

## Identification of a Promiscuous T-Cell Epitope in *Mycobacterium tuberculosis* Mce Proteins

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**The characterization of *Mycobacterium tuberculosis* antigens inducing CD4<sup>+</sup> T-cell responses could critically contribute to the development of subunit vaccines for *M. tuberculosis*. Here we performed computational analysis by using T-cell epitope prediction software (known as TEPITOPE) to predict promiscuous HLA-DR ligands in the products of the *mce* genes of *M. tuberculosis*. The analysis of the proliferative responses of CD4<sup>+</sup> T cells from patients with pulmonary tuberculosis to selected peptides displaying promiscuous binding to HLA-DR in vitro led us to the identification of a peptide that induced proliferation of CD4<sup>+</sup> cells from 50% of the tested subjects. This study demonstrates that a systematic computational approach can be used to identify T-cell epitopes in proteins expressed by an intracellular pathogen.**

*Mycobacterium tuberculosis* survives phagocytosis and replicates within macrophages. Following infection with *M. tuberculosis*, both healthy subjects and patients with active tuberculosis develop T-cell responses against mycobacterial antigens. In mice CD4<sup>+</sup> T cells (5, 22, 24, 32) and gamma interferon (IFN- $\gamma$ ) (8, 11), as well as IFN- $\gamma$  receptor in humans (16, 23), were shown to be essential to the generation of a protective immune response to *M. tuberculosis*. Recently, depletion of CD4<sup>+</sup> cells was shown to result in reactivation of latent *M. tuberculosis* infection in mice in spite of unaltered expression of IFN- $\gamma$ , suggesting that CD4<sup>+</sup> cells could have roles in addition to IFN- $\gamma$  production in controlling *M. tuberculosis* infection (25). Therefore, the identification of antigens capable of eliciting CD4<sup>+</sup> T-cell responses and the characterization of immunodominant T-cell epitopes are of primary importance for the development of subunit vaccines for tuberculosis. The role of CD8<sup>+</sup> T cells in immunity to *M. tuberculosis* is less defined (10). CD8<sup>+</sup> cytotoxic T cells capable of lysing infected macrophages as well as reducing the viability of intracellular mycobacteria through a granulysin-dependent mechanism were described (29). Moreover, epitopes from mycobacterial antigens capable of eliciting cytotoxic CD8<sup>+</sup> T-cell responses were recently identified (17, 21, 6) as well as CD8<sup>+</sup> T cells specific for mycobacterial antigens presented through an alternative major histocompatibility complex class I (MHC-I) processing pathway(s) (4, 19). MHC-II-restricted responses against *M. tuberculosis* have been more extensively characterized, and a number of *M. tuberculosis* antigens for CD4<sup>+</sup> T cells have been identified to date. Recently, an immunodominant family of *M. tuberculosis* antigens recognized by T cells from healthy PPD (purified protein derivative)-reactive subjects was isolated (1). Expanding the knowledge of the human T-cell repertoire to

peptide epitopes derived from *M. tuberculosis* antigens is also of potential interest for immunodiagnostic applications.

Activation of CD4<sup>+</sup> T cells is dependent upon the presentation of peptides from disease-related protein antigens in the context of MHC-II molecules. The MHC genes are the most polymorphic present in the genome of every species analyzed. Most of the differences in the products of individual MHC-II alleles are localized in the peptide binding groove and determine the peptide binding properties of the different MHC molecules. In this study, the products of the mycobacterial cell entry (*mce*) genes were submitted to analysis by T-cell epitope prediction software (TEPITOPE), which enables the computational identification of promiscuous and allele-specific HLA-DR ligands (31). The *mce1* gene was originally defined as an element conferring invasiveness to a nonpathogenic strain of *Escherichia coli* (2). Moreover, an *mce*-deficient *Mycobacterium bovis* BCG mutant exhibited reduced ability to invade nonphagocytic cells (9). The analysis of the complete genome of *M. tuberculosis* revealed the existence of four *mce* gene homologues very similarly organized in operons containing eight genes (7). Five peptides predicted by TEPITOPE as potential HLA-DR ligands and based on the sequence of the Mce2 protein were tested for induction of proliferation of CD4<sup>+</sup> cells isolated from *M. tuberculosis*-infected subjects bearing 12 different haplotypes. This analysis led us to the identification of a peptide inducing CD4<sup>+</sup> cell proliferation in 50% of the tested subjects, indicating that the application of the TEPITOPE software to mycobacterial antigens could lead to the identification of promiscuous epitopes eliciting MHC-II-restricted responses during infection with *M. tuberculosis*.

