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HLA class II restricted helper and suppressor clones reactive with *Mycobacterium leprae*

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Introduction

More than 10 years ago we decided to start a search for HLA-linked factors controlling the course of *M. leprae* infections (1). We choose an infectious disease because – in contrast to the diseases known to be associated with HLA at that time – there the etiological agent is known. We choose leprosy because of its remarkable spectrum of clinical symptoms paralelling the cell-mediated immune reactivity of the host to the bacillus (2). This choice appeared to be an extremely lucky one for two reasons. The first is that HLA molecules were shown to regulate antigen presentation to T cells (3), which apart from being important in leprosy appeared to be easy to clone in vitro (4). The second reason is that recently a remarkable progress has been made in the characterisation and synthesis of *M. leprae* antigens (5, 6). Thus we and others are now in a rather priviliged position to be able to study in detail the role of HLA products in the presentation of well defined medically relevant antigens. Such studies may contribute to the definition of both mechanisms and potential epitopes involved in protective immunity, immunopathology and suppression following an infection with *M. leprae*.

In this paper we will review our recent studies and present new data on restriction and antigen specificity of *M. leprae* reactive helper and suppressor clones.

M. LEPRAE REACTIVE HELPER T CELL (T_H) CLONES

M. leprae reactive helper T (T_h) cells are probably responsible both for acquired protective immunity to the bacillus and delayed type hypersensitivity which may result in immunopathology (2, 7). In order to obtain detailed information on the *M. leprae* epitopes recognized by T_H cells and the HLA restriction determinants involved we decided to clone *M. leprae* reactive T_H from the peripheral blood of leprosy patients.

One of the major technical obstacles in cloning T lymphocytes is the limited availability of autologous or HLA identical peripheral blood mononuclear cells, which are needed for restimulating established clones. This holds true especially for patients. Recently it was shown that Epstein-Barr virus transformed human B cell lines (EBV-BLCL) can process and present soluble antigens to T cells (8). We have shown that this is also the case for *M. leprae* antigens (9). Such EBV-BLCL constitute an endless, continuous and homogeneous source of antigen presenting cells (APC's), which not only made T cell cloning much more feasible but also enabled us to study in detail the HLA molecules and epitopes that are used to present *M.*

leprae antigens. The method we used to generate *M. leprae* reactive T_H clones has been described previously (10). In brief, we isolated peripheral blood mononuclear cells (PBMNC's), generated EBV-BLCL and restimulated the PBMNC with Dhamendra lepromin. T cell blasts were then cloned by limiting dilution on a feeder cell mixture consisting of irradiated autologous EBV-BLCL and PBMNC from random donors together with Dharmendra lepromin.

The *M. leprae* reactive clones thus obtained were of the $T3^+T4^+T8^-$ phenotype and strongly HLA-DR positive. Upon addition of Dharmendra- or armadillo-derived lepromin and autologous APC's they strongly proliferated (measured as incorporation of radiolabeled thymidine) and produced-γ-interferon (IFN-γ). Based upon their membrane markers, IFN-γ production, class II restricted proliferative responses to *M. leprae* antigens and the inability to suppress other *M. leprae* reactive T cells (vide infra) these T cell clones were defined as T_H cells.

None of the *M. leprae* reactive T_H clones reacted with unrelated antigens such as tetanus toxoid and candida. To assess whether these clones might recognize *M. leprae* specific determinants, 20 different mycobacterial preparations were presented to them (4). Four patterns of reactivity were observed: 1) *M. leprae* specific (roughly 25 % of the clones), 2) cross-reacting with one or two other mycobacteria, mainly *M. vaccae* and *M. lepraemurium* (another 25 %) 3) reactive with the majority of but not all mycobacteria tested (5-10 %), and 4) reactive with all mycobacterial preparations tested, sometimes with the exception of *M. nonchromogenicum* (nearly half the number of clones tested). Thus roughly 50 % of the T_H clones was absolutely or almost *M. leprae* specific.

