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Low T lymphocyte responsiveness to *Mycobacterium leprae* antigens in association with HLA-DR3

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SUMMARY

The type of leprosy which develops after infection with *Mycobacterium leprae* is influenced by the presence or absence of HLA-DR3, as has been demonstrated in an ethnic group originating from Surinam. In the present study we investigated in this same ethnic group the role of HLA-DR, and of HLA-DR3 in particular, in monocyte–T cell interactions during leprosy specific proliferative responses *in vitro*. HLA-DR3 heterozygous T cells from tuberculoid leprosy patients were cultured with antigen and either HLA-DR3 positive or HLA-DR3 negative homozygous HLA-DR compatible allogeneic monocytes as antigen presenting cells (APCs). T cell proliferative responses with DR3 homozygous monocytes as APCs, were observed to be decreased as compared to T cell proliferative responses with DR3 negative homozygous monocytes as APCs. Furthermore, although the leprosy specific monocyte–T cell interactions were shown to be restricted for HLA-DR, in the anti-MLW-1 response HLA-DR3 appeared to function as a restricting element poorly or not at all. These observations may imply, that an *in vitro* correlate has been found for an (HLA associated) genetic control of leprosy *in vivo*.

Keywords *Mycobacterium leprae* T cell proliferation HLA

INTRODUCTION

Since the discovery of the major histocompatibility complex (MHC) as a highly polymorphic and ubiquitous immunogenetic system, the central question has been: what is the biological function of the products encoded by the system? In man, an answer to this question has been sought by studying the role of the products of the HLA system in various diseases. Especially the notion that these products could well play a crucial role in cellular interactions in the immune response, has stimulated the search for relationships between HLA antigens and diseases that have an immunological component.

One of the diseases studied to this end is leprosy, an infectious disease with a varying clinical picture. Both by segregation studies in families and by association studies a role for HLA antigens has been shown in leprosy, particularly with regard to the clinical type of the disease that develops upon infection. (de Vries, van Eden & van Rood, 1981; Sergeantson, 1982).

Recently we have carried out an HLA association study among an ethnic group originating from Surinam who migrated to the Netherlands during the past 10–15 years. In this ethnic group a role for HLA-DR3 or an HLA-DR3 associated factor in determining the type of leprosy was shown

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(van Eden *et al.*, 1982). Whereas HLA-DR3 was found to be nearly absent among patients with lepromatous leprosy, the frequency of HLA-DR3 was found to be increased among patients with polar tuberculoid leprosy as compared to healthy controls. Since especially the cellular immune response determines the type of leprosy in an individual patient (varying from a specific non-responsiveness in lepromatous leprosy to specific responsiveness in tuberculoid leprosy; Godal, 1978), these data suggested that HLA-DR3 itself or an associated factor influences the leprosy specific cellular immune response.

The present study was undertaken to investigate the role of HLA-DR, and HLA-DR3 in particular, in the leprosy specific interaction between antigen presenting cells and immune T lymphocytes *in vitro*.

Various *in vitro* studies have already shown that T lymphocytes recognize foreign antigens presented on the cell membrane in conjunction with MHC determinants (Rosenthal & Shevach, 1973). This phenomenon of so called MHC restriction has also been demonstrated for HLA determinants in man. By measuring proliferative responses of allogeneic mixtures of antigen presenting cells (APCs) and immune T cells following the addition of specific antigens *in vitro*, it was observed that the degree of antigen-induced proliferation was dependent on the presence of HLA-DR specificities shared between the antigen presenting cell and the T cell (Thorsby, Berle & Nousiainen, 1982). In other words, the primed T cell has a double specificity, firstly for the specific antigen and secondly for one of the self-HLA-D(R) specificities. The exact mechanisms responsible for this double specificity of the T cell for foreign and self determinants are not known at present. Nevertheless, this phenomenon of MHC restriction has important implications. Mainly from experiments carried out in the mouse, it has become apparent that the same molecules involved in the phenomenon of MHC restriction are imposing the Ir gene phenotype and that both phenomena reflect an identical biological process (Benacerraf, 1980). Therefore, it seems likely that also in man restriction specificities of T cells may influence the functional capacities of T cells in the immune response. Similarly, the HLA-DR3 associated control in leprosy, may arise from the role of HLA-DR3 (or a closely linked gene product) as a restricting molecule in leprosy specific cellular interactions.

