP, DQ and DR each consist of an α- and β-chain which are both coded by a separate HLA class II gene: DP_α and DP_β, DQ_α and DQ_β, and DR_α and DR_β, respectively. The situation is, however, more complicated. For instance, there are usually two DR_β genes per

haplotype which code for two different DR molecules ($DR_{\alpha\beta I}$ and $DR_{\alpha\beta III}$). The polymorphism of the class II genes, like that of the class I genes, is mainly expressed on the outer domains of the molecule and the general structure in all probability is quite similar to class I.

A major function of MHC class I and II molecules is the presentation of antigens to T cells [12]. During the past 3 years several important questions concerning the molecular basis of this process have been answered [39, 41, 42]. It is now almost generally agreed that, for most protein antigens, some kind of processing is necessary to allow binding to MHC molecules. This processing probably involves unfolding and/or cleavage of the protein to peptides [2]. Although as yet binding of peptides to MHC class I molecules has not been shown, all data point to the notion that there are no fundamental differences between class I and class II molecules in the binding and presentation to T cells of antigenic peptides [20, 39]. If it is true that certain antigens are preferentially presented by class I and other antigens by class II molecules, then that may be due to differential trafficking of antigens and the two types of MHC molecules in the antigen-presenting cell [20]. The clear functional "isotypic" differences between class I and II molecules, that is the preferential restriction of CD8-positive cytotoxic T cells by class I and of CD4-positive helper T cells by class II molecules may be explained by the binding of CD8 and CD4 to non-polymorphic sites on, respectively, class I and II, and does not seem to be related to differential binding of certain peptides to class I or II molecules. Because binding to MHC is necessary for recognition by T cells, the quantitative expression of these MHC molecules clearly has a regulatory role in the induction and focusing of the immune response [51]. Finally, the polymorphism of MHC class I and II molecules is biologically significant. Binding of peptides to MHC molecules shows a certain degree of specificity, that is: certain peptides bind better to the products of certain alleles. This differential binding correlates with and, therefore, may be the explanation for the MHC-controlled inter-individual differences in antigen-specific immune reactivity. In other words: MHC class I and II molecules are immune response (Ir-) gene products.

An an example of HLA class II Ir-gene control of the immune response relevant for the course of mycobacterial disease, we will review mostly unpublished data on the T cell response to the mycobacterial 65-kDa heat shock protein. This protein is a major immunogen of *Mycobacterium leprae* and *Mycobacterium tuberculosis* and may be involved in the pathogenesis of rheumatoid arthritis [53, 56]. Until now ten T cell epitopes have been defined on this protein using T cell clones, deletion mutants of the gene coding for this protein and synthetic peptides [29, 49] (W. C. A. van Schooten et al., submitted for publication). The T cell recognition of one of these epitopes has been studied in detail. This epitope is *M. leprae* specific, the minimal peptide consists of nine amino-acids (Anderson et al., to be published), and its recognition by a T cell clone is restricted by HLA-DR2 [38]. Using a large battery of peptides with a systematic series of substitutions at each position, it was shown that residues 3–8 are all crucial for binding to HLA-DR2 and/or recognition by the T cell receptor (Anderson et al., submitted for publication). That a DR2 molecule is actually involved in the presentation of this peptide was formally demonstrated with the use of transfectants [55]. In fact nine out of the ten epitopes on the 65-kDa protein are recognized by DR-restricted

T cells. The dramatic Ir-gene control of the T cell response to this 65-kDa mycobacterial protein is shown in Fig. 4: each epitope is exclusively presented by one particular HLA-DR allele (Van Schooten et al., submitted for publication).

How such HLA class II Ir-gene control may explain the associations between particular HLA class II alleles and leprosy type is illustrated in Fig. 5. This hypothesis also has obvious implications for vaccine development. We are currently testing this hypothesis, and this review ends with the results so far obtained. *M. leprae*-reactive helper T (Th) cell are probably responsible both for acquired protective immunity to the bacillus and delayed-type hypersensitivity (DTH) which may result in immunopathology [22]. In our hypothesis the latter is caused by another subset of T cells recognizing a DTH-inducing epitope restricted via a particular HLA class II allele. Detectable Th cell reactivity against *M. leprae* but not other mycobacteriae is absent in (polar) lepromatous leprosy patients. This *M. leprae*-specific unresponsiveness may be a consequence of *M. leprae*-reactive suppressor T (Ts) cells, although these cells have been notoriously difficult to demonstrate [7]. These Ts cells might recognize so-called suppressor epitopes on *M. leprae* also restricted via a particular class II allele. For a better characterization of these Th and Ts cells and the HLA molecules involved in the presentation of particular *M. leprae* epitopes to them, we have cloned *M. leprae*-reactive Th and Ts cells. *M. leprae*-reactive Th cells were cloned, which were OKT3 and OKT4 positive, showed *M. leprae*-specific proliferation and gamma-interferon production and did not suppress other *M. leprae*-reactive T cells [37]. With the use of monoclonal antibodies directed against different HLA class I and II molecules, we could show that *M. leprae* antigens were nearly always presented to these Th clones by HLA-DR class II molecules [38]. To test the hypothesis of whether the above-mentioned association of HLA-DR3 with TT leprosy might be due to a HLA-DR Ir-gene, we investigated whether HLA-DR3 molecules differed from

