Immunology 1991 **74** 1–7

Analysis of human T-cell epitopes in the 19,000 MW antigen of *Mycobacterium tuberculosis*: influence of HLA-DR

A. FAITH,*† C. MORENO,* R. LATHIGRA,* E. ROMAN,* M. FERNANDEZ,* S. BRETT,*‡
D. M. MITCHELL,† J. IVANYI* & A. D. M. REES*
**MRC Tuberculosis and Related Infections Unit, Hammersmith Hospital, and*
†*Department of Medicine, St Mary's Hospital Medical School, Paddington, London*

Accepted for publication 2 May 1991

SUMMARY

The potential number of T-cell epitopes in the 19,000 molecular weight (MW) antigen has been investigated using overlapping peptides which comprise the complete sequence. Sixteen potential epitopes could be deduced from the responses to these peptides by polyclonal T cells derived from 22 antigen-responsive donors. The majority of epitopes were not predicted by either of the major paradigms, the Rothbard motif and the amphipathic helix. A hierarchy of epitopes was indicated by the responses, which ranged from strong and frequent in the N-terminal region, to moderate or weak elsewhere. Some epitopes were restricted by single HLA-DR determinants, or families of determinants sharing structural features in common, whilst the two N-terminal peptides were recognized by donors with a diversity of DR types. The high degree of T-cell recognition of the N-terminal region may be of relevance to the design of a sub-unit vaccine capable of priming T cells against *Mycobacterium tuberculosis*.

INTRODUCTION

The identification of T-cell epitopes which are associated with protective immunity is important to the design of sub-unit vaccines. This is particularly true for diseases such as tuberculosis (TB), where the causative organisms are facultative intracellular parasites and immunity is, therefore, dependent on T-cell effector function.[1] Using monoclonal antibodies, a range of mycobacterial antigens, varying in molecular weight (MW) from 12,000 to 71,000 (reviewed by ref. 2), has been identified. Further characterization of most of these antigens has been achieved by cloning of the genes encoding these proteins.[2] Human T cells have been shown to react with six antigens of *Mycobacterium tuberculosis*,[3–7] for which complete or partial sequence information is available.

Two major groups of mycobacterial protein antigens have been provisionally identified, on the basis of function, sequence data and sub-cellular localization.[2] One group, the heat-shock proteins, are essential, constitutive proteins, whose synthesis is enhanced in response to stress stimuli, such as temperature increase or oxidative injury.[8] The heat-shock proteins, such as the 65,000 and 71,000 MW antigens of *M. tuberculosis*, are highly conserved within, and across species, which has led to the hypothesis that the response of T cells to determinants shared between self and extrinsic heat-shock proteins may play a role in autoimmune disease.[9]

A second category of antigens includes the 19,000 and 38,000 MW antigens of *M. tuberculosis*, which are secreted by mycobacteria during growth.[2] The amino acid sequence of the 38,000 MW antigen possesses 30% homology with a phosphate-binding and transport protein, PstS, from *Escherichia coli*, which is active during phosphate starvation.[10] No homology with any known protein has been described, as yet, for the 19,000 MW antigen (R. Lathigra, unpublished data).[11] Recent evidence has, however, indicated that the 19,000 MW and 38,000 MW antigens are related. Both molecules possess potential signal peptides preceding cysteine residues, at positions 22 and 24, respectively. These cysteine residues form part of a consensus motif for lipoylation, matching that observed in the immature forms of other bacterial lipoproteins, and the mature 19,000 and 38,000 MW antigens have been shown to be modified by the addition of a covalent lipid tail.[12]

‡ Present address: Dept. of Cell Biology, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.

Abbreviations: BCG, Bacille–Calmette–Guerin; HLA, human lymphocyte antigen; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells; PEC, pleural effusion cells; TB, tuberculosis; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis

Correspondence: Dr D. M. Rees, MRC Tuberculosis and Related Infections Unit, Hammersmith Hospital, DuCane Rd, London W12 0HS, U.K.