In *Table 1* we have summarized the results of 14 T_H clones which were studied for reactivity against 3 of the 5 *M. leprae* proteins known to contain *M. leprae* specific epitopes seen by murine monoclonal antibodies (5).

Table 1. Helper T cell clones from patients are reactive with the known M. leprae proteins

Reactive with 65K and/or 36K:	n = 11
Reactive with only 12K:	n = 1
Not reactive with 65K, 36K, or 12K:	n = 2

It is clear that the majority of the clones recognize one or more of the proteins tested, and from results to be described below we infer that maybe all *M. leprae* reactive T_H clones might turn out to be reactive with one or more of the 5 known *M. leprae* proteins. Of course this does not imply that these T_H cells recognize the same epitopes as the mouse immunoglobulins. In fact we have evidence for the opposite: $T_H$cell responses cannot be blocked by the relevant monoclonal antibodies.

One of the major breakthroughs research has been the cloning and expression of *M. leprae* DNA followed by screening with the above mentioned monoclonal antibodies recognizing *M. leprae* specific epitopes (6). Thus basically unlimited quantities of well-defined *M. leprae* proteins and peptides will become available in the near future. Some of our T_H clones, obtained from patients were tested against *E. coli* lysates containing recombinant *M. leprae* proteins or at least peptides reactive with monoclonal antibodies against respectively 65K,

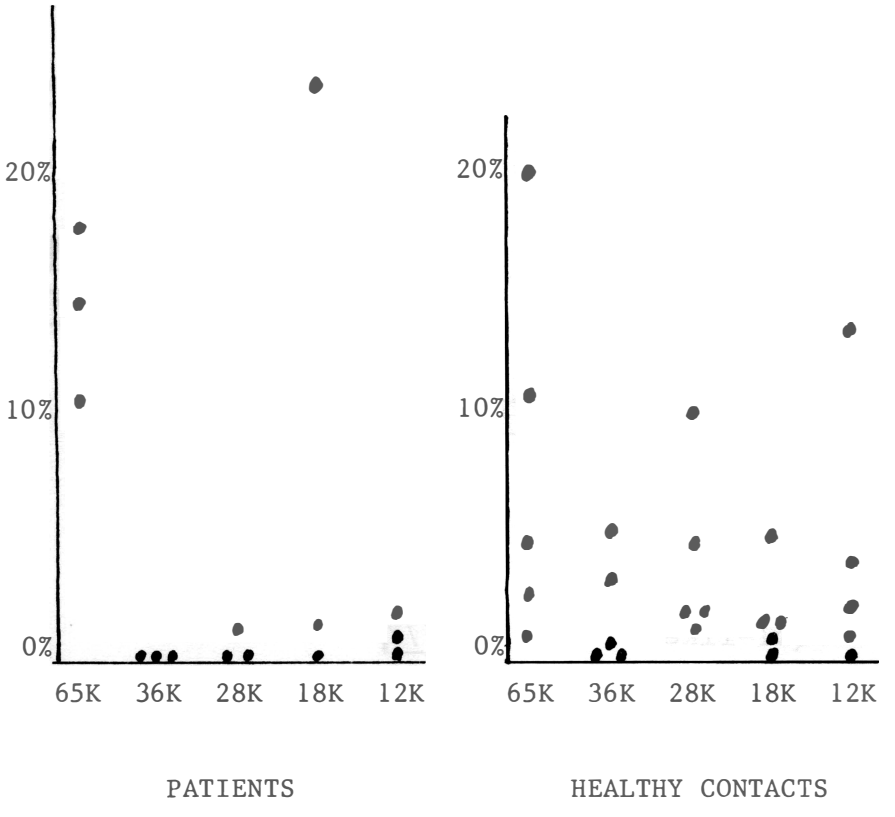

Figure 1: T cell reactivity with recombinant M. leprae proteins.

Expressed as percentage of Δ counts/min. to Dharmendra lepromin. 3 T_H cell lines from tuberculoid patients and 5 T_H cell lines from healthy contacts were tested with the *E. coli* lysates containing resp. 65K, 36K, 28K, 18K and 12K proteins (ref. 6).