### MATERIALS AND METHODS

**Study subjects.** Patients were recruited at Consorzio Antitubercolare "Istituto Villa Marelli," Milan, Italy, and Azienda Ospedaliera "S. Maria degli Angeli," Pordenone, Italy, and were typed for HLA-DR at the Tissue Typing Laboratory of S. Raffaele Hospital, Milan, Italy. All sera employed in Western blotting were from tuberculin-positive and human immunodeficiency virus-seronegative subjects. Individuals defined as *M. tuberculosis* positive were a group of patients with

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postprimary pulmonary tuberculosis who were sputum positive by at least two of the following criteria: direct observation, cultural isolation, and PCR (Amplicor MTB test; Roche), whereas *M. tuberculosis*-negative subjects had recovered from postprimary pulmonary tuberculosis and were free of symptoms as well as sputum negative as determined by direct observation, cultural isolation, and PCR for at least 6 months. Peripheral blood mononuclear cells (PBMCs) for immortalization of autologous antigen-presenting cells (APCs) and analysis of CD4<sup>+</sup> T-cell response to *mce2* peptides were from tuberculin-positive, human immunodeficiency virus-negative subjects with postprimary pulmonary tuberculosis ( $n = 22$  [15 males and 7 females]; average age, 30.2 years) who had been treated with antituberculous chemotherapy for at least 1 month. Four PPD-negative healthy donors were used as negative controls.

**T-cell epitope prediction.** The sequences of the *Mce* proteins were subjected to HLA-DR ligand prediction by the TEPITOPE software to identify promiscuous HLA-DR ligands (13). We used a version of TEPITOPE incorporating 25 virtual matrices covering most of the human HLA class II peptide binding specificity in the Caucasian population. We set the TEPITOPE prediction threshold at 3% (31) and picked peptide sequences predicted to bind the most common HLA-DR alleles (DRB1\*0101, DRB1\*0301, DRB1\*0401, DRB1\*0701, DRB1\*0801, DRB1\*1101, and DRB1\*1501). The peptides were chosen based on the predicted binding as well as the degree of similarity between the four *mce* members and were synthesized according to the sequence of the *Mce2* protein. Peptide no. 4, which is present in two proteins and showed poor prediction of promiscuous binding, was used as a nonpromiscuous control.

**Cloning, expression, and immunoblot of Mce2-Myc fusion protein with sera from *M. tuberculosis*-infected subjects.** The *mce2* gene was obtained from *E. coli* (NM554 strain) transformed with the cosmid MTCY19H5 (kindly provided by S. Cole) by PCR amplification with the following primers: forward 5'-CCATGGT GCCAACGCTGGTGACG and reverse 5'-AAGCTTTCATTAATTCAGATC CTCTCTGAGATGAGTGTCTTGTCTGGGTTGATCGTGTCTCTCC containing the sequence (underlined) encoding the *c-myc*-derived epitope. The *mce-myc* fragment was cloned in pCR2.1 (Invitrogen, Groningen, The Netherlands) that was used to transform Top 10 *E. coli*. Purified plasmid DNA was digested with *Hind*III and *Nco*I and cloned in pSE420 (Invitrogen) under the control of the *trp-lac* (*trc*) promoter. Isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) at 1 mM was used to induce the resulting transformants. The Mce2-Myc protein was selectively detected, by immunoblotting with an anti-Myc monoclonal antibody (MAb) in the subcellular fraction containing membranes and inclusion bodies of IPTG-induced transformants, as a band migrating with an apparent molecular mass of 45 kDa (not shown). To immunoprecipitate the Mce2-Myc protein, IPTG-induced bacteria were lysed in 1% sodium dodecyl sulfate (SDS) at 95°C for 20 min. After centrifugation at 20,800  $\times$  g for 15 min, the supernatant was diluted to 1:10 with 1% NP-40 in Tris-EDTA buffer and was immunoprecipitated with 9E10 anti-Myc MAb (CRL-1279; American Type Culture Collection) followed by Sepharose-coupled protein G. The immunoprecipitates were resolved in SDS-12% polyacrylamide gel electrophoresis under reducing conditions and were blotted onto nitrocellulose membranes. Immunoblots were performed with sera from *M. tuberculosis*-infected patients that were revealed by horseradish peroxidase-conjugated antibodies specific for human immunoglobulin G (IgG) and anti-Myc MAb as a control that was revealed by horseradish peroxidase-conjugated antibodies specific for mouse IgG. The enhanced chemiluminescence detection system (Amersham) was used to reveal the binding. Positive sera were reactive with the recombinant Mce2-Myc protein in Western blotting at 1:100 and 1:1,000 dilutions and were negative on anti-Myc-immunoprecipitated lysates from noninduced bacteria at 1:100 dilution.