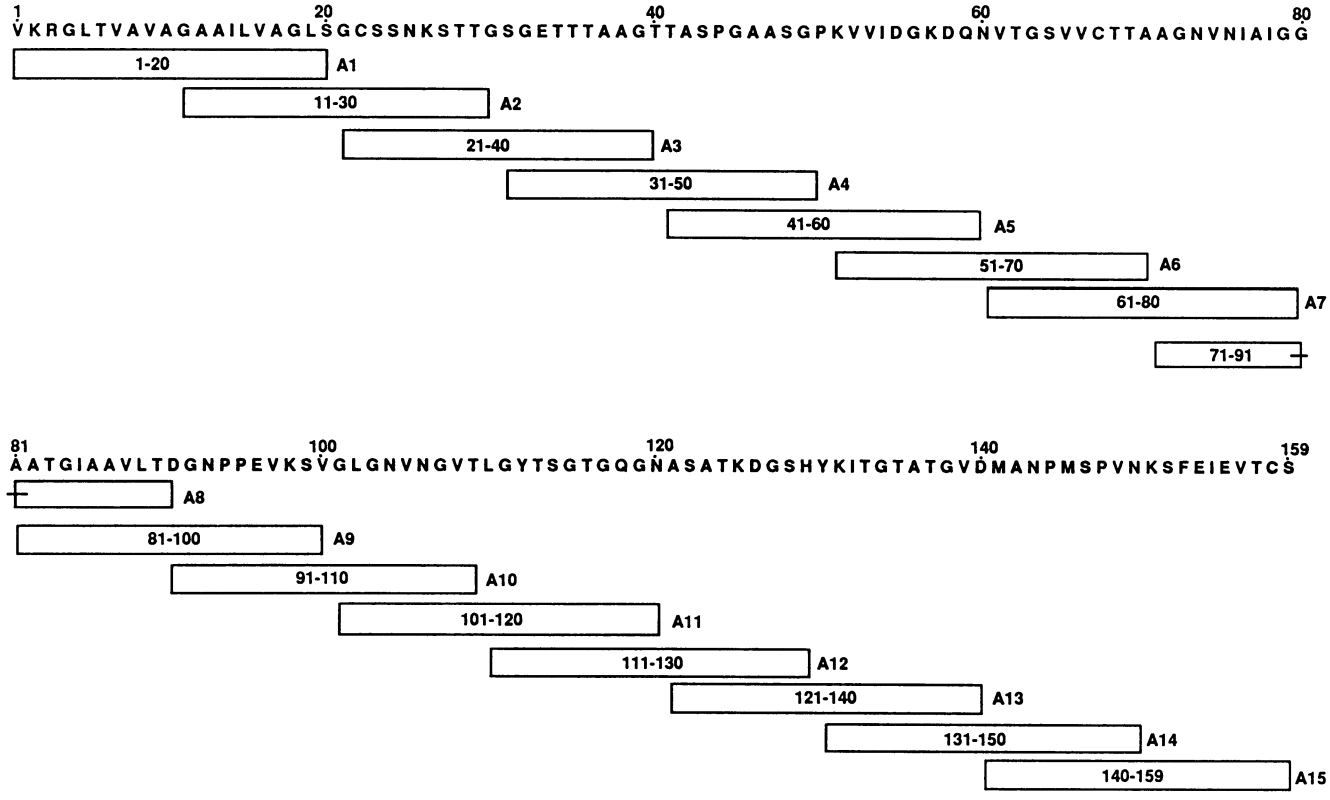

Figure 1. The sequence of the 19,000 MW mycobacterial antigen[11] is shown in single letter coding. Peptides A1–A15, corresponding to the sequences indicated, were synthesized according to the methodology of ref. 21, and were then purified to homogeneity by high-performance liquid chromatography. Open boxes show the sequences overlapped by each peptide.

Both the 19,000 and 38,000 MW proteins have been shown to stimulate the T cells of both normal subjects and TB patients,[4,7,13,14] indicating that recognition of the native proteins does not distinguish a protective from a pathogenic response. T-cell determinants, however, consist of processed fragments of the intact antigen, displayed on antigen-presenting cells as short, linear sequences.[15] It is, therefore, possible that within a protein there may be T-cell epitopes that specifically mediate protection or contribute to pathogenesis. To aid identification of sequences of polypeptides which are recognized by T cells, predictive theories have been developed. One proposes that T-cell determinants are located in sequences which can form an amphipathic helix,[16] while another suggests that a particular motif of hydrophobic and polar amino acids is a conserved feature of peptides which carry T-cell epitopes.[17] The peptide which includes a Rothbard motif in the 19,000 MW antigen was found to be selectively recognized by donors with HLA-DR1, and subtypes of HLA-DR4 and 6.[7]

The identification of potentially protective or pathogenic epitopes may depend on exploring the role of all the T-cell epitopes within a molecule, but predictive methods are not yet powerful enough to be able to describe all T-cell epitopes (reviewed by ref. 18). We have, therefore, used peptides which cover the entire sequence of the 19,000 MW antigen, in conjunction with polyclonal T-cell populations, to locate determinants within this molecule.

As T cells recognize a complex formed between a peptide, and a limited number of MHC class II determinants,[19] the HLA-type of each donor has also been determined in order to define the genetic restriction of T cells responsive to each epitope. The relationship between responsiveness and HLA-type or disease status was then assessed.

MATERIALS AND METHODS

Donors

Pleural effusions or peripheral blood were obtained from eight patients with TB and six with sarcoidosis, with informed consent. Peripheral blood was also obtained from 23 healthy normals, including laboratory personnel and blood donors. Peripheral blood mononuclear cells (PBMC) and pleural effusion cells (PEC) were isolated by centrifugation on a Ficoll–Hypaque (Pharmacia, Uppsala, Sweden) density gradient.

Antigen preparations

A soluble extract of *M. tuberculosis* (MTSE), strain H37RV, was prepared by mechanical disruption.[20] The recombinant 19,000 MW (clone Y4147) (19R), derived from a λgt11 library, was prepared as described previously.[20] The 19,000 MW antigen was sequenced from the DNA insert of a λgt11 clone (Y3293).[11] The sequence, in single letter amino acid coding, is shown in Fig. 1.