36K, 28K, 18K and 12K *M. leprae* proteins. The first results were disappointing because few clones reacted, but on the other hand interesting because the positive reactions were only with the 65K containing lysate. We thought this might be of potential interest because Mustafa et al. using the same preparations had shown that $T_H$clones generated from healthy vaccinated subjects only reacted with the 18K containing lysate (11). This observation suggested that *M. leprae* reactive T_H cells from patients might recognize other epitopes than those from healthy contacts or successfully vaccinated subjects. In other words: this might provide a clue to the definition of epitopes providing protective immunity. In order to approach this question we decided to study bulk T_H cell lines from several individuals rather than T_H clones from one or at most a few individuals. The first results from T_H cell lines obtained from 3 different (tuberculoid) leprosy patients and 5 healthy contacts are shown in Fig. 1. Although clearly more data would be neccessary, these data already suggest several interesting conclusions. In the first place they do not give further support to the idea the 18K protein would contain epitopes of particular importance for protective immunity. Secondly, the 64K recombinant protein containing lysate seems to be the best in vitro stimulator of (activated) T_H

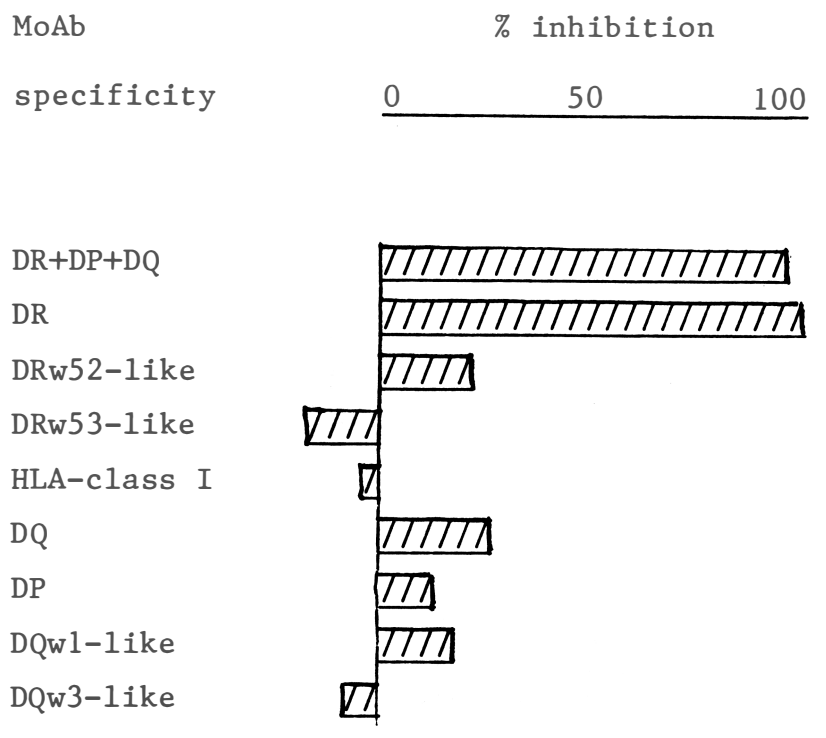

Figure 2: M. leprae antigens are presented to helper T cells by DR molecules.

Inhibition of M. leprae induced response in the presence of autologous APC. Results from a representative T_H clone restricted by the DR4/Dw13 haplotype are shown. The results are expressed as percentage inhibiton of the T_H response in the absence of monoclonal antibody (MoAb). Standard deviations did not exceed 10%.

cells. Whether this also implies that the 65K is the most immunogenic protein or contains the most important T_H epitopes is too early to conclude given the lack of characterisation of at least some of the other recombinant proteins as well as other (possibly suppressive) components of the lysates (see other contributions in this issue). Finally, T_H lines from patients tend to proliferate more consistently to the 65K lysate than similar lines from healthy contacts. This might suggest that patients do recognize different determinants compared with healthy contacts.