**DR peptide binding assay.** The *Mce2* peptides together with a control peptide derived from the sequence of Epithelial V-like antigen (12) were synthesized with a multiple peptide synthesizer (model 396; Advanced Chem Tech, Louisville, Ky.) using fluorenylmethoxycarbonyl chemistry and solid-phase synthesis. The ability of unlabeled *Mce2*-derived peptides to compete with a biotinylated indicator peptide was determined using an enzyme-linked immunosorbent assay (ELISA)-based competition assay, as described earlier (13). The following biotinylated indicator peptides were used: GFKA<sub>7</sub> for DRB1\*0101 (DR1) and DRB1\*0701 (DR7), GIRA<sub>2</sub>YA<sub>4</sub> for DRB1\*1501 (DR2), IAYDA<sub>5</sub> for DRB1\*0301 (DR3), YPKFVKQNTLKA<sub>2</sub> for DRB1\*0401 (DR4), tetanus toxin peptide 830-843 for DRB1\*1101 (DR5), and GYRA<sub>6</sub>L for DRB1\*0801 (DR8). The relative binding affinity of the predicted ligands for the different HLA-DR molecules was determined as the 50% inhibitory concentration (IC<sub>50</sub>) (i.e., the concentration of competitor peptide required to inhibit binding of the biotinylated indicator peptide by 50%) and was compared with the promiscuous HA<sub>307-319</sub> peptide from influenza hemagglutinin.

**Immortalization of autologous APCs and analysis of CD4<sup>+</sup> T-cell response to Mce2 peptides.** To immortalize autologous APCs, PBMCs isolated on a Ficoll density gradient were resuspended in RPMI 1640 medium (Gibco-BRL, Gaithersburg, Md.) supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 50 U of penicillin-streptomycin/ml, 50 nM  $\beta$ -mercaptoethanol, 10% heat-inactivated fetal calf serum, 30% culture supernatant of the Epstein-Barr virus (EBV)-producing cell line B95.8 (generous gift of Paola Panina-Bordignon), and cyclosporine A (Novartis Pharma, Basel, Switzerland) at 300 to 600 ng/ml. After 3 days of culture, the supernatant was replaced with normal medium and cells were cultivated for 2 weeks. After an appropriate period of culture, cells were frozen. To analyze CD4<sup>+</sup> T-cell responses to *Mce2* peptides, PBMCs were resuspended at 10<sup>6</sup> cells/ml in complete RPMI 1640 medium supplemented with 10% human AB<sup>+</sup> serum and 10  $\mu$ g of whole-cell lysate of *M. tuberculosis* H37Rv (kindly provided by John T. Belisle, Colorado State University, Fort Collins, Colo.)/ml and cultivated for 3 days. Stimulated cells were expanded in medium with interleukin 2 (20 U/ml) to propagate T cells for stimulation with EBV-immortalized autologous B cells (27). Then 4  $\times$  10<sup>5</sup> CD4<sup>+</sup> T cells and 2  $\times$  10<sup>5</sup> autologous, irradiated (6,000 rads) B cells were distributed in triplicate in 96-well plates and were stimulated with the various peptides at 1 and 10  $\mu$ M concentrations. After 72 h, cell proliferation was assessed by [<sup>3</sup>H]thymidine incorporation and scintillation counting. The proliferative response was expressed as stimulation index (SI), the ratio between counts per minute of triplicate wells from CD4<sup>+</sup> cells cultured with peptide and counts per minute for cells cultured without peptide. Cells displaying an SI/PPD-negative SI ratio of >2.5 were calculated to be significantly stimulated by a paired Student *t* test ( $P < 0.05$ ).

