A peptide mimetic of the mycobacterial mannosylated lipoarabinomannan: characterization and potential applications

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Mannosylated lipoarabinomannan (ManLAM), a complex lipoglycan, is a major component of *Mycobacterium tuberculosis*, the agent of tuberculosis (TB), and is an antigen used for serological diagnosis of TB. Screening random phage-display peptide libraries with anti-ManLAM mAb CS40 for peptide epitope mimics (mimotopes) led to the isolation of a panel of peptides. One of these peptides (B11) was characterized as a ManLAM mimotope: it bound the anti-ManLAM CS40 mAb and competed with ManLAM for antibody binding. Mice immunized with keyhole limpet haemocyanin-conjugated B11 peptide in a proper adjuvant developed antibodies that recognized ManLAM. Competition experiments demonstrated that the B11 peptide inhibited binding of mAb CS40 to ManLAM in a concentration-dependent manner. The data indicated that the affinity of CS40 mAb to B11 (K_D 1.33×10^{-8}) is higher than its affinity to ManLAM (K_D 3.00×10^{-7}). The sera of TB patients, as well as the sera of mice experimentally infected with *M. tuberculosis*, contained significant levels of antibodies that recognized both the B11 peptide and ManLAM. The specificity and sensitivity of the ELISA B11-based test were similar to those of the ELISA ManLAM-based test, indicating that the B11 antigen could be a good substitute for ManLAM serology for the diagnosis of TB.

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INTRODUCTION

Carbohydrate antigens of micro-organisms are targets of the immune system in a variety of infectious diseases and as such have been suggested as candidates for diagnosis and immunization. A major obstacle in using carbohydrates as antigens is the difficulty involved in obtaining or synthesizing complex carbohydrate ligands. A possible alternative to the use of carbohydrates would be the development of protein or peptide mimics that are specifically recognized by anti-carbohydrate antibodies (Abs) and can thus serve as surrogate diagnostic markers in serological tests. Peptides are well defined and easier to synthesize than complex microbial polysaccharides.

Combinatorial phage-display peptide libraries have been used for identifying peptide epitope mimics (mimotopes) of microbial polysaccharides (Shin *et al.*, 2002). An additional application of these peptidomimetics could be as components in a vaccine in which the peptides would elicit an immune response that could cross-react with the corresponding sugar targets of the pathogen (Tang *et al.*, 2003).

Mannosylated lipoarabinomannan (ManLAM) is a lipoglycan, a polysaccharide with a phosphatidyl-*myo*-inositol anchor. ManLAM is a major cell-surface component of *Mycobacterium tuberculosis*, the agent of tuberculosis (TB) (Chatterjee *et al.*, 1992). The polysaccharide ManLAM has diverse biological activities in the development of mycobacterial pathogenesis and in the interaction with macrophages *in vitro* that are different from the biological actions induced by other lipoarabinomannans present in non-pathogenic mycobacteria (Briken *et al.*, 2004). ManLAM binds to macrophages via mannose-binding receptors such as the mannose receptor and DC-SIGN, and immunomodulates various cytokines and nitric oxide secretion

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Abbreviations: i.p., intraperitoneally; KLH, keyhole limpet haemocyanin; ManLAM, mannosylated lipoarabinomannan; MPL-TDM, monophosphoryl lipid A + trehalose dimycolate; s.c., subcutaneously; SPR, surface plasmon resonance; TB, tuberculosis.

(Prinzis *et al.*, 1993; Tailleux *et al.*, 2003). ManLAM is a large complex molecule that is difficult to analyse and elucidate which of its components are responsible for the specific activities of the molecule functions. Mimotopes of specific epitopes might be good candidates for addressing two major issues in TB: efficient diagnosis (Antunes *et al.*, 2002; Ciesielski, 1995) and effective vaccination (Castanon-Arreola & Lopez-Vidal, 2004). In TB patients, high levels of anti-ManLAM Abs can be detected, making ManLAM a candidate for serological diagnosis (Hamasur *et al.*, 2001). Indeed, a commercial kit, MycoDot, based on antibody recognition of the ManLAM is available (Hamasur *et al.*, 2001). The main disadvantage of MycoDot is its low sensitivity (Ratanasuwan *et al.*, 1997). Another disadvantage of using ManLAM for this test is the complex procedure required for its purification.