HLA class II molecules are neccessary for and regulate the presentation of antigens to T_H cells (12). The polymorphism of these HLA class II molecules may thus result in genetically controlled differences in T cell dependent immune responses. This might also be the case in leprosy: HLA class II linked genes are known to control the type of leprosy which develops upon infection (rather than the susceptibility to leprosy per se) as well as the in vivo cell mediated immune reactivity against *M. leprae* and related mycobacteria (13, 14). Since $T_H$cells are of main importance in determining the cellular immune reactivity to *M. leprae*, the polymorphism of HLA class II gene products might thus be responsible for differences in presentation of *M. leprae* antigens to T_H cells and thus control leprosy type.

In order to study which HLA molecules and epitopes are important in the presentation of *M. leprae* antigens to T_H cells, we have performed extensive panel and inhibition studies with fully HLA class II typed allogeneic antigen presenting cells and well defined HLA class II specific monoclonal antibodies (manuscript submitted for publication). The results of the blocking studies show in the first place that by far the majority (34 out of 36 T_H clones) of restriction determinants (RD's) for *M. leprae* reactive T_H clones are situated on DR (and not DP or DQ) molecules *(fig. 2)*. These data obtained for clones from three leprosy patients were confirmed in studies with plyclonal T_H lines obtained from 22 patients. Thus HLA-DR molecules seem to play a major role in the presentation of *M. leprae* antigens to T_H cells. Since the expression of DR molecules usually is much stronger than that of DP and DQ molecules, this observation suggests that the capacity to present *M. leprae* (and probably other antigens) correlates with the quantitative expression of the different HLA class II molecules. This rule was confirmed, when we studied in more detail the restriction molecules on DR4 positive cells, which express two types of DR molecules called $\alpha\beta_1$ and $\alpha\beta_3$. The $\alpha\beta_1$ molecules carry the DR4 and Dw specificities and the $\alpha\beta_3$ molecules a supertypic determinant (DRw53) recognized by a monoclonal antibody. As shown in fig. 2 blocking studies mapped the RD's only on the $\alpha\beta_1$ (DRw53 negative) molecules, which are known to be highest in expression.

In order to study the fine specificity of the RD's for *M. leprae* antigens we performed large panel studies using selected allogeneic fully HLA class II typed APC's to present *M. leprae* antigens. A remarkable heterogeneity of the capacity to present *M. leprae* antigens was observed. For instance six different *M. leprae* reactive T_H clones restricted via epitopes on the DR4-Dw13 containing DR$\alpha\beta_1$ molecule showed 5 different patterns of responsiveness to the allogeneic APC's and *M. leprae*. Two patterns correlated with the DR4 and Dw13 allospecificity, whereas the other three apparently correlated with different Dw13 related epitopes. Because recently several DRβ_1 alleles of the specificities studies by us have been sequenced, we were in a unique position to analyse the relation between structure and function of HLA class II molecules. The DR$\alpha\beta_1$ chains of Dw4, 13 and 14 positive individuals seem to differ for only 1-3 different nucleotides, which are likely to be situated on the outer face of the first domain of the DRβ_1 molecule. Thus, like allo-reactive T_H clones (15) our *M. leprae* reactive T_H clones can distinguish between 1 till 3 amino acids difference between DR$\alpha\beta_1$ molecules. Moreover, they can apparently distinguish between the combination of as far as we know identical DR$\alpha\beta_1$ molecules and probably different *M. leprae* proteins or peptides. This leads to two important conclusions, at least one of which has practical implications. The first is that differences in processing of *M. leprae* (whatever that means) may contribute to the resulting T_H repertoire. The second is that at least the DR4-Dw13 positive HLA DRβ_1 molecule contains a large number of potential restriction determinants, and that most probably conformational changes of class II molecules contribute to the expression of them.

