Characterization of B cell epitopes on the 16K antigen of *Mycobacterium tuberculosis*

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SUMMARY

To characterize the antigenic parts of the 16K protein of *Mycobacterium tuberculosis*, overlapping peptides according to the amino acid sequence of the 16K protein were synthesized. In total, 14 peptides of 20 amino acids in length with an overlap of 10 amino acids and two additional decapeptides (amino acids 31–40 and 61–70) were tested with eight anti-16K MoAbs and human sera. The common recognition site of MoAbs F67-8 and F67-16 was LRPTFDTRLM (amino acids 31–40) and of MoAbs F159-1 and F159-11 DPDKDVDIMV (amino acids 61–70). However, for binding of the MoAbs to these peptides additional amino acids were required at either the N- or C-terminus, suggesting that some kind of conformation is required. The recognition sites of the MoAbs F23-41, F23-49, F24-2 and TB68 could not be identified using the peptides, indicating that the MoAbs only bound to conformational epitopes and not to peptides which may contain parts of these epitopes. The MoAbs bound to β-galactosidase fusion proteins comprising parts of the 16K protein, indicating that some kind of native conformation is present on the recombinant proteins. Sera from 14 of 19 patients with tuberculosis and none from 19 controls reacted with the purified 16K protein. Sera from four of these 14 patients reacted with two overlapping peptides (amino acids 71–100). Apparently, antibodies in patients' sera against the 16K protein are predominantly directed against conformational epitopes.

Keywords *M. tuberculosis* B cell epitopes 16K antigen serology

INTRODUCTION

Tuberculosis is responsible for 2–3 million deaths annually [1], mostly in developing countries, but in urban regions of the USA tuberculosis is also increasing [2]. Biochemical, immunological and molecular biological characterization of *Mycobacterium tuberculosis* has led to the identification of several antigens which may be important for the development of new diagnostic methods or vaccines [3].

Recently we have described the nucleotide sequence of the 16K protein of *M. tuberculosis*, which has homology with various antigens belonging to the alpha-crystallin family of low molecular weight heat shock proteins [4]. The 16K protein has often been referred to as the 14K protein [5]. However, since the molecular mass of 16 277 D corresponds to the apparent mol. wt of 16K [6], we have named this antigen the 16K protein [4]. The 16K protein has been used for the identification of mycobacteria belonging to the *M. tuberculosis* complex from early cultures [7,8]. Expression of the protein seems to be related to the age of the culture [8]. Eight MoAbs directed against at least four

different epitopes on the 16K protein reacted specifically with mycobacteria from the *M. tuberculosis* complex, indicating that the 16K protein contains several *M. tuberculosis* complex-specific B cell epitopes [4]. Promising results have been produced in an ELISA in which the 16K protein was used as antigen [9] and in a competition assay using the MoAb TB68 against the 16K antigen [10,11].

Identification of B cell epitopes on the 16K protein which are specific for the *M. tuberculosis* complex may make possible the synthesis of peptides comprising these regions which could then be used in serodiagnostic assays. Such peptides have the advantage that they can be easily and reproducibly synthesized, whereas isolation from cultures is difficult and cumbersome. Using the PEP-scan method with peptides of 10 amino acids we could identify a murine B cell epitope (MoAb SA-12) on the 10K protein of *M. tuberculosis*, but we were unable to identify a human B cell epitope with this method [12]. Human antibodies produced after mycobacterial infections probably bind to conformational epitopes [12,13]. Since neighbouring residues may alter the antigenic conformation of a peptide [14–16], additional residues in longer peptides may contribute to a structure which more closely resembles the conformation of the epitope in its natural state.

The purpose of this study was to characterize murine and human B cell epitopes on the 16K antigen using the purified protein, β-galactosidase fusion proteins and overlapping peptides 20 amino acids in length.

MATERIALS AND METHODS

Monoclonal antibodies

The murine MoAbs F23-41, F23-49, F24-2 [6], F67-8, F67-16, F159-1, F159-11 [4] and TB68 [17] are directed against the 16K antigen of *M. tuberculosis* and are specific for the *M. tuberculosis* complex. MoAb SA-12 is directed against the 10K [18], F67-2 against the 65K [19] and F116-5 against the 24K antigen [20] of *M. tuberculosis*.

Human sera

Patients with tuberculosis. Samples of sera were obtained from 19 patients with a positive culture of *M. tuberculosis* who were either untreated or had been treated for less than 2 months. Fifteen patients had pulmonary tuberculosis and four had extrapulmonary tuberculosis.

Control subjects. Samples of sera were obtained from 18 Caucasian control subjects and one individual from India; four bacille Calmette–Guerin (BCG)-vaccinated individuals (including one person from India); five skin test positive persons, without a history of BCG vaccination or previous tuberculosis; eight skin test negative, not BCG-vaccinated, healthy individuals; two patients with *M. avium* infection, diagnosed by positive cultures from the sputum and blood.

Peptides

Sixteen overlapping peptides of 10 or 20 amino acids were synthesized according to the 144 amino acid sequence of the 16K antigen (Fig. 1) [4]. Solid-phase synthesis of the peptides was carried out manually via Fmoc technology [21] using a trialkoxy-diphenyl-methylester resin [22] and Castro's reagent (pyBOP) or coupling procedures [23]. The reagents for the synthesis were purchased from Novabiochem, Nottingham, UK. After cleavage with trifluoroacetic acid and deprotection, the peptides produced by this method were amidated at the carboxy terminus. Purification of the peptides was done chromatographically as described previously [24]. Sequence and purity was verified by reverse phase HPLC