## RESULTS

### Prediction of HLA-DR-restricted epitopes in *Mce* proteins and binding of selected *Mce2* peptides to soluble HLA-DR.

The identification of allele-specific motifs in antigenic peptides together with the structural definition of HLA-peptide complexes provided the basis for the development of methods to predict MHC-II-binding peptides (15, 30). TEPITOPE was applied to the four members of the *mce* family and led to the identification of peptides predicted to bind to HLA-DR (shaded sequences in Fig. 1). We synthesized four peptides with strong prediction for promiscuous binding to HLA-DR that were identified in the same region of at least three members of the *mce* family. A fourth peptide (P4 in Fig. 1) with poor prediction of promiscuous binding was synthesized as a nonpromiscuous control. To the nonamer selected by TEPITOPE as the core HLA-binding motif, two amino acids were added at both ends (peptides 1 and 4) or two at the N terminus and three at the C terminus (peptide 2) based on the sequence homology between the four *mce* members. These extensions of the nonamer increase the efficiency of in vitro peptide presentation to CD4<sup>+</sup> T cells. Peptide 3 was synthesized as a 16-mer to include the four nonamers selected by TEPITOPE in the *Mce* sequences with the addition of two amino acids at the N terminus of the *Mce2* nonamer for the reason mentioned above. The increased lengths of predicted epitopes in the regions corresponding to peptide 5 were due to the presence of more than one hypothetical HLA-binding frame. The synthesized 13-mer was chosen in the *Mce2* sequence with the inclusion at the N terminus of glutamic acid and glycine to limit the presence of nonpolar residues. The five peptides were tested for binding to soluble HLA-DR molecules using an ELISA-based competition assay (13). Table 1 shows the relative binding capacity (IC<sub>50</sub> in micromolars) for each peptide, calculated as the concentration of competitor peptide required to inhibit 50% of the binding of an allele-specific biotinylated peptide (indicator peptide). The lowest IC<sub>50</sub>s with the different haplotypes were obtained with peptides 2 and 3.