Finally, we have obtained preliminary evidence that allelic differences between DR molecules are correlated with differences in antigen specificity of DR-restricted T_H clones: DR3-restricted T_H clones from one tuberculoid leprosy patient recognized significantly more often than DR2-restricted T_H clones *M. leprae* specific determinants. Note that DR3 is associated with tuberculoid leprosy in the population from which this patient originates. Of course such data will have to be confirmed in larger material and in different individuals. They may serve however as an example to show how the study of HLA class II Ir genes and their possible expression as restriction elements for antigen presentation to T_H cells may contribute to define potential epitopes involved in protective immunity and immunopathology following infection with *M. leprae*.

M. LEPRAE REACTIVE SUPPRESSOR T CELL (T_S) CLONES

Whereas several important mysteries in cellular immunology, like the T cell receptor, have been solved during recent years, this does not seem to be the case for suppressor T cells. Therefore several immunologists assume that they do not exist in order to keep their schemes simple. We also like simple schemes, but we have recently cloned T cells from a borderline lepromatous leprosy patient which specifically suppress but do not kill autologous T_H cells reactive with mycobacteria (16). This leaves us with two possibilities: either we have to show that we are dealing with an in vitro artefact or we have to show that such cells are important and – complicated or not – how they have to be fitted into at least the immunology of leprosy. At this stage we still favor the latter possibility.

Several investigators have implicated $T_S$cells in the pathogenesis of the *M. leprae* specific T cell unresponsiveness observed in lepromatous leprosy patients. However, studies on this subject have been notoriously difficult to interpret for at least two reasons: in the first place the assays used to measure suppression were antigen non-specific or at least indirect and secondly heterogeneous cell population were used. Two recent reports (16, 18) may have the solution for both problems.

We have used the following approach: we observed that in contrast to T cell lines from tuberculoid leprosy patients activated by *M. leprae* and propagated in IL-2, similar lines of (borderline) lepromatous leprosy consistantly failed to show a proliferative response against *M. leprae* antigens presented by autologous or allogeneic HLA class II matched APC. This lack of proliferation by thus cultured T cells was even observed when the PBMNC of such borderline lepromatous patients did proliferate to *M. leprae*. We selected one such a borderline patient arguing that this might enable us to study both T_H and possible $T_S$responses towards *M. leprae*. To our intense pleasure it appeared that the *M. leprae* non-responsive T cell line specifically suppressed the *M. leprae* response of autologous PBMNC's. Cloned T cells derived from this T cell line did not proliferate with *M. leprae* in the presence of APC but did suppress the response to *M. leprae* and other mycobacteria but not unrelated antigens. We could also generate T_H clones from the same patient, some of which were also suppressed by the T_S clones. All T_S clones were T3 and most were positive for a T cell receptor framework determinant defined by monoclonal antibody WT31.

At present we do not know the mechanism of the specific suppression we observed. We do know that the T_S clones are radiation sensitive. Trivial explanations like IL-2 consumption are ruled out by the antigen specificity, which is even observed when PBMNC are stimulated simultaneously with *M. leprae* and unrelated antigen. Also in such experiments only the *M. leprae* response is suppressed, indicating specificity at least in the effector phase. As shown in *fig. 3,* we have also excluded cytotoxicity of either APC or responding T cells.

An important question is whether the $T_S$are activated by *M. leprae* antigens or by idiotypes of T_H cells. If specialised epitopes (so-called suppressor epitopes) would be responsible for activating T_S cells and not T_H cells, suitable skin test and vaccine preparations might be developed which would contain only helper and no suppressor epitopes (18). On the other hand, if the suppression would be anti-idiotypic such a simple approach will probably not be useful (19). Thus far we have not obtained evidence for activation of T_S clones by (idiotypes of) T_H clones. On the other hand we did obtain evidence that the T_S clones are activated by *M. leprae* antigens presented by APC in an HLA class II restricted fashion. This activation is blocked by anti-T3 antibodies and (in most cases) by the anti-T cell receptor antibody WT31.