Synthetic peptide synthesis

The 19,000 MW peptides (A1–15) were 20 residues in length and overlapped in sequence by 10 amino acids (Fig. 1). The peptides were synthesized according to the 'Tea bag' method, described previously,[21] and were cleaved by a single step using hydro-

fluoric acid, and extraction with ether.[22] The peptides were then further purified by gel filtration through Sephadex G-15, equilibrated with 25% acetic acid or, alternatively, with 50% acetonitrile in water, depending upon the solubility of the peptide. Homogeneity and purity was confirmed by reverse-phase high-performance liquid chromatography (Dionex 4500, Camberley, Surrey, U.K.) using a Zorbax ODS column (Jones Chromatography Ltd, Hengoed, Mid-Glamorgan, U.K.). For each series of synthesis some of the peptides were analysed for amino acid content (Alta Bioscience, Birmingham, U.K.)

T-cell proliferation assay

PEC and PBMC were suspended (5×10^5/ml) in microtitre trays (Flow Laboratories, Irvine, Ayrshire, U.K.) in RPMI-1640 (Gibco, Life Technologies Ltd, Paisley, Renfrewshire, U.K.) supplemented with 2 mM L-glutamine, 100 IU of penicillin-streptomycin/ml and 10% A^+ human serum. Antigen (0·3–20 μg/ml) or peptide (0·3–30 μg/ml) was added at the initiation of these cultures. After 4 or 7 days incubation, for PEC and PBMC, respectively, the cultures were pulsed with 1·0 μCi or tritiated methyl thymidine ([^{3}H]TdR; Amersham International, Amersham, Bucks, U.K.) for 16 hr and harvested onto glass fibre filters. Proliferation, as correlated with [^{3}H]TdR incorporation, was measured by liquid scintillation spectroscopy. The results are expressed as the mean counts per minute (c.p.m.) ± standard error of the mean (SEM) for triplicate cultures. The frozen–thawed cells from each donor were assessed on three separate occasions.

HLA typing

HLA typing was performed on PBMC using 80 antisera and the two-colour fluorescence test.[23]

RESULTS

Reproducibility of T-cell responses to 19,000 MW peptides

In preliminary experiments it was noted that polyclonal proliferative T-cell responses to the 19,000 MW peptides (A1–15; shown schematically in Fig. 1) were often weak, and reproducibility (SEM of triplicate cultures) was poorer than following stimulation with the intact antigen. A representative experiment (Fig. 2) shows the responses of PBMC from a normal donor to the A series of peptides, the recombinant 19,000 MW antigen, and MTSE, determined in three separate assays. The SEM for peptide responses could exceed 80%, as shown for A9 in Fig. 2a. This was thought to represent strongly proliferative responses by a low frequency of T cells. The frequency of T cells recognizing peptide determinants was probably limiting in cultures, at or near 1 cell/well. It was, therefore, necessary at the outset to establish criteria for assessing the reproducibility of responses to peptides. When T-cell responses were measured on three separate occasions, certain peptides were stimulatory in all three assays, as for example, A1, A2, A8 and A9 (Fig. 2). Responses which were reproducible in three separate assays were graded, as shown in Fig. 3, in order to represent the degree of proliferation, Although the level of proliferation to a particular peptide varied between assays, the relative degree of responsiveness, as for example between A1 and A2 (Fig. 2), was fairly constant. Certain peptides, such as A13, were stimulatory in two out of three assays (Fig. 2a, c) and these were included in a separate category, shown in Fig. 3. These results were not graded as to strength of response. Responses observed in only one out of three assays, such as to A7 and A15 in Fig. 2c, were not considered reproducible.

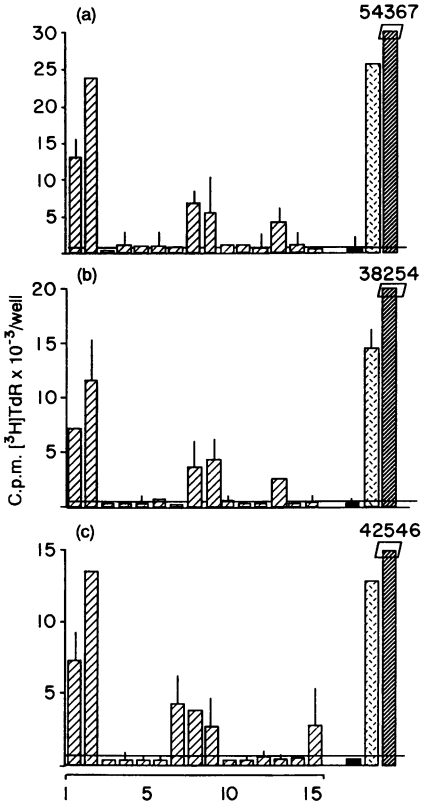

Figure 2. PBMC (5×10^5/ml) derived from donor AF, were cultured with peptides A1–A15 (▨); R19 antigen (▨); λgt11 control lysogen (■); and MTSE (■). Proliferation was measured in three separate assays, and optimal responses from a range of peptide and antigen concentrations (0·3–30 μg/ml) are shown. The response ([^{3}H]TdR incorporation at 7 days) is expressed as c.p.m. Except where indicated with error bars, the SEM was less than 10%. The horizontal line is proliferation in the absence of any stimulus.