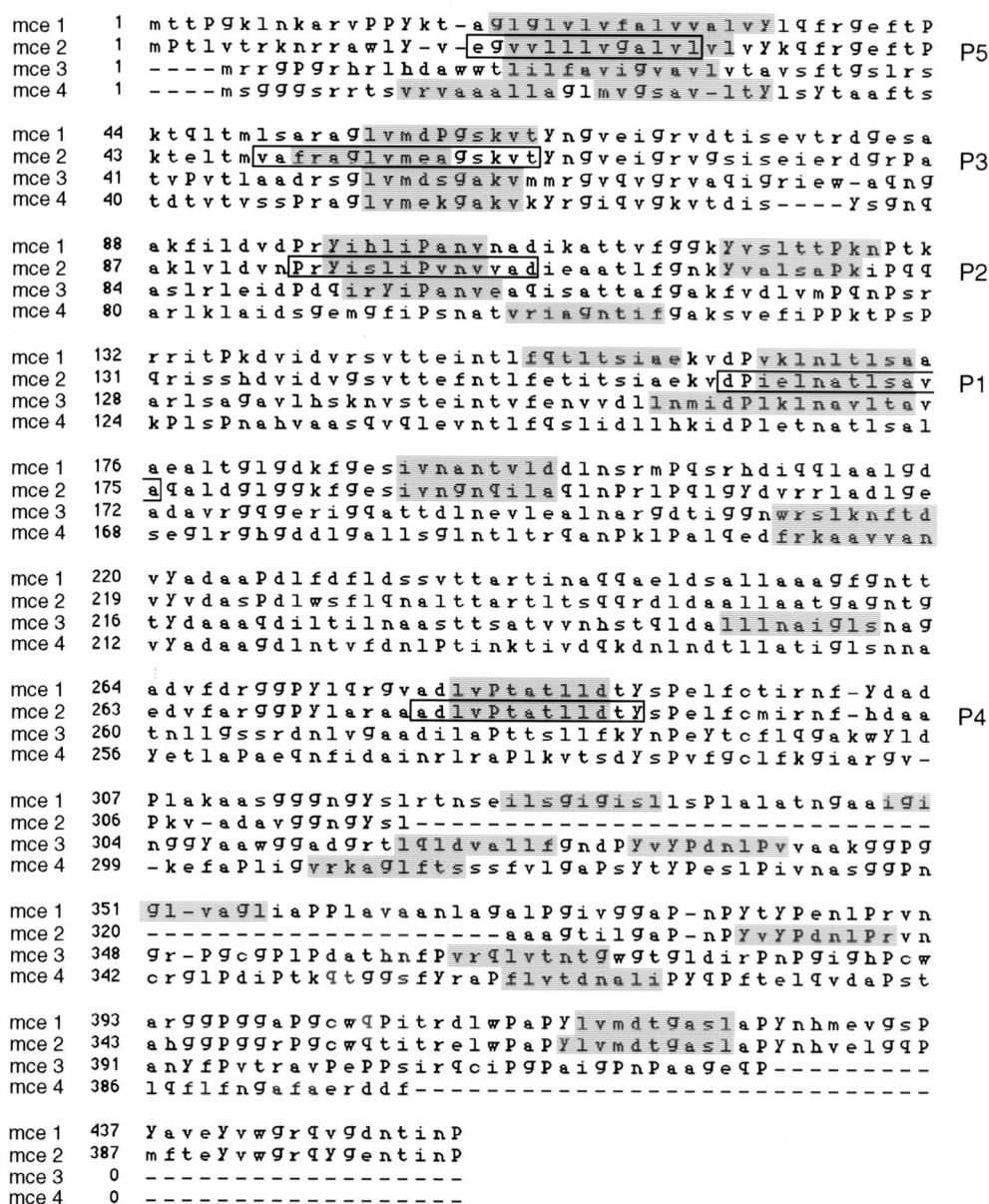


FIG. 1. Sequence alignment of Mce proteins. Shading indicates the peptides selected by TEPITOPE as ligands for the most common HLA alleles. The boxed peptides identified by the indicated numbers were synthesized and used in the HLA-DR-binding assay as well as the CD4+ T-cell proliferation assay.

**IgG responses to Mce2 in *M. tuberculosis*-infected individuals.** The Mce2 protein was expressed in *E. coli* transformants upon induction with IPTG as a fusion protein with a c-Myc epitope tag. To detect human Mce2-specific IgGs, bacteria were lysed and the lysates were subjected to immunoprecipitation with anti-Myc MAb. The immunoprecipitates were resolved by SDS-12% polyacrylamide gel electrophoresis and were transferred onto nitrocellulose membranes. Figure 2A shows the selective detection by a positive serum of the 45-kDa band corresponding to the Mce2-Myc protein in the anti-Myc immunoprecipitate from the lysate of IPTG-induced transformants. Mce2-specific IgGs were detected predominantly in

treated individuals who recovered from pulmonary tuberculosis and who were negative for *M. tuberculosis* by cultural analysis (73% of tested subjects). In contrast, patients with active infections were mostly negative in this assay (90% of tested subjects) (Fig. 2B). Thus, the detection by immunoblotting of IgGs recognizing Mce2-Myc correlated with the recovery from *M. tuberculosis* infection following pharmacological treatment.

**Proliferative responses of CD4+ T cells to predicted immunogenic peptides.** The HLA-DRs of PPD-negative healthy donors and *M. tuberculosis*-infected subjects were identified by molecular typing of the HLA-DRB locus. The relative distributions of the various alleles in the tested population are

TABLE 1. HLA-DR-binding assay of Mce2-derived peptides<sup>a</sup>

Peptide	IC <sub>50</sub> (μM) for:						
	DRB1*0101 (DR1)	DRB1*1501 (DR2)	DRB1*0301 (DR3)	DRB1*0401 (DR4)	DRB1*1101 (DR5)	DRB1*0701 (DR7)	DRB1*0801 (DR8)
1 (aa 163–175)	30	15	0.45	0.8	2	0.025	100
2 (aa 95–108)	0.02	0.008	8	2	0.4	0.025	0.15
3 (aa 49–64)	0.8	1	100	8	1	0.4	10
4 (aa 278–290)	>100	>100	25	>100	>100	0.6	0.2
5 (aa 17–29)	20	1	30	>100	18	0.07	100
HA <sub>307–319</sub>	0.12	4	8	1.5	0.5	0.3	4