In a previous paper we have reported an experimental setting, which enabled us to study successfully the HLA-DR restriction of the interactions between allogeneic combinations of monocytes and T cells in PPD (tuberculin) specific proliferative responses *in vitro* (van Eden *et al.*, 1983a). In the present study the same experimental setting was used to investigate the leprosy specific proliferative responses of T cells obtained from Surinam patients with tuberculoid leprosy, being heterozygous for HLA-DR and carrying HLA-DR3. Comparing the responses obtained with DR3 homozygous monocytes and the responses obtained with DR-non-3 homozygous DR compatible monocytes as APCs, it was observed, that the proliferative responses with HLA-DR3 homozygous monocytes were of a lower level than those with DR-non-3 homozygous monocytes. Furthermore, it was demonstrated for the first time that leprosy specific proliferative responses are HLA-DR restricted. The observations made with regard to the particular role of HLA-DR3 in the HLA-DR restricted leprosy specific proliferative responses, indicate that an *in vitro* correlate has been found for an *in vivo* operative, HLA associated, genetic control in leprosy.

MATERIALS AND METHODS

Donors. T lymphocytes were obtained from 15 patients. The patients were selected on the following criteria. (1) They had to belong to the ethnic group, originating from Surinam, in which it had been shown that HLA-DR3 controlled the type of leprosy (van Eden *et al.*, 1982). (2) They had to suffer from tuberculoid leprosy. (3) They had to be heterozygous for HLA-DR and positive for HLA-DR3. The HLA-DR typings of the patients selected were as follows: eight patients were DR3/7, two patients were DR3/2 and the other patients were DR3/1, 3/4, 3/5, 3/w6 and 3/w9.

Monocytes were obtained from healthy caucasoid donors. Twenty-two donors were homozygous for HLA-DR. The majority of these donors were the offspring of first cousin marriages. For each HLA-DR specificity present among the T cell donors, HLA-DR homozygous monocytes were available as APCs. Twenty-four additional donors were heterozygous for HLA-DR. These donors

were either laboratory staff members, or volunteers undergoing plasmapheresis for the collection of HLA typing sera.

T lymphocyte isolation. Mononuclear cells were obtained from 80–100 ml heparinized peripheral blood samples by gradient centrifugation on Percoll (Pharmacia, Uppsala; sp. gr. 1·077 g/ml) and subsequently depleted for monocytes (see below). The remaining cell fraction was rosetted with AET (2-aminoethylisothiouronium) treated sheep red blood cells, layered on Percoll (specific gravity 1·077 g/ml) and spun for 25 min at 1300*g*, 4°C. the pellet was treated with ammonium chloride buffer, pH 7·4, to haemolyse the sheep red blood cells and washed several times. This T cell enriched cell population contained less than 1% esterase staining cells and did not show proliferative responses to antigens without the addition of monocytes. The T cells tested were never cryopreserved before use.

Monocyte isolation. Monocytes were isolated from heparinized buffy coats, obtained by manual lymphopheresis. The isolation and freezing procedure for monocytes was adopted from de Boer *et al.* (1981) with some minor modifications and has been described previously (van Eden *et al.*, 1983a). In short: mononuclear cells, obtained by centrifugation on Percoll (sp. gr. 1·077 g/ml), were depleted for platelets by low speed centrifugation in wash medium and removal of the supernatant several times. After subsequent incubation of the remaining cell fraction in 20 ml 25 mM Tris-HCL buffer, supplemented with 5% (vol./vol.) inactivated human serum, at 37°C, 10^7 cells were resuspended in 2 ml ice cold Percoll solution with a sp. gr. of 1·063 g/ml. After centrifugation for 10 min, 1500*g* at 4°C, monocytes are collected from the interphase. This monocyte enriched cell fraction was found to contain more than 90% esterase staining cells. After isolation, the monocytes were either freshly used in the experiments (the autologous monocytes) or frozen with DMSO (dimethylsulphoxide) 10% (vol./vol.) and stored in liquid nitrogen until use. Before use in the experiments the monocytes were thawed and incubated in RPMI medium containing 20% (vol./vol.) inactivated human AB serum during 30 min to recover. After this, viability, as checked by eosine dye exclusion, was generally more than 80%. Functional tests showed that, also after cryopreservation, more than 97% of the monocytes were able to phagocytose and that they had a killing efficiency of 90% within 2 h (van Furth, van Zwet & Leijh, 1978). Before use the monocyte fraction was irradiated with 2000 rad from a ^{137}Cs source.