Due to its presence on the bacterial surface, ManLAM has also been suggested as a vaccine candidate (Glatman-Freedman *et al.*, 2004). However, purified ManLAM, as other polysaccharides, is a poor immunogen (Hamasur *et al.*, 1999) unless conjugated to a carrier protein (Glatman-Freedman *et al.*, 2004). Peptide mimotopes of polysaccharides may be more efficient for elicitation of Abs than polysaccharides (Beninati & Teti, 2000; Grothaus *et al.*, 2000; Maitta *et al.*, 2004; Prinz *et al.*, 2004).

Therefore, in this work, we screened phage-display libraries in order to find ManLAM mimotopes. We describe here a peptide (B11) that is a ManLAM mimic.

METHODS

Materials. ManLAM from *M. tuberculosis* strain H37Rv and non-mannose-capped lipoarabinomannan-binding mAb CS35 (Chatterjee *et al.*, 1992; Kaur *et al.*, 2002) and ManLAM-binding mAb CS40 (Chatterjee *et al.*, 1992; Navoa *et al.*, 2003) were kindly provided by Drs Brennan and Belisle (Colorado State University, Fort Collins, CO, USA; NIH grant AI-75320).

Peptide synthesis. Peptides were synthesized by Professor Fridkin at the Weizmann Institute, Rehovot, Israel, at the interdepartmental facility of the Hebrew University Faculty of Medicine, Jerusalem, Israel, and at Sigma (Rehovot, Israel) using a solid-phase technique. For conjugation to thiol-reactive keyhole limpet haemocyanin (KLH; Pierce), B11 peptide was synthesized with an additional N-terminal cysteine residue. B11 peptide, with or without cysteine, bound mAb CS40 in ELISAs.

Biopanning, phage screening assay and peptide sequencing. Random phage-display peptide libraries, constructed using the fth1 type 88 vector (Enshell-Seijffers *et al.*, 2001), were biopanned as described previously (Enshell-Seijffers, 2002). Clones were screened for recognition by mAbs CS35 and CS40 using dot-blot analysis and analysed as described previously (Enshell-Seijffers *et al.*, 2003).

Selected peptide sequence inserts were aligned by CLUSTAL W using MACVECTOR 7.0 (Oxford Molecular).

Direct ELISA. Direct ELISA tests were performed by coating 96-well ELISA plates overnight at 4 °C with 50 µl per well of solutions containing either 10^9 phages ml⁻¹, 5–20 µg peptide ml⁻¹ or 5 µg ManLAM ml⁻¹. Plates were washed twice in PBS and blocked with

PBS/1 % BSA. After washing twice in PBS, serum or mAb (50 µl per well) was then added and incubated for 1 h at 37 °C. Plates were then washed four times. Alkaline phosphatase-labelled goat anti-mouse Ig (Sigma) was added for 90 min at 37 °C, followed by *p*-nitrophenyl phosphate (KPL). The A_{405} was measured using an ELISA reader (ELX-800UV; Bio-Tec Instruments). All samples were tested in triplicate.

For detection of IgM and IgG, sera were diluted 1 : 50 and 1 : 200 in PBS/0.5 % BSA. Human serum ELISA was performed according to Hetland *et al.* (1998). Sera were provided by Dr Spector (Hadassah University Hospital, Jerusalem, Israel), Professor Marchal (Pasteur Institute, Paris), Dr Acosta (Finlay Institute, Havana, Cuba) and Dr Mendez (Universita Veracruzana, Mexico) after appropriate Helsinki Committee approvals.