Hierarchy of T-cell epitopes defined by responses to 19,000 MW peptides

In order to evaluate the potential number of T-cell epitopes present in the 19,000 MW molecule, polyclonal T cells of a number of HLA-typed individuals, derived from both normal and disease groups, were assessed for their responses to the series of overlapping peptides (Fig. 3). Each of the 15 peptides tested was recognized by cells from at least one individual, with most donors responding to several of the peptides. The most frequent and strongest responses were found to be directed at the N-terminal peptides, A1 and A2, with 17/19 donors recognizing one or both of these peptides. The other peptides stimulated generally weaker responses which could be divided into two groups. The first group consisted of peptides recognized by three or more donors, including the overlapping pairs, A6/A7 and A11/A12, and the individual peptides A7, A13 and A15. The second group was recognized by single, or at most two, donors, and included peptides A4, A6, A10, A11, A12, and A5/

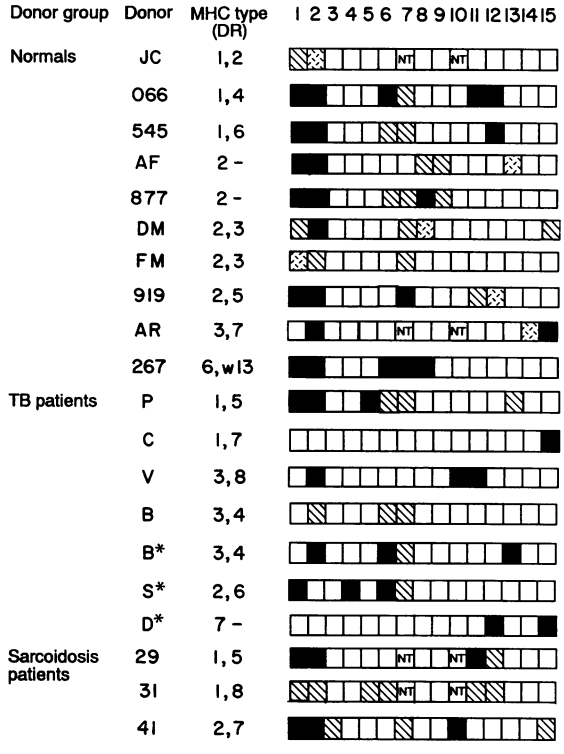

Figure 3. PBMC and PEC (5×10^5/ml) were cultured with peptides A1–A15, and proliferative responses determined as follows. For individual assays each mean c.p.m. in test (+peptide) was expressed as a ratio of the mean c.p.m. of control (−peptide)+1 SEM. The mean ratio was then taken for the three separate assays. The results were then expressed as: >8-fold mean c.p.m.+1 SEM of unstimulated cells (■); 6–8-fold mean c.p.m.+1 SEM of unstimulated cells (■); 3–5-fold mean c.p.m.+1 SEM of unstimulated cells (▨); <3-fold mean c.p.m.+1 SEM of unstimulated cells (□). These responses were reproducible in three separate assays. Responses which were reproducible in 2/3 assays are shown ungraded (▨). Optimal responses at a dose range (0·3–30 μg/ml) are given. Proliferation was measured after culture for 4 or 7 days, for PEC and PBMC, respectively. *Represents responses of PEC. NT, untested peptides.

A6, A8/A9, A10/A11, A14/A15. There was very limited recognition by donor T cells of two regions of the molecule, covered by A3/A4 and A9/A10.

In each assay the response to the peptides was compared to proliferation induced by the 19R antigen (Figs 2, 4, 5 and data not shown). Antigen responders comprised 29 out of 37 donors (80%) tested. Twenty-two out of 27 antigen responders also recognized one or more of peptides A1–A15. Antigen non-responder donors, in contrast, also failed to recognize any of the peptides (Table 1 and data not shown). The sum of peptide responses was often found to exceed proliferation to the intact antigen, although a valid comparison could not be made as the antigen preparation used was not purified to homogeneity.

HLA-DR associations of responses to the 19,000 MW peptides

Some similarities in the pattern of response could be seen for those donors with shared HLA specificities (Fig. 3). Three out of eight individuals, sharing DR2, responded to A7, while 3/5 individuals with either DR5 or DR8 determinants responded to

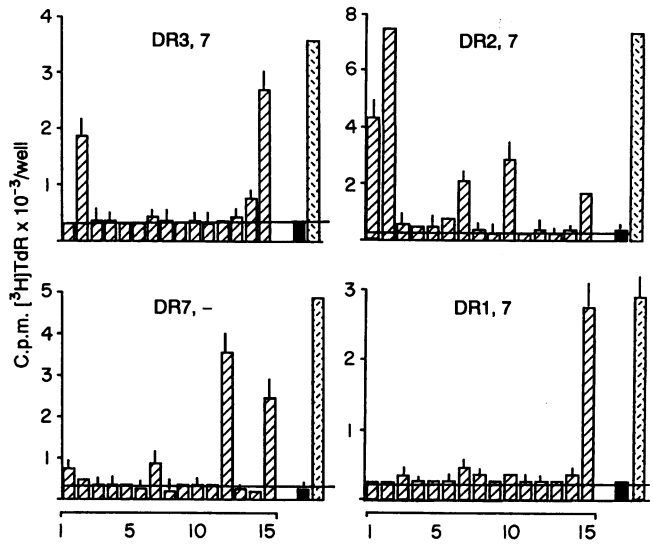

Figure 4. PBMC (5×10^5/ml) derived from four HLA typed donors, sharing DR7, were cultured with the peptides A1–A15 (▨); R19 antigen (▨); and λgt11 control lysogen (■). The optimal response from a range of peptide and antigen concentrations (0·3–30 μg/ml) is shown. The response ([^{3}H]TdR incorporation at 7 days) is expressed as c.p.m. Except where indicated with error bars the SEM was <10%. The horizontal line is the proliferation in the absence of any stimulus.