Cell culture technique. Cultures were carried out in flat bottomed 96 well microtitre plates (Greiner, W. Germany). To a volume of 0·1 ml of antigen solution in plain culture medium or plain medium *per se* as negative control, 0·05 ml of a suspension of 2,000 rad irradiated monocytes (2×10^5/ml) and 0·05 ml of a suspension of T cells (2×10^6/ml) were added. The cell suspensions contained 20% (vol./vol.) human, pooled, inactivated AB serum in the medium, thus providing a final serum concentration in the culture of 10% (vol./vol.). The culture medium was freshly prepared Iscoves Modified Dulbecco's medium, supplemented with 100 u/ml penicillin and 100 μg/ml streptomycin. All combinations of cells and antigen in several concentrations were set up in triplicate and incubated for 6 days at 37°C in a humidified 5% CO_2 environment. Eighteen hours before terminating, 2 μCi of ^{3}H-thymidine (sp. act. 5 Ci/mmol) (Radiochemical Centre, Amersham, England) was added. Samples were harvested on glass fibre filters using a semi-automatic device. ^{3}H-thymidine incorporation was assessed by liquid scintillation counting.

HLA-DR typing. Eighty platelet absorbed sera were used in the two-colour fluorescence test for the typing or DR specificities on B cells (van Leeuwen & van Rood, 1980).

Antigens. Two *Mycobacterium leprae* derived antigenic preparations were used: one preparation, lepromin, consisted mainly of whole bacilli, extracted from human lepromas according to the method of Dharmendra (1942). This preparation was kindly provided by Dr M. Abe (National Institute of Leprosy Research, Tokyo, Japan). The lepromin was tested in final concentrations, equivalent to 5×10^4, 1×10^5, 5×10^5 and 1×10^6 bacteria per ml. The second preparation, MLW-1 (*M. leprae* cell wall antigen 1) was purified from armadillo grown *M. leprae* (Closs *et al.*, 1982). The cellular immune response *in vitro* against the MLW-1 antigen was shown to correlate remarkably well with leprosy or exposure to leprosy (Closs *et al.*, 1982). MLW-1 was tested in concentrations of 0·03, 0·10, 0·30, 1·0 μg/ml. As a control antigen, purified protein derivative (PPD) of tuberculin (Statens Serum Institute, Copenhagen, Denmark) was used in concentrations of 0·50, 1·67, 5·00 and 16·70 μg/ml.

Analysis of results. Analysis of the antigen specific response of allogeneic mixtures of cells is complicated, since the alloreactive response coincides with the antigen specific response. In a number of reports correction factors were introduced to correct for the allogeneically induced part of the response (Bergholtz & Thorsby, 1977; Hansen *et al.*, 1978; Bright & Munro, 1981). However, these correction factors have only been partly satisfactory and could give conflicting results if applied to the same set of data (Bright & Munro, 1981). To obviate the need for a correction factor for the alloreactivity, we calculated a summary measure of the antigen-dependent part of the response by a regression analysis of the response on dose of antigen added. At each antigen dose, the mean response (counts per minute) of the triplicate was taken. For these mean values, the slope of the regression equation was determined. For standardization purposes, relative slopes were calculated, denoted RRC (relative regression coefficient), by taking the ratio of the slope of a specific cell combination to the slope of the response of the T cells with their autologous monocytes. Differences in the distributions of the RRCs were evaluated by Wilcoxon's two sample test. Differences in the distributions of the RRCs for individual T cells between the combinations with HLA-DR3 and HLA-DR-non-3 homozygous monocytes were evaluated by Wilcoxon's test for paired samples.