ELISA competition assay. An ELISA competition assay was performed according to Kaur *et al.* (2002). Briefly, Immulon 4HBX plates were coated with 50 µl per well of a solution of 5 µg ManLAM ml⁻¹ and blocked as described above. mAb CS40 (diluted 1 : 2000) was mixed with various numbers ($0-1 \times 10^{16}$ ml⁻¹) of phage particles in TBS/1 % BSA or various amounts (0–200 µg ml⁻¹) of synthetic peptide in PBS/1 % BSA. This solution was incubated for 60 min at room temperature and 50 µl was transferred to the ELISA plates and incubated for 1 h at 37 °C. After washing in PBS, the ELISA procedure was performed as described above.

Surface plasmon resonance (SPR). Experiments were all performed using BIAcore 3000 at 30 °C in PBS (at a 20 µl min⁻¹ flow rate). ManLAM was immobilized on a CM5 sensor chip according to the BIAcore aldehyde coupling protocol, using continuous flow (5 µl min⁻¹). The surface was activated by 0.05 M *N*-hydroxysuccinimide and 0.2 M *N*-ethyl-*N'*-(dimethylaminopropyl)-carbodiimide and the introduction of a hydrazine group was done by injection of 5 mM hydrazine, followed by deactivation of the unreacted esters by injection of 1 M ethanolamine (pH 8). The ligand was injected at a concentration of 100 µg ml⁻¹ in 10 mM glycine buffer (pH 1.5). The immobilization level was 400–600 resonance units. Stabilization of the bound ligand was done by injection of 0.1 M cyanoborohydride in 0.1 M acetate (pH 4) at 2 µl min⁻¹. Activated Fc using the same procedure, without binding the ligand, served as a control. For evaluating the K_D of the mAb CS40–ManLAM interaction, different CS40 concentrations (0–250 nM) were tested for binding of ManLAM. For evaluating the K_D of the mAb CS40–B11 peptide interaction, a competition assay (Montalto, 2001) was performed as follows. mAb CS40 (125 nM) plus different concentrations of B11 peptide (0–150 µM) were pre-incubated in PBS (30 min at 37 °C) and then injected manually over the cell flow. Comparative inhibition of mAb CS40 binding to ManLAM was evaluated, comparing B11 peptide with A1 peptide (Fig. 1) and B11-glycine, a control peptide synthesized with three glycine amino acids replacing the three aromatic amino acids (W, W and Y) of the B11 peptide

A1	rcgWEADDKNQHGEgcaa
B11	rgISLTEWSMWYRHaa
C1	rcgEEGPWSTHVGRTcaa
C3	rgcWGNEGGDHLQPVcaa
F7	rgSLKIRWELKMYQEcaa
G3	rgcAVERWEKHTWSEcaa

Fig. 1. Phage-displayed peptides recognized by mAb CS40. Six peptide sequences (upper case) are given, flanked by residues derived from the fth1 vector (lower case). Sequence inserts were aligned by CLUSTAL W using MACVECTOR 7.0.

sequence (Fig. 1). A competition assay was performed as described above, by pre-incubating mAb CS40 (250 nM) with 50 μ M of each of the peptides before injection.

Regeneration in all assays between each injection was done by injection of 10 μ l 1 mM NaOH. BIAEVALUATION 4.0 (BIAcore) was used to calculate the K_D values using the solution-affinity model with general fit parameters.

Immunization protocols. Immunizations were all performed in specific-pathogen-free female BALB/c mice, 6–8 weeks old. Five or 50 μ g KLH-conjugated peptide was administered twice at 3-week intervals subcutaneously (s.c.) and intraperitoneally (i.p.). The adjuvants monophosphoryl lipid A + trehalose dimycolate (MPL-TDM) (Sigma), incomplete Freund's adjuvant (Sigma) and dimethyldioctadecylammonium bromide (Fluka) were tested in s.c. immunization. Immunization i.p. was performed using the MPL-TDM adjuvant system (Sigma).

Control groups were immunized with KLH in the relevant adjuvant. For detection of serum-specific anti-ManLAM antibodies, mice were bled from the tail vein. All animal experiments were performed in accordance with the regulations of the animal experimentation ethics committee of the Hebrew University Hadassah Medical School.