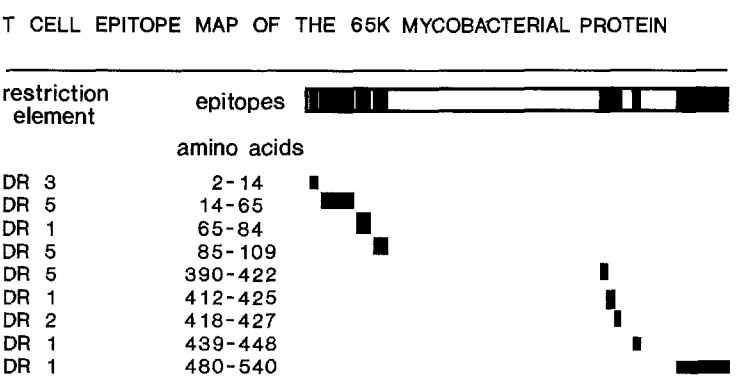


Fig. 4. T cell epitope map of the 65-kDa mycobacterial protein. The restriction element of 65-kDa reactive T cell clones and lines was established with the use of inhibition studies with monoclonal anti-HLA monoclonal antibodies and a panel of HLA-typed allogeneic antigen-presenting cells. The T cell epitopes were mapped using deletion mutants of the 65-kDa gene and synthetic peptides. [29, 49] (Van Schooten et al., submitted for publication)

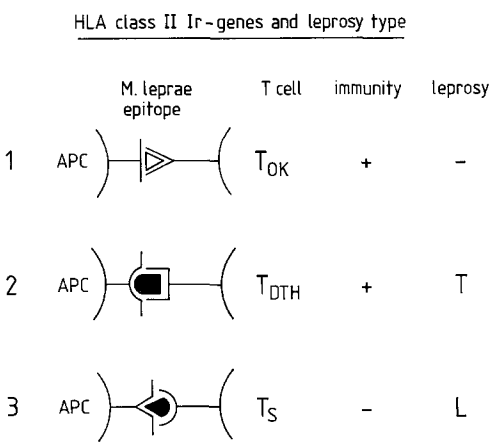


Fig. 5. HLA class II Ir-genes and leprosy type. Antigen-presenting cells (*APC*) of three individuals differing for HLA class II present different *M. leprae* epitopes to functionally different T cells: T_{OK} is a helper T cell that confers protective immunity, the same is true for T_{DTH} which, however, also causes type IV immunopathology [delayed-type hypersensitivity (DTH)-reaction] seen in tuberculoid leprosy, and *Ts* in a suppressor T cell responsible for the *M. leprae*-specific non-responsiveness seen in lepromatous leprosy

other HLA-DR molecules in the presentation of *M. leprae* and other mycobacterial antigens to Th cells. This appeared to be the case indeed (T. H. M. Ottenhoff et al. and J. B. A. G. Haanen et al., in preparation).

We have also succeeded in cloning T cells from the peripheral blood of a borderline lepromatous leprosy patient, which specifically suppressed autologous Th cells reactive with mycobacteria [36]. These Ts clones are also activated by *M. leprae* antigens presented by HLA class II molecules, probably in most cases DR molecules [17]. Interestingly, all Ts clones raised from this patient could only proliferate to *M. leprae* antigens when presented by DR molecules on cells from haplo-identical family members, in contrast to the Th clones from this patient that proliferated to *M. leprae* when presented by HLA-DR matched antigen-presenting cells. These data suggest that differences between DR molecules may control whether certain epitopes are presented to helper or suppressor T cells. So far no DQw1-associated Is gene and/or product has yet been demonstrated convincingly which might explain the association of HLA-DQ1 with lepromatous leprosy. However, in some instances *M. leprae*-specific suppression could be abolished with a monoclonal antibody directed against DQw molecules (Li Shuguang et al., unpublished observations; T. Sasazuki and T. H. M. Ottenhoff, personal communication), suggesting DQ-restricted suppression.

Conclusions

1. Immune response (Ir-) genes are polymorphic genes coding for differences in immune reactivity between individuals.
2. HLA class I and II molecules are the products of Ir-genes.
3. The course of an infection with *M. leprae* and *M. tuberculosis* is controlled by HLA class II-linked genes and associated with certain HLA class II types.
4. HLA class II Ir-genes regulating the T cell response against *M. leprae* antigens may explain the data mentioned in 3.

5. Most probably similar conclusions may be drawn for tuberculosis.
6. The study of anti-mycobacterial T cell clones from patients and healthy contacts may offer important contributions to the search for the mechanisms responsible for and epitopes inducing protective immunity, immunopathology and suppression during the course of an infection with pathogenic mycobacteria.
7. The study of epitope specificity, preferential restriction (Ir-gene control) and function of anti-mycobacterial T cells might result in the development of more effective (sub-unit) vaccines against leprosy and tuberculosis.

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