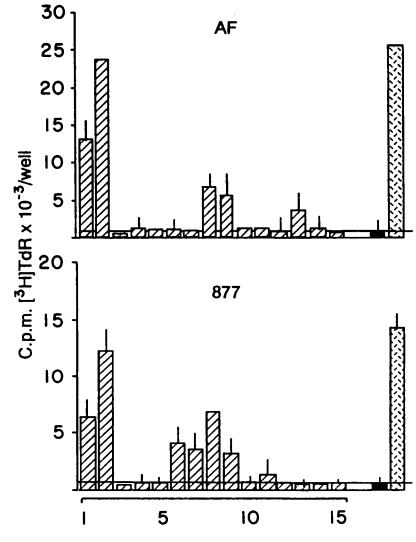

Figure 5. PBMC (5×10^5/ml) derived from two HLA typed donors, homozygous for DR2, were cultured with the peptides A1–A15 (▨); R19 antigen (▨); and λgt11 control lysogen (■) The optimal response from a range (0·3–30 μg/ml) of peptide and antigen concentrations is shown. The response ([^{3}H]TdR incorporation at 7 days) is expressed as c.p.m. Except where indicated with error bars the SEM was <10%. The horizontal line is the proliferation in the absence of any stimulus.

overlapping peptides A11 and A12. Recognition of the overlapping peptides, A6 and A7, was made by 6/10 of donors with DR1, DR4 or DR6. Although one DR2/DR3 donor responded to the carboxy-terminus peptide, A15, this peptide appeared to be more strongly associated with DR7, as all four donors with this determinant responded to A15. A comparison of the responses of these four DR-heterozygous donors is shown in Fig. 4. Clearly, apart from recognition of peptide A15, these donors showed different patterns of peptide responses. The only other example of a shared response was to peptide A2 by two of the four donors. Comparison of the responses of donors sharing both DR determinants, as for example AF and 877 (Fig. 5), also showed differences as well as similarities. Both donors recognized peptides A1, A2, A8 and A9, but cells from 877 responded to A6 and A7, while cells from AF recognized A13.

Promiscuity of the T-cell response to the N-terminal peptides of the 19,000 MW antigen

In order to explore further the apparent promiscuity of the responses to the N-terminal peptides, A1 and A2, a panel of HLA-typed donors was examined for responses to these peptides alone (Table 1). The possibility that A1 and A2 were stimulating cells in a non-specific manner was considered unlikely, because three antigen non-responder donors (Table 1) did not recognize these peptides. Of the 10 antigen responder individuals, 7/10 and 10/10 recognized A1 and A2, respectively. Recognition of A1 was made by individuals with DR1/5, DR2, DR4 and DRw6/DRw13 determinants. Responses to A2 were obtained from donors with HLA determinants DR1/5, DR2, DR4, DRw6/DRw13 and DR7.

Table 1. Promiscuity of T-cell responses to the N-terminal peptides of the 19,000 MW antigen

Donor MHC class II	Stimulation index 19R	Peptide A1 1–20	Peptide A2 11–30
DR1,2	3·8 (0.6)	3·5 (0·6)	3·6 (0·5)
DR1,5	26·0 (2·4)	79·0 (14·4)	140·8 (18·4)
DR2	15·8 (3·8)	13·8 (1·5)	41·8 (8·8)
DR2,3	10·8 (2·2)	9·5 (1·9)	14·9 (2·6)
DR2,7	4·8 (1·2)	5·5 (1·2)	20·8 (3·2)
DR3	15·3 (3·3)	1·2 (0·3)	31·3 (11·8)
DR3,7	5·8 (1·9)	1·6 (0·4)	10·1 (1·8)
DR4,7	22·4 (2·6)	8·2 (1·9)	36·4 (6·4)
DRw6, w13	20·2 (5·1)	12·7 (2·9)	24·5 (2·8)
DR7	5·9 (1·3)	2·0 (0·4)	12·9 (3·6)
DR2,5*	0·6 (0·1)	0·6 (0·1)	0·5 (0·1)
DR2,4*	1·8 (0·3)	1·2 (0·1)	1·6 (0·3)
DR3,7*	1·1 (0·2)	1·3 (0·3)	1·2 (0·1)

PBMC (5×10^5/ml), derived from a panel of HLA-typed donors, were cultured in the presence of either no stimulus, the recombinant 19,000 MW antigen (19R) or the synthetic peptides A1 and A2 (whose sequences are shown in Fig. 1), The responses ([^{3}H]TdR incorporation at 7 days) were determined at the optimal antigen or peptide concentration (range 0·3–30 mg/ml). The results are shown as the mean stimulation index (SI ± 1 standard deviation):

$$SI = \frac{\text{c.p.m. of culture with antigen}}{\text{c.p.m. of culture without antigen}}.$$

Stimulation indices > 3 were taken as significant.