RESULTS

T cells obtained from 15 patients suffering from polar tuberculoid leprosy were investigated for their proliferative response against Dharmendra lepromin, MLW-1 and PPD. The patients were selected for being HLA-DR heterozygous and carrying HLA-DR3.

Monocytes of seven different donors, who were homozygous for HLA-DR3 were available. A varying random selection of two to four out of these seven monocyte donors were used, as APCs, in each experiment. In addition, monocytes collected from one to four donors being homozygous for the other non-DR3 DR specificity, present in the T cell donor, were used for antigen presentation in the same experiments.

With the addition of autologous monocytes, all patient T cells showed responses against Dharmendra lepromin and MLW-1, whereas for 11 patients also T cell responses were obtained against PPD. No antigen-dependent proliferation of the T cells was observed, without the addition of monocytes. The relative regression coefficients of the responses obtained in such cultures were as a rule less than 0·01.

The T cell proliferative responses of two individuals upon stimulation with the MLW-1 antigen in one single experiment are shown in Table 1. The mean ^{3}H-thymidine incorporation of the triplicates in ct/min $\pm$ s.e. are shown for different antigen concentrations and monocyte T cell combinations tested. In addition both the RCs (slopes) and the RRCs (relative slopes) are indicated. For both individuals the responses obtained with DR3 homozygous monocytes as APCs were of relatively low levels.

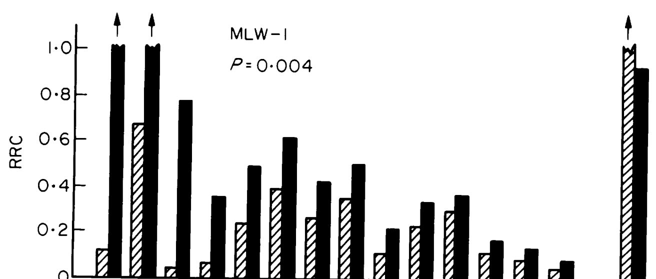

Fig. 1. RRCs of responses of T cells obtained from 15 DR3 heterozygous individual T cell donors, co-cultured with either DR3 ▨ or DR-non-3 (■) homozygous allogeneic DR compatible monocytes as APCs for MLW-1 antigen. *P*-values were calculated from Wilcoxons test for paired samples (two sided).

Table 1. T cell proliferative responses of two individual T cell donors against MLW-1 antigen, presented by autologous and allogeneic monocytes.

Donor	MLW-1 antigen	T cells alone	T cells + monocytes autologous: DR2/3	DR2	T cells + monocytes allogeneic			
					DR2/2	DR3/3	DR3/3	DR7/7
1	0·03 μg/ml	2,015 ± 329	95,545 ± 1,085	2,271 ± 634	1,346 ± 99	2461 ± 296	5,583 ± 1,714	6,433 ± 414
	0·10 μg/ml	983 ± 296	129,057 ± 2,176	6,901 ± 2,272	2,341 ± 500	4,081 ± 1,341	9,948 ± 4,658	3,820 ± 469
	0·30 μg/ml	1,305 ± 413	143,171 ± 2,470	13,823 ± 2,597	8,866 ± 724	3,205 ± 1,072	13,270 ± 931	8,481 ± 4,002
	1·00 μg/ml	530 ± 164	124,810 ± 18,352	30,410 ± 3,218	16,580 ± 6,017	5,213 ± 1,345	8,790 ± 4,452	11,630 ± 4,776
	no antigen	2,427 ± 767	4,486 ± 304	451 ± 3	997 ± 461	956 ± 317	2,426 ± 1,025	3,070 ± 511
	RC	−1,105	12,608	27,511	15,430	2,218	838	6,603
	RRC	−0·08	1	2·18	1·22	0·17	0·07	0·52
2	0·03 μg/ml	1,998 ± 541	60,874 ± 211	2,706 ± 682	1,266 ± 360	1,318 ± 397	5,678 ± 3,294	17,533 ± 6,485
	0·10 μg/ml	735 ± 374	92,522 ± 544	5,191 ± 3,000	1,180 ± 401	1,750 ± 510	1,750 ± 400	35,003 ± 4,323
	0·30 μg/ml	2,235 ± 530	118,856 ± 7,687	6,590 ± 1,491	3,190 ± 930	2,335 ± 148	3,375 ± 330	56,988 ± 5,690
	1·00 μg/ml	538 ± 229	114,386 ± 5,378	9,603 ± 1,129	1,891 ± 825	2,561 ± 500	4,658 ± 1,812	55,606 ± 6,868
	no antigen	988 ± 471	868 ± 169	3,871 ± 2,078	783 ± 74	655 ± 109	120 ± 24	1,128 ± 84
	RC	−1,064	38,831	6,050	530	1,187	828	30,007
	RRC	−0·03	1	0·16	0·01	0·03	0·02	0·77