Experimental *M. tuberculosis* infection. Specific-pathogen-free female BALB/c mice were inoculated intravenously in the tail vein with 5×10^5 c.f.u. of *M. tuberculosis* strain H37Rv (a kind gift from Professor Marchal, Pasteur Institute, Paris) (Hovav *et al.*, 2003). Naïve mice ($n=6$) and mice 30 days ($n=6$) and 3 months ($n=4$) after *M. tuberculosis* infection were bled and tested for the presence of IgG specific for ManLAM and B11 peptide.

Statistics analysis. Data were analysed by Student's *t*-test. *P* values of <0.05 were considered to be significant.

RESULTS AND DISCUSSION

Sequence selection by phage display technology

mAb CS40 (Chatterjee *et al.*, 1992; Navoa *et al.*, 2003) and mAb CS35 (Chatterjee *et al.*, 1992; Kaur *et al.*, 2002) were used to screen the phage display libraries for ManLAM and non-mannose-capped lipoarabinomannan mimotopes, respectively, as described in Methods. Six different peptides were isolated using mAb CS40 (Fig. 1) but no relevant peptides were detected with mAb CS35. As in other carbohydrate mimotopes (Kieber-Emmons *et al.*, 1999; Prinz *et al.*, 2004; Young *et al.*, 1997), tryptophan (W) is a conserved residue present in all selected sequences (Fig. 1). Among the sequences selected, B11 peptide was unique as it was the only peptide with three aromatic residues, which may be important in maintaining the peptide activity, even when produced synthetically and out of context of the phage. This propensity for multiple aromatics in high-affinity polysaccharide mimotopes has been reported previously by others (Park *et al.*, 2004; Prinz *et al.*, 2004; Westerink *et al.*, 1995).

The relative efficiency of binding of the selected recombinant phage clones to mAb CS40 was performed by ELISA. The most efficient clone competing with ManLAM was B11 (data not shown). It has been shown previously that

higher-affinity mimetics make mimotopes with better immunological characteristics (Fuchs *et al.*, 2003; Young *et al.*, 1997). Thus we examined the immunogenicity of the B11 phage clone. We found that mice immunized twice with the B11 phage clone developed specific ManLAM-binding IgG Abs (data not shown). Therefore, a synthetic sequence corresponding to the sequence present in the clone B11 was synthesized and tested for its binding and immunogenic properties.

Binding properties of synthetic B11 peptide to mAb CS40

To determine whether the B11 synthetic peptide was capable of binding mAb CS40 and inhibiting binding of mAb CS40 to the natural antigen ManLAM, we performed direct ELISAs, competitive ELISAs and SPR analysis. The binding properties of B11 were compared with those of two control peptides: (i) a sequence corresponding to clone A1 (Fig. 1), a phage clone that bound the mAb in direct ELISAs but did not compete in binding of mAb CS40 in competitive ELISAs, as described in Methods; and (ii) a control peptide, B11-glycine, designed to test the importance of the aromatic amino acids in binding to mAb CS40. B11-glycine peptide was synthesized with three glycine amino acids replacing the three aromatic amino acids (W, W and Y) of the B11 peptide sequence. We found that the synthetic peptides (B11, A1 and B11-glycine) bound mAb CS40 in direct ELISAs (data not shown) with different efficiencies. However, in competitive ELISAs, only B11 competed with ManLAM in binding to the mAb ($\sim 90\%$ decrease in A_{405} with 50 μ g peptide ml^{-1}). This reduction was not seen with the control peptide A1 (Fig. 2).

When evaluating the ability of the B11 peptide to inhibit binding of mAb CS40 to ManLAM in SPR comparative analysis, we found that B11 showed the highest inhibition of the three synthetic peptides (Fig. 3). For this assay, we primarily tested the binding of mAb CS40 to ManLAM. ManLAM was immobilized on a sensor chip and, as expected, mAb CS40 (250 nM) bound immobilized ManLAM (Fig. 3a). The inhibition of mAb CS40 binding to the immobilized ManLAM using B11 was compared with inhibition using the control peptides A1 and B11-glycine (Fig. 3) using a competitive binding assay (Adamczyk *et al.*, 2000; Montalto, 2001). For this, each of the three peptides (50 μ M each) was pre-incubated with mAb CS40 for 30 min at 37 °C. After incubation, binding of free mAb to the immobilized ManLAM was measured and compared with binding of the mAb alone. All three peptides reduced binding of mAb CS40 to ManLAM, indicating that they bound the mAb (Fig. 3a). The highest inhibition was measured with the B11 peptide ($\sim 60\%$). The control peptides B11-glycine and A1 had a lower inhibition of ~ 30 and $\sim 20\%$, respectively (Fig. 3b). The comparative binding of B11 and B11-glycine indicated the importance of the aromatic amino acids in binding to mAb CS40.