* Represents antigen non-responder donors.

Disease associations of responses to the 19,000 MW peptides

Although no dramatic associations of disease status with peptide responses were observed (Fig. 3), it was interesting that the usually strong recognition of the N-terminal peptides, A1 and A2, by the majority of normal donors and sarcoidosis patients, was not so obviously reflected in the responses by TB patients. Responses to A1 and A2 were made by 2/7 and 3/7 TB patients, respectively, and the strength of these responses was generally weaker than that made by normals and sarcoidosis patients, even though responses to MTSE were comparable (data not shown).

For a number of the TB patients, B, D, A and S, the responses of pleural effusion cells are shown (Fig. 2). Paired peripheral blood and pleural effusion samples were only available from donor B, whose T cells from both compartments responded to peptides A2 and A6/A7, suggesting that comparable response profiles could be obtained from the two sources of cells.

DISCUSSION

In this study multiple T-cell epitopes have been detected throughout almost the entire region of the 19,000 MW molecule. This might perhaps be expected in a molecule which is completely extrinsic, in that no homology with any known protein has been reported (R. Lathigra, unpublished data), and, therefore, no regions of the molecule were likely to induce self-tolerance.[24] It does, however, suggest that human T-cell responses to such a molecule are very diverse. Nevertheless, every region of the molecule was not found to be recognized equally. Instead, there appeared to be a hierarchy in the responses, with the N-terminal region inducing strong proliferation, whereas the peptides corresponding to other regions of the antigen stimulated moderate to weak responses. Studies of the human and murine responses to other extrinsic antigens have also detected multiple but distinct T-cell epitopes.[25,26] In some reports a similar focusing of the response on a limited number of immunodominant epitopes was also observed.[25,27]

Sixteen potential epitopes could be deduced from the responses to either overlapping, or individual peptides of the 19,000 MW antigen. Of these, five contained features of an amphipathic helix[16] and one, at the N-terminal end, the haplotype-specific Rothbard motif.[7] Synthetic peptides overlapping all these regions were recognized by donor T cells. The majority of epitopes, including two of the most stimulatory within peptides A6/A7 and A11/A12, were not, however, predicted by either method. Similar results have been reported in other studies of human[28] and murine[29] responses to mycobacterial antigens. It is probable therefore, that the characteristics of some T-cell determinants may be found in other features of peptide structure.[30]

The influence of HLA type on responses to the deduced epitopes was found to be somewhat complex. Some epitopes were recognized in the context of a single DR type as, for instance, the responses to peptide A7 by several DR2 individuals. This epitope appeared to be unique to A7, as peptides A6 and A8, which overlapped A7, were not recognized by these DR2 donors. In contrast, the response to the epitope defined by the overlapping peptides A6 and A7, was made by donors with DR1, 4 and 6. Homologous sequences in the part of B_1 domain forming the α-helical wall of subtypes of these molecules have been found to permit cross-recognition by alloreactive and antigen-specific[31] T-cell clones. DR5 and DR8 also share sequence at key residues in the antigen-combining site,[31] and three out of four donors, responding to the epitope defined by the overlapping peptides A11 and A12, had these HLA determinants. It was clear, however, that donors sharing one, or even both, DR determinants showed differences as well as similarities in their patterns of recognition. The overall impression is, therefore, one of a highly diverse, partly shared, and partly individual, response to epitopes of the antigen. The correlation between the ability of a peptide to bind to MHC class II molecules and induce T-cell responses,[19,32] when taken together with the wide array of peptides that appear able to interact with each MHC class II combining site,[19] is consistent with competition by processed peptides for binding, which would partly explain the heterogeneous nature of the response to the 19,000 MW antigen. The individual responses to particular peptides, may, however, reflect either the influence of other MHC class II molecules, or the effect of other factors, such as the relative avidity of the T-cell receptor[33] and differential antigen processing,[34] together with the role of thymic selection on the T-cell repertoire.[24]

The generation of different determinants following processing may occur when the individual is exposed to the antigen in different forms. A live infection with the accumulation of the 19,000 MW antigen during intracellular growth may result in some differences in the range of peptides presented to the patient with TB, and the individual protected against the disease by vaccination or natural immunity. The heterogeneity of the T-cell response and the paucity of patients and controls with identical MHC class II determinants made such a comparison difficult. There was, however, some evidence of diminished responses by TB patients compared to normals, to the N-terminal peptides.