So, if the T_S clones described by us are relevant for the in vitro obserbed *M. leprae* specific suppression, we may now start to look for suppressor determinants on *M. leprae* antigens. The easiest situation would be that suppression is induced by non-protein determi-

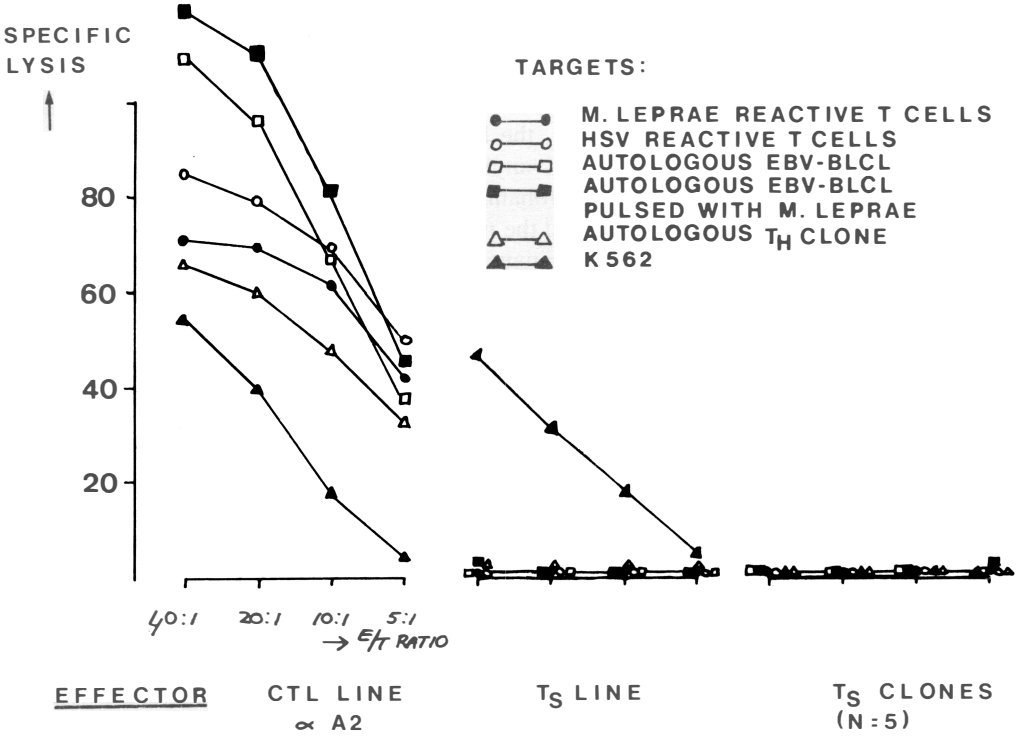

Figure 3: T_S clones are not cytotoxic.

E/T ratio = effector/target ratio.

nants, like the suppression described for *M. leprae* specific phenolic glycolipid (20). In contrast, we did obtain preliminary evidence that suppressor determinants are present on *M. leprae* proteins, e.g. the 36K protein. Studies are now in progress which will answer the question whether helper and suppressor determinants on one *M. leprae* molecule may be dissociated or not.

As mentioned before, we obtained evidence that activation of T_S clones is restricted by determinants on HLA class II molecules. Mixing experiments of allogeneic T_H and $T_S$clones yielded the same conclusion and moreover suggested that additional constraints apart from HLA class II restriction determinants are placed on suppression to occur. I hear Avrian Mitchison ask now: are your suppressor cells restricted by DQ? My tentative answer to this question is that at least some seem to be restricted by DR rather than DQ.

Of course most of the studies presented in this paper are far from final and a lot of work still needs to be done. We are sure that the clever study of *M. leprae* reactive T_H and T_S clones and lines from both patients and healthy contacts may offer important contributions to the search for mechanisms responsible for and epitopes involved in protective immunity, immunopathology and suppression during the course of an infection with *M. leprae*. We realise that for most of these studies the HLA restriction and the existence of Ir (and Is?) genes is only a nuisance. However, the study of HLA class II Ir and Is genes in this increasingly well defined system may – apart from being fun for a few immunogeneticists – also offer leads in the search aimed at better treatment and prevention of leprosy.

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