Fig. 2. Competitive binding of synthetic peptides against ManLAM by ELISA. Inhibition of mAb CS40 binding to ManLAM was analysed by incubating the antibody with increasing amounts of peptides B11 (■) and A1 (○). Microtitre plate wells were coated with ManLAM (5 µg per well). After blocking, mAb CS40 (diluted 1 : 2000) was added to wells with or without increasing amounts of peptide. Binding of mAb CS40 to ManLAM was detected using an alkaline phosphatase-conjugated secondary Ab. The data represent the mean of triplicate wells. Binding of 100% was measured at an absorbance value of 0.2 nm. Representative results shown are for one of three independent experiments.

These findings demonstrated that the synthetic B11 peptide specifically binds mAb CS40, indicating that B11 is a promising mimic of ManLAM.

SPR determination of the affinity of synthetic B11 peptide to mAb CS40

The affinity of the B11 peptide to mAb CS40 was measured by SPR analysis. For this evaluation, we first calculated the affinity of mAb CS40 to ManLAM (Fig. 4). Measuring the binding of mAb CS40 to immobilized ManLAM over a range of concentrations (0–250 nM) (Fig. 4a) enabled calculation of the K_D value (3.00×10^{-7}) (Fig. 4b), using BIAEVALUATION software version 4.0. As low-molecular-mass analytes (<1 kDa) do not give sizeable relative responses under direct binding conditions using BIAcore (Adamczyk *et al.*, 2000; Montalto, 2001), we chose to calculate the affinity of B11 to mAb CS40 by solution competition experiments. For this, during the binding phase of the experiment, a solution of mAb CS40 or solutions of mAb CS40 plus different concentrations of B11 peptide were passed over the chip and the ability of B11 peptide to bind mAb CS40 was measured by the reduction

Fig. 3. Competitive binding of synthetic peptides against ManLAM by SPR analysis. The ability of B11 peptide to inhibit mAb CS40 binding to ManLAM was compared to that of the control peptides A1 and B11-glycine, as measured by competitive SPR analysis. (a) Binding of mAb CS40 alone (1; 250 nM) to immobilized ManLAM was compared with the binding of mAb CS40 (250 nM) after pre-incubation (30 min, 37 °C) with 50 µM peptide A1 (2), B11-glycine (3) or B11 (4). Flow parameters and binding conditions are outlined in Methods. The results are shown as an overlay of the sensorgrams, zeroed on the y-axis to the mean baseline before injection. The start injection time for each sample was set to zero on the x-axis. (b) Percentage inhibition of mAb CS40 binding to ManLAM by B11 peptide and the control A1 and B11-glycine peptides, as calculated at the time of maximal binding (39 s after injection).

Fig. 4. SPR analysis of mAb CS40 binding to ManLAM. SPR was performed at 30 °C as described in Methods. (a) Binding of a range of mAb CS40 concentrations [125 μ M (1), 62.5 μ M (2), 31.25 μ M (3), 7.8 μ M (4), 3.9 μ M (5) and 0 μ M (PBS only) (6)] to ManLAM, shown in an overlay of the sensorgrams, as described in the legend of Fig. 3. Flow parameters and binding conditions are described in Methods. (b) Solution-affinity analysis of various mAb CS40 concentrations injected over immobilized ManLAM. The slope was recorded 76 s after injection. To generate the calibration curve, a non-linear regression plot of the initial binding rate was plotted using a four-parameter fit. The K_D of 3.00×10^{-7} was established as described in Methods. Representative results shown are for one of three independent experiments.

in binding of mAb CS40 to ManLAM (Fig. 5a). The amount of mAb CS40 available to bind ManLAM was plotted against B11 peptide concentration (Fig. 5b). The K_D for the CS40–B11 interaction was determined to be 1.33×10^{-8} using BIAEVALUATION software.