Data are presented as mean ct/min of triplicate cultures ± s.e. In addition (Relative) regression coefficients (slopes) of response on antigen dose are shown, (R)RC.

Such antigen-dependent T cell proliferative responses of the HLA-DR3 and the HLA-DR-non-3 sharing monocyte–T cell combinations were analysed for each individual T cell donor and for the three antigens tested separately. With only one exception, for all individual T cell donors the mean MLW-1 responses as measured by RRCs obtained with DR3 homozygous monocytes as APCs were of a lower level than the mean responses obtained with DR-non-3 homozygous monocytes as APCs. The mean RRCs for each individual T cell donor for MLW-1 responses

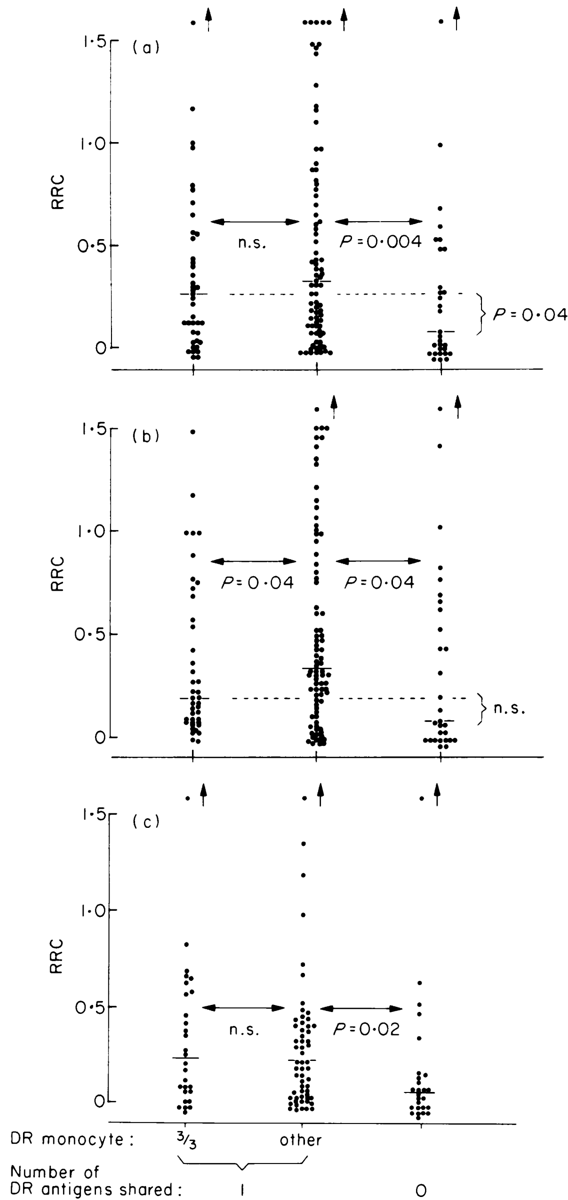

Fig. 2. RRCs of monocyte–T cell combinations, grouped into 1 DR sharing and 0 DR sharing cell combinations, tested for responses to Dharmendra lepromin (a) and MLW-1 (b) and PPD (c). The 1 DR sharing group was subdivided into cell combinations with DR-3 homozygous monocytes and others. Median values were indicated. *P* values were calculated from Wilcoxons two sample test (one sided).

obtained with either DR3 homozygous monocytes or with DR-non-3 homozygous monocytes are shown in Fig. 1. As evaluated by a Wilcoxon test for paired samples the difference between the DR3 and DR non-3 sharing combinations was significant ($P = 0{\cdot}004$). T cells from all but four patients showed lower responses upon stimulation with Dharmendra lepromin, when cultured in the combination with HLA-DR3 sharing monocytes than in the combination with HLA-DR-non-3 sharing monocytes ($P = 0{\cdot}04$). For cultures stimulated with PPD, no such systematic differences between responses obtained with HLA-DR3 homozygous monocytes and with HLA-DR-non-3 homozygous monocytes were observed.