<sup>a</sup> Synthesized Mce2-derived peptides are listed. Peptides were tested by ELISA for the competition of binding to HLA-DR of an indicator peptide and were compared to a promiscuous HLA-DR ligand from influenza hemagglutinin (HA<sub>307–319</sub>). The HLA-DR binding is expressed as the micromolar concentration of the competitor peptide able to inhibit the binding of the biotinylated indicator peptide by 50% (IC<sub>50</sub> in micromolars). aa, amino acids.

shown in Fig. 3A. T cells contained in the PBMC fractions from healthy donors ( $n = 4$ ) and *M. tuberculosis*-infected subjects ( $n = 22$ ) were stimulated with whole-cell lysates of H37Rv mycobacteria and propagated in interleukin 2. Stimulation of H37Rv-specific CD4<sup>+</sup> T cells with Mce2-derived peptides was performed using previously EBV-immortalized autologous B cells as APCs. Proliferative responses displaying an SI/PPD-negative SI ratio of >2.5 were considered specific for a given peptide ( $P < 0.05$  and <0.001). Peptides 2 and 3 were the best inducers of proliferative responses with different alleles (Fig. 3B and 4), whereas no significant proliferations were observed when CD4<sup>+</sup> cells were stimulated with a control peptide. The percentages of proliferative responses were negatively correlated with the mean IC<sub>50</sub>s detected in the HLA-DR-binding assay (Fig. 3C). Peptide 3, which induced 50% of proliferative responses with a mean IC<sub>50</sub> of 17.3, was excluded from such an analysis because of the increased length (16 amino acids) with respect to the other four peptides. The extended length was determined by the inclusion of contiguous homologous Mce regions to incorporate the slightly shifted nonamer in the Mce2 sequence and the predicted nonamers in the other Mce members (Fig. 1). This should allow the detection of more anti-Mce CD4<sup>+</sup> T-cell responses (see discussion). The proliferative re-

sponses could be correlated to the binding of the peptide in vitro to a particular HLA-DR apart from DR13, which was not employed in such an assay. The efficiency of peptide 3 in eliciting CD4<sup>+</sup> proliferative responses suggests that it could be a promiscuous epitope generated by the natural processing of *mce* proteins.

## DISCUSSION

Several lines of evidence indicate that activation of *M. tuberculosis*-specific CD4<sup>+</sup> T cells is critical for the control of mycobacterial infection. Analysis of human sera in immunoblots with recombinant Mce2-Myc protein detected Mce2-specific IgGs predominantly in subjects treated for pulmonary tuberculosis who were negative for *M. tuberculosis* isolation. Immunoglobulin isotype switching generally involves the recruitment of CD4<sup>+</sup> helper T cells (28). Since the immunoblot assay likely detects also IgGs induced by other members of the Mce family, this result suggests that the Mce2 protein and/or other Mce members elicit an MHC-II-restricted response.

HLA-DR constitutes the dominant isotype of human MHC-II (33). TEPITOPE incorporates 25 virtual matrices covering the majority of human HLA-DR specificities and

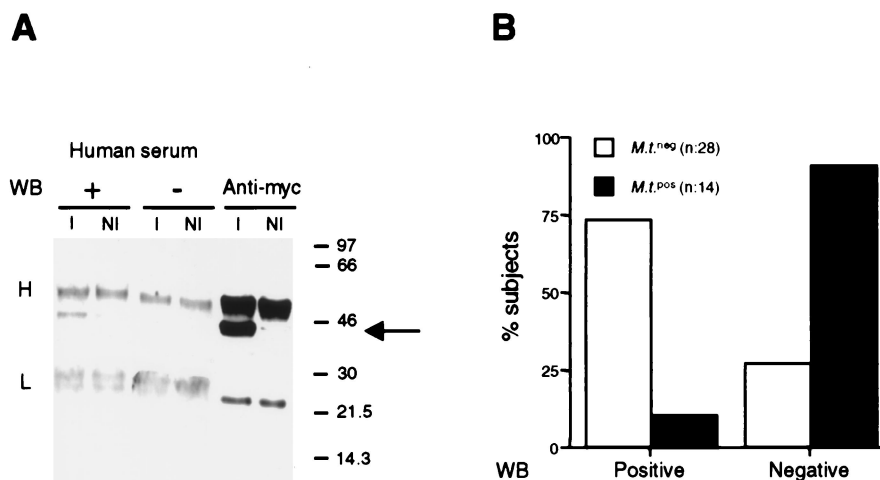


FIG. 2. (A) Western blots (WB) with human positive (+) and negative (-) sera as well as with anti-Myc MAb of anti-Myc immunoprecipitates from lysates of noninduced (NI) and IPTG-induced (I) *E. coli* transformants expressing the Mce2-Myc fusion protein. Heavy (H) and light (L) chains of the immunoprecipitating anti-Myc MAb as well as the Mce2-Myc protein (arrow) are indicated. Numbers on the right are molecular sizes in kilodaltons. (B) Relative distributions of *M. tuberculosis*-positive (black bars) and -negative (white bars) subjects with respect to IgG reactivity to Mce2-Myc protein in immunoblot.

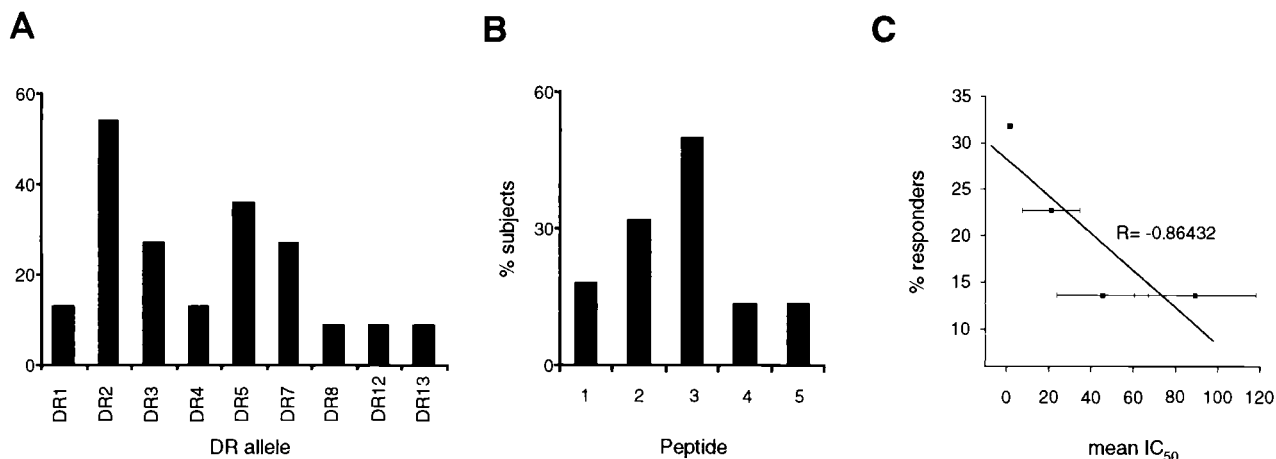


FIG. 3. (A) Relative representations of HLA-DR alleles in subjects tested for CD4<sup>+</sup> T-cell proliferative responses. (B) Percentages of subjects responding to the different Mce2-derived peptides. (C) Correlation between percentages of proliferative responses obtained in the CD4<sup>+</sup> T-cell stimulation assay and mean IC<sub>50</sub>s detected in the HLA-DR-binding assay with peptides 1, 2, 4, and 5.

enables the systematic prediction of peptide ligands for a broad range of HLA-binding specificities (10). It was successfully employed to identify HLA-DR ligands derived from tumors (20, 31) and endogenous proteins involved in autoimmune diseases (14). We investigated whether promiscuous HLA-DR ligands could be identified in the sequences of the Mce protein family. We used a stringent threshold setting, which was proven to be effective in predicting up to 80% of an in vitro-selected peptide repertoire (31). The HLA-DR-binding assay with the five selected peptides corresponding to the Mce2 sequence confirmed the validity of such an approach. Indeed, peptides 2 and 3, which displayed the lowest IC<sub>50</sub>s, were predicted to be the most promiscuous ligands. Peptide 5 demonstrated high IC<sub>50</sub>s with most MHCs in spite of the promiscuous binding prediction, and this might be due to the high hydrophobicity of such a peptide. Nevertheless, we employed peptide 5 in the CD4<sup>+</sup> T-cell proliferation assay, because it was

shown that hydrophobic peptides could represent promiscuous T-cell epitopes and could be efficiently presented in vitro (26).

In vivo antigen processing and HLA class II binding are complex multistep processes that can be influenced by unpredictable mechanisms, i.e., the susceptibility to proteolysis of a given antigen, the specificity of the proteolytic enzymes involved during processing, and the stability of the generated peptides. We assessed the capacity of the selected peptides to be presented in vivo in the course of *M. tuberculosis* infection by testing the proliferation of CD4<sup>+</sup> T cells from patients with pulmonary tuberculosis upon stimulation with autologous APCs loaded with the Mce2 peptides as well as a control, unrelated peptide. Such an analysis revealed that the percentage of proliferative responses was inversely correlated to the mean IC<sub>50</sub> obtained in the HLA-binding assay. Peptide 3 displayed a significant increase in the percentage of proliferative responses with respect to the expected value by the correlation

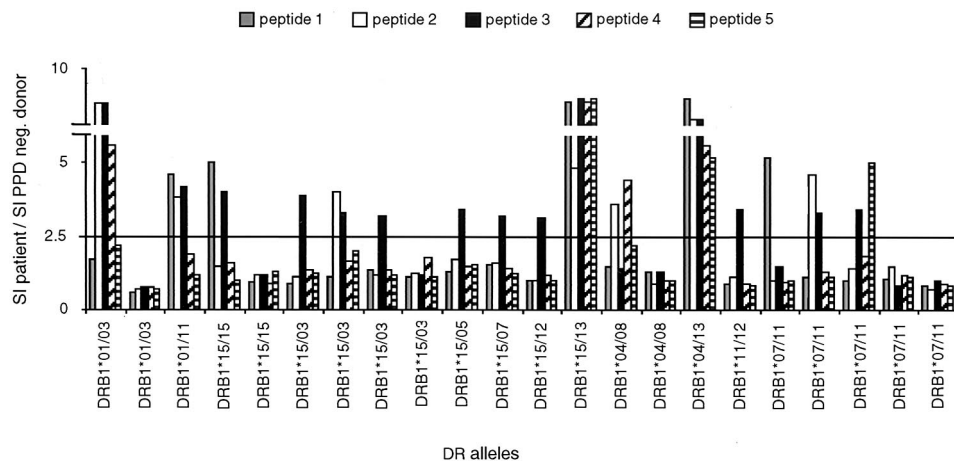


FIG. 4. Proliferative responses (expressed as ratio of SI of patient/SI of PPD-negative donor) to Mce2-derived peptides of typed patients with pulmonary tuberculosis. A paired Student *t* test was used to determine the *P* value that was <0.05 or <0.001 for cellular responses above the marked value of 2.5.

between mean IC<sub>50</sub>s and percentages of T-cell responses obtained with the other four peptides. This could be due to both the extended length with inclusion of more potential epitopes from the four Mce proteins and/or some preferential presentation of nonamers contained in peptide 3 to CD4<sup>+</sup> cells in vivo. Two DRB1\*04/08 patients did not respond to peptide 3 in the antigen presentation assay, whereas no restriction to particular haplotypes was evident in responses to peptide 2. Indeed, both DR4 and DR8 were not predicted to be optimal ligands for peptide 3 in the HLA-DR-binding assay, and peptide 2 displayed the most promiscuous pattern in such an assay.

The Mce proteins were predicted to bear signal sequences or hydrophobic stretches at the N terminus, suggesting that they could be either secreted or surface exposed (7), consistent with the proposed role of the Mce1 protein in invasion of host cells (2). The results presented in this study indicate that Mce proteins are immunogenic. Moreover, a promiscuous T-cell epitope could be identified by TEPITOPE in a region of homology of the four Mce proteins. Subunit vaccines consisting of mycobacterial protein antigens represent a potential safe and specific tool for the prevention of tuberculosis. A potential limit of subunit vaccines is limited persistence in vivo, resulting in the inability to induce strong primary responses with long-lived memory (18). However, it was recently shown that in mice previously immunized with BCG, memory CD4<sup>+</sup> T cells could be boosted by a protein antigen (3). Since BCG is widely administered to children, immunization with BCG could be beneficial for obtaining more specific and protective secondary responses with subunit vaccines. The identification of promiscuous binding to HLA is an ideal prerequisite for the design of subunit vaccines. Since the complete genome of *M. tuberculosis* is available, TEPITOPE could constitute a valuable tool for the identification of immunogenic peptides from *M. tuberculosis* proteins selectively implicated in the various pathogenetic aspects of tuberculosis.

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