Immunogenic properties of synthetic B11 peptide

To test whether B11 synthetic peptide was a mimotope and thus induced ManLAM-binding IgG and IgM, mice ($n=5$ per group) were immunized with KLH-conjugated and

non-conjugated peptide using various doses, routes of administration and adjuvants, as described in Methods. IgM and IgG ManLAM-binding Abs were detected only in the group immunized s.c. with 50 μ g conjugated peptide emulsified in MPL-TDM adjuvant. All groups showed high background levels of non-specific binding to ManLAM, as is commonly found in IgM ELISAs (Ochsenbein *et al.*, 1999). Specific IgM was detected 2 weeks after the first injection ($P < 0.01$ vs the control groups) (Fig. 6a). IgG ($P < 0.05$ vs the control groups) was detected 3 weeks after immunization (Fig. 6b). No Abs were detected in mice

Fig. 5. Inhibition of binding of mAb CS40 to ManLAM by B11 peptide. To measure the affinity of the mAb CS40 to B11 peptide, the inhibition of mAb CS40 binding to ManLAM by the B11 peptide was measured by SPR. (a) A range of B11 concentrations [0 μ M (PBS only) (1), 6 μ M (2), 12.5 μ M (3), 25 μ M (4), 50 μ M (5) and 75 μ M (6)] were pre-incubated (30 min, 37 °C) with mAb CS40 (125 nM) before injection over immobilized ManLAM. Flow parameters and binding conditions are outlined in Methods. Sensorgrams were overlaid as described in the legend to Fig. 3. (b) To measure the mAb CS40–B11 interaction, the amount of free mAb CS40 in solution was determined from the calibration curve and plotted against B11 peptide concentrations. A K_D of 1.33×10^{-8} was established as described in Methods. Representative results shown are for one of three independent experiments.

Fig. 6. Anti-ManLAM Ab titres in mice immunized with B11 synthetic peptide. ManLAM-binding IgM (a) and IgG (b) in the sera of mice ($n=5$) immunized twice (50 μ g per mouse) with KLH-conjugated B11 peptide in MPL-TDM (filled bars) or with KLH in MPL-TDM as a control (dotted bars) or with saline (naïve mice, open bars) were detected by ELISA as described in Methods. In (a), levels of IgM titres are shown after the first immunization at two dilutions (1 : 50, 1 : 200). In (b), IgG levels are shown after each immunization. Representative results shown are for one of three independent experiments. The A_{405} value of each sample in triplicate was read 30 min after addition of substrate and the mean $\pm$ SD was determined. Significance was determined by a one-tailed, unpaired Student's t -test: *, $P < 0.01$; **, $P < 0.05$.

immunized with free B11 peptide, as has been described for other free peptides (Taouji *et al.*, 2004; Wan *et al.*, 2001) and peptide mimotopes (Charalambous & Feavers, 2000). ManLAM-binding IgG was detected when mice were immunized s.c. with the conjugate in MPL-TDM adjuvant (50 μ g per mouse), whereas no Abs were detected with the other adjuvants tested (incomplete Freund's adjuvant and dimethyldioctadecylammonium bromide i.p.). This correlates with results in the literature showing that administration of low-immunogenic synthetic peptides in MPL-TDM is more efficient by the s.c. route (Leenaars *et al.*, 1998) and that administration of synthetic peptide in MPL-TDM adjuvant is more potent than administration in incomplete Freund's adjuvant (Honma *et al.*, 1999).

IgG B11 peptide-binding Abs in *M. tuberculosis*-infected mice

To further evaluate the mimotope characteristics of the peptide, we compared the levels of Ab binding to B11 peptide with binding levels to ManLAM in the serum of mice experimentally infected with *M. tuberculosis*. Thirty days ($n=6$) and 3 months ($n=4$) after infection,

both anti-ManLAM and B11 peptide-binding Abs were detected in the sera of BALB/c mice. The levels of antibody binding to ManLAM and B11 peptide were similar (Fig. 7). B11-binding Abs increased 3 months post-infection compared with 1 month post-infection; this was also seen for anti-ManLAM Abs.