In contrast to the more restricted, generally weaker recognition elsewhere, the N-terminal region of the molecule stimulated T cells from most normals and the sarcoidosis patients tested, regardless of HLA type. One possible explanation for the apparent promiscuity of the recognition of this region was the presence of multiple epitopes, each restricted by different HLA types. This was suggested by the observation that cells from some donors responded to only one of the N-terminal peptides. Several reports have indicated that an immunodominant region may be composed of multiple, overlapping epitopes recognized by T cells with different fine specificities.[27,35] In a previous study, the peptide containing the Rothbard motif at the N-terminus of the 19,000 MW antigen (residues 1–12)[7] was found to stimulate polyclonal T-cell responses in donors with DR1, DR4, DRw13 and DRw14. This peptide, was, however, extended to include three residues not present in the predicted translation of the 19,000 MW nucleotide sequence,[11] which could have altered the pattern of response. The A1 peptide (residues 1–20), however, also stimulated donors with DR1, 4 and 6 determinants, suggesting similar patterns of recognition of the Rothbard motif in the two peptides. The A1 peptide was also recognized by DR2 and DR5 donors, indicating the presence of a second epitope, possibly located in the non-overlapping sequence of the two peptides.

It has been suggested that a sub-unit vaccine may be composed of T-cell epitopes within a limited number of regions of an antigen, and recognized by a high proportion of recipients.[36] An important criterion for such a vaccine is that it should be capable of stimulating donors with the diversity of HLA-DR types present in the population. The N-terminal region of the 19,000 MW protein appears to fulfill this criterion, and may therefore have an application in the priming of T cells for recognition of *M. tuberculosis*.

ACKNOWLEDGMENTS

We should like to thank Dr R. Lechler and Dr D. B. Young for critical reading of the manuscript; Nick Davey, Immunology Dept., Hammersmith Hospital, for HLA-typing; Richard Campbell, Wellcome Research Laboratories, for cleaving of the synthetic peptides; and Dr M. Contreras, National Blood Transfusion Centre, Edgeware, for providing blood donors. The support of the Chest, Heart and Stroke Association is acknowledged by A. Faith.

REFERENCES

1. Mackaness G.B. (1969) The influence of immunologically committed lymphoid cells on macrophage activity *in vivo*. *J. exp. Med.* **129,** 973.
2. Young D.B., Garbe T., Lathigra R. & Abou-Zeid C. (1990) Protein Antigens: structure, function and regulation. In: *Molecular Biology of the Mycobacteria* (ed. J. McFadden), p. 1. Surrey University Press.
3. Britton W.J., Hellquist L., Basten A. & Inglis A.S. (1986) Immunoreactivity of a 70kD protein purified from *M. Bovis* BCG by monoclonal antibody affinity chromatography. *J. exp. Med.* **164,** 695.
4. Young D.B., Kent L., Rees A., Lamb J. & Ivanyi J. (1986) Immunological activity of a 38kDa protein purified from *M. tuberculosis*. *Infect. Immun.* **54,** 177.
5. Munk M.E., Schoel B. & Kaufmann S.H.E. (1988) T cell response of normal individuals towards recombinant protein antigens of *M. tuberculosis*. *Eur. J. Immunol.* **18,** 1835.
6. Huygen K., Palfliet K., Jurion F., Hilgers J., Ten Berg R., Van Vooren J.P. & De Bruyn J. (1988) H-2 linked control of γ-interferon production in response to a 32kDa antigen of *M. Bovis BCG*. *Infec. Immun.* **56,** 3198.
7. Lamb J.R., Rees A.D.M., Bal V., Ikeda H., Wilkinson D., De Vries R.R.P. & Rothbard J.B. (1989) Prediction and identification of an HLA-DR restricted T cell determinant in the 19kDa protein of *M. tuberculosis*. *Eur. J. Immunol.* **18,** 973.
8. Pelham H.R.B. (1986) Speculations on the functions of the major heat-shock proteins. *Cell,* **46,** 959.
9. Young D.B., Ivanyi J., Cox J.H. & Lamb J.R. (1987) The 65kDa antigen of mycobacteria—a common bacterial protein? *Immunol. Today,* **8,** 215.
10. Anderson A.B. & Hansen E.B. (1989) Structure and mapping of antigenic domains of protein antigen b, a 38kDa protein of *M. tuberculosis*. *Infect. Immun.* **57,** 2481.
11. Ashbridge K.R., Booth R.J., Watson J.D. & Lathigra R.B. (1989) Nucleotide sequence of the 19kDa antigen gene from *M. tuberculosis*. *Nucl. Acids Res.* **17,** 1249.