In addition to HLA-DR homozygous monocytes also HLA-DR heterozygous monocytes were tested as APCs in the majority of experiments. However, no systematic differences in response levels were observed, between the cultures containing heterozygous monocytes sharing HLA-DR3 and monocytes sharing HLA-DR-non-3 with the T cell (data not shown).

To analyse the T cell responses for HLA-DR restriction, the 151 distinct allogeneic monocyte–T cell combinations tested, were grouped into 1 DR and 0 DR sharing cells combinations. Due to the protocol followed no 2 DR sharing cell combinations were tested. The RRCs of the responses are shown in Fig. 2. The 1 DR sharing group was subdivided into cell combinations with HLA-DR3 homozygous monocytes as APCS (1) and others (2). The latter cell combinations (2 = others) were combinations with homozygous monocytes sharing DR non-3 with the T cells and combinations with heterozygous monocytes sharing either DR3 or DR-non-3 with the T cells. These different combinations were pooled, since no differences between the distinct subgroups were observed.

For Dharmendra lepromin (Fig. 2a), the RRCs of the responses obtained for the 0 DR sharing cell combinations were less than those obtained for both subgroups of 1 DR sharing cell combinations ($P = 0{\cdot}04$ (1) and 0·004 (2), respectively). The RRCs of the responses obtained for the two subgroups within the 1 DR sharing group were of identical levels.

For MLW-1 (Fig. 2b), the RRCs of the 1 DR sharing cell combinations in which the APCs were DR 'other', were observed to be higher than those for the 1 DR sharing combinations with DR3 homozygous monocytes (1) ($P = 0{\cdot}05$) and the 0 DR sharing combinations ($P = 0{\cdot}04$). Moreover, the RRCs in the latter two groups were observed not to differ significantly.

For PPD (Fig. 2c), a pattern similar to that obtained for Dharmendra lepromin was observed. The RRCs for 0 DR sharing cell combinations were less than those for both subgroups of 1 DR sharing cell combinations ($P = 0{\cdot}01$ (1) and 0·02 (2), respectively). The RRCs of the responses obtained for both 1 DR sharing subgroups were of identical levels.

DISCUSSION

We investigated the co-operation between T cells obtained from tuberculoid leprosy patients, being HLA-DR3/DR-non-3 heterozygous, and allogeneic monocytes as APCs in leprosy specific proliferative T cell responses. Tuberculoid leprosy patients have been demonstrated to respond *in vitro* to leprosy antigens when unseparated mononuclear cell populations are tested (Godal, 1978). In the present study it was observed that the proliferative T cell responses directed to leprosy antigens presented by HLA-DR3 homozygous monocytes were of a lower level than the responses directed to leprosy antigens presented by HLA-DR-non-3 homozygous monocytes. Although four T cell donors were found not to be responsive to the non-leprosy antigen PPD we were able to test a sufficient number of cell combinations to suppose that such a DR3-dependent differential responsiveness did not exist with regard to PPD.

By analysing the response results of all cell combinations tested, including the cell combinations with HLA-DR heterozygous monocytes as APCs, the HLA-DR restriction of the monocyte–T cell interactions in the leprosy specific proliferative responses was investigated. Both for Dharmendra lepromin and for the MLW-1 antigen, the RRCs of the responses obtained from cell combinations not sharing any HLA-DR determinant were found to be less than the RRCs of responses obtained from cell combinations sharing one HLA-DR determinant. This observation indicates that the interaction between APCs and immune T cells in the leprosy specific response was HLA-DR restricted. However, the responses channeled through HLA-DR3, as measured in cell combinations

with HLA-DR3 homozygous monocytes as APCs, were found to be almost of the same low level as the responses in the 0 DR sharing combinations, when MLW-1 was used as a stimulant.

From these data it is concluded that HLA-DR3 is either a poor restricting element in leprosy specific monocyte–T cell interactions or no restricting element at all.

The presence of DR3 restricted responsiveness for Dharmendra lepromin, and not for MLW-1 (Fig. 2a & b) is certainly not incompatible with our hypothesis that the DR3 associated defect is true leprosy specific. The Dharmendra lepromin, is known to contain a variety of different antigenic determinants, many of them being cross-reactive with determinants on other mycobacteria, whereas in the MLW-1 antigen the number of cross-reactive determinants is relatively small. Therefore, the DR3 restricted response directed against Dharmendra lepromin may have represented responses directed against the cross-reactive determinants. The low level of DR3 restricted leprosy specific responses was not observed when leprosy antigens were presented to the T cell by HLA-DR heterozygous monocytes, sharing only one DR antigen with the T cell. The explanation for this could be that, due to the greater complexity of the repertoire of class II products in the DR heterozygous antigen presenting cell, the DR3 associated defect in DR restricted recognition is by-passed somehow. Alternatively, it could be that the induction of poor responsiveness is recessive on the level of the APC. This seems to be in contrast to the low PPD specific response in MB1 sharing combinations (van Eden *et al.*, 1983b).

In a previous paper (van Eden, Elferink & de Vries, 1983a) we have presented some, although weak, evidence for the *in vitro* expression of an HLA-DR2 associated factor which had been shown to predispose to tuberculoid leprosy. The data suggested the presence of a higher proliferative T cell responsiveness to Dharmendra lepromin among the healthy individuals carrying HLA-DR2, than among the healthy individuals lacking HLA-DR2. Unfortunately, the MLW-1 antigen was not tested in that study. This fact, in addition to the fact that, in contrast to the present study, the phenomenon was observed among healthy individuals, hampers the proper comparison of the present data with the previous data.

In another paper (van Eden *et al.*, 1983b), we have reported on the relationship between the presence of HLA-DR3 and patterns of *in vivo* skin test responses towards mycobacterial antigens among British volunteers. From the reported high frequency of HLA-DR3 among individuals responding to all mycobacterial species tested—individuals who are supposed to recognize mycobacterial 'common antigens'—one could speculate that the mycobacterial antigens recognized in association with HLA-DR3 were preferentially the common antigens. If this was the case, the present *in vitro* data could reflect the fact that the MLW-1 preparation in particular, does contain very few mycobacterial common determinants and that the antigenic determinants present in this preparation are therefore poorly recognized by the T cell in conjunction with HLA-DR3. As explained before, this would be applicable to Dharmendra lepromin to a lesser extent. Since the skin test data were collected from individuals belonging to a population different from the population studied in the present study, we should be cautious in making such generalizations, however.

It is tempting to speculate on the mechanisms by which the presently described DR3 related defect in leprosy specific responsiveness could contribute to the development of tuberculoid leprosy *in vivo*. In this respect it is quite remarkable that the tuberculoid leprosy associated marker, DR3, seems to control low responsiveness rather than high responsiveness, whereas tuberculoid leprosy is the relative high responder type of the disease. At this moment, however, we have no good explanation for this. Therefore, our conclusion for the time being should be broad, namely that the presence of HLA-DR3 seems to modify leprosy specific responses *in vitro* and that the present data indicate an *in vitro* correlate of immune mechanisms operative in and obviously relevant to the *in vivo* situation. However, in order to approach the exact mechanisms underlying the predisposition to tuberculoid leprosy, we feel that the nature of the mechanisms underlying the observed *in vitro* phenomena should first be investigated more extensively. To this end attention should be paid to the precise determination of the *M. leprae* derived antigenic determinants that are recognized by the T cell, preferably by studying the fine specificity of T cell lines or clones. Furthermore, the functional properties of responding T cells should be investigated and finally the process of antigen presentation in relation to HLA determinants should be studied, preferably in antigen presenting cells obtained from leprosy patients.

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