IgG anti-peptide Abs in human TB patients

TB patients develop high levels of anti-ManLAM Abs (Hamasur *et al.*, 2001). Therefore, we used an ELISA to test whether the sera of TB patients contain Abs that can also bind B11 peptide. Similar to results found for ManLAM, we found significantly higher titres of B11 peptide-binding Abs in the sera of active pulmonary TB patients ($n=40$) compared with the sera of healthy individuals ($n=36$) (Fig. 8a). When defining a positive response as an A_{405} value +2 SD higher than the mean for negative-control subjects, specificity ($\sim 90\%$) and sensitivity ($\sim 50\%$) were found to be similar to those described for the MycoDot kit (Ratanasuwan *et al.*, 1997) (Fig. 8b). Twenty-one serum samples from the TB patients were positive for ManLAM and 20 were also positive for the B11 peptide, suggesting that the B11 peptide and ManLAM share a common epitope.

Using mimotopes for diagnosis has been suggested in a few studies such as that describing the polysaccharide mimotope of the *Salmonella* Vi antigen (Tang *et al.*, 2003). Obtaining such mimotopes has rarely been successful (Youn *et al.*, 2004). The results presented in this work

Fig. 7. Anti-ManLAM and B11-binding antibody titres in mice. Detection of serum IgG in mice that bound ManLAM (filled bars) or B11 (open bars) was performed by ELISA. The plates were coated with 5 μ g ManLAM or B11 peptide per well, as described in Methods. Results are shown for naïve mice ($n=6$) and *M. tuberculosis* (Mtb)-infected mice 30 days ($n=6$) and 3 months ($n=4$) post-infection. The median value is represented by a triangle. The A_{405} value of each sample in triplicate was read 30 min after the addition of substrate and the mean $\pm$ SD was determined. Significance was determined compared with the negative-control group by a one-tailed, unpaired Student's t -test: *, $P < 0.01$.

Fig. 8. Anti-ManLAM and B11-binding antibody titres in human sera. (a) Detection of IgG in human sera that bound ManLAM (●) and B11 (○) was performed by ELISA. The plates were coated with 5 μ g ManLAM or B11 peptide per well, as described in Methods. Sera from human healthy individuals ($n=36$) and TB patients ($n=40$) were tested. The median value is represented by a triangle. The results are shown as the mean A_{405} value of each triplicate sample read 20 min after the addition of substrate. Significance was determined against the negative-control group by a one-tailed, unpaired Student's t -test. *, $P < 0.0001$. (b) A positive response was defined as an A_{405} value + 2 SD higher than the mean for negative-control subjects. The specificity was 91.6 % for B11 and 88.8 % for ManLAM, and the sensitivity was 50 % for B11 and 52.5 % for ManLAM. The SD values for the ManLAM-coated plates were 0.057 for the healthy individuals and 0.226 for the TB patients. The SD values for the B11 peptide-coated plates were 0.035 for the healthy individuals and 0.112 for the TB patients.

indicate the possibility of using the B11 mimotope in combination with a serological diagnosis kit. Using a peptide for this purpose has major benefits compared with the commonly used TB diagnostic tests. Serological diagnosis is rapid and requires only a single visit to the clinic, in contrast to clinical isolation of the slow-growing *M. tuberculosis*, which takes ~3–4 weeks (Chan *et al.*, 2000). A synthetic peptide can be inexpensive to produce, well-defined and highly reproducible (Shin *et al.*, 2002), whilst purifying ManLAM from the virulent *M. tuberculosis* or from the bacillus Calmette–Guérin vaccine is a fastidious procedure. In addition, ManLAM from different batches may differ in antigenic properties.

Thus we conclude that the B11 peptide is an efficient and promising mimotope of ManLAM and shows potential for the clinical diagnosis of TB.

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