12. YOUNG D.B. & GARBE T. (1991) Lipoprotein antigens of *M. tuberculosis*. *Res. Microbiol* (in press).
13. REES A.D.M., SCOGING A., DOBSON N., PRAPUTPITTAYA K., YOUNG D., IVANYI J. & LAMB J.R. (1987) T cell activation by anti-idiotypic antibody. *Eur. J. Immunol.* **17,** 197.
14. OFTUNG F., MUSTAFA A.S., HUSSON R., YOUNG R.A. & GODAL T. (1987) Human T cell clones recognise 2 abundant *M. tuberculosis* protein antigens expressed in *E. coli*. *J. Immunol.* **138,** 927.
15. UNANUE E.R. & ALLEN P.M. (1987) The basis for the immunoregulatory role of macrophages and other accessory cells. *Science*, **236,** 551.
16. DELISI C. & BERZOFSKY J.A. (1985) T-cell antigenic sites tend to be amphipathic structures. *Proc. natl. Acad. Sci. U.S.A.* **82,** 7048.
17. ROTHBARD J.B. & TAYLOR W.R. (1988) A sequence pattern common to T-cell epitopes. *EMBO J.* **7,** 93.
18. ROTHBARD J.B. (1989) Antigen recognition by T helper and T killer cells. In: *T Cells* (eds M. Feldman, J. Lamb and M. J. Owen), p.125. Wiley-Interscience, New York.
19. BUUS S., SETTE A., COLON S., MILES C. & GREY H.M. (1987) The relation between MHC restriction and the capacity of Ia to bind to immunogenic peptides. *Science*, **235,** 1353.
20. LAMB J.R., IVANYI J., REES A.D.M., ROTHBARD J.B., HOWLAND K., YOUNG R.A. & YOUNG D.B. (1987) Mapping of T-cell epitopes using recombinant antigens and synthetic peptides. *EMBO J.* **6,** 1245.
21. HOUGHTEN R.A., DEGRAW S.T., BRAY M.K., HOFMAN S.R. & FRIZZELL N.D. (1986) Simultaneous multiple peptide synthesis. *Biotechniques*, **4,** 522.
22. HOUGHTEN R.A., BRAY M.K., DEGRAW S.T. & KIRBY C.J. (1986) Simplified procedure for carrying out simultaneous multiple hydrogen fluoride cleavages of protected peptide resins. *Int. J. Peptide Res.* **27,** 673.
23. VAN LEEUWEN A. & VAN ROOD J. (1980) Two colour fluorescence test. In: *Histocompatibility Testing* (ed. P. Terasaki), p. 278. UCLA Tissue Typing Laboratory, Los Angeles.
24. KAPPLER J., STAERZ U., WHITE J. & MARRACK P. (1988) Self-tolerance eliminates T cells specific for Mls-modified products of the MHC. *Nature*, **332,** 35.
25. LAMB J.R., ECKELS D.D., LAKE P., WOODY J.N. & GREEN N. (1982) Human T cell clones recognise chemically synthesised peptides of influenza haemagglutinin. *Nature*, **300,** 66.
26. MILICH D. (1989) Synthetic T and B cell recognition sites: implications for vaccine development. *Adv. Immunol.* **45,** 195.
27. SINIGAGLIA F., GUTTINGER M., KILGUS J., DORAN D.M., MATILE H., ETLINGER H., TRZECIAK A., GILLESSEN D. & PINK J.R.L. (1989) A malaria T-cell epitope recognised in association with most mouse and human MHC class II molecules. *Nature*, **336,** 778.
28. OFTUNG F., MUSTAFA A.S., SHINNICK T.M., HOUGHTEN R.A., KVALHEIM G., DEGRE M., LUNDIN K.E.A. & GODAL T. (1988) Heterogeneity among T-cell clones recognising an HLA DR4, Dw4 restricted epitope from the 18kDa antigen of *M. leprae*. *J. Immunol.* **141,** 2749.
29. HARRIS D.P., BACKSTROM B.T., BOOTH R.J., LOVE S.G., HARDING D.R. & WATSON J.D. (1989) The mapping of epitopes of the 18kDa protein of *M. leprae* recognised by murine T cells in a proliferation assay. *J. Immunol.* **143,** 2006.
30. SETTE A., ADORINI I., COLON S.M., BUUS S. & GREY H.M. (1989) Capacity of intact proteins to bind to MHC class II molecules. *J. Immunol.* **143,** 1265.
31. LOMBARDI G., SIDHU S., BATCHELOR J.R. & LECHLER R.I. (1989) Allorecognition of DR1 by T cells from a DR4/DRw13 responder mimics self-restricted recognition of endogenous peptides. *Proc. natl. Acad. Sci. U.S.A.* **86,** 4190.
32. KILGUS J., ROMAGNOLI P., GUTTINGER M., STUBER D., ADORINI L. & SINIGAGLIA F. (1989) Vaccine T-cell epitope selection by a peptide competition assay. *Proc. natl. Acad. Sci. U.S.A.* **86,** 1629.
33. LEHMANN P.V., CARDINAUX F., APPELLA E., MULLER S., FALCIONI F., ADORINI L. & NAGY Z.A. (1989) Inhibition of T-cell response with peptides is influenced by both peptide-binding specificity of MHC molecules and susceptibility of T-cells to blocking. *Eur. J. Immunol.* **19,** 1071.
34. BRETT S.J., CEASE K.R. & BERZOFSKY J.A. (1988) Influences of antigen processing on the expression of the T-cell repertoire. *J. exp. Med.* **168,** 357.
35. CEASE K.B., BERKOWER J., YORK-JOLLEY J. & BERZOFSKY J.A. (1986) T cell clones showing different fine specificities for synthetic peptides. *J. exp. Med.* **164,** 1779.
36. BERZOFSKY J.A., BENSUSSAN A., CEASE K.B., BOURGE J.F., CHEYNIER R., LURHUMA Z., SALAUN J.-J., GALLO R.C., SHEARER G.M. & ZAGURY D. (1988) Antigenic peptides recognised by T lymphocytes from AIDS viral envelope-immune humans. *Nature*, **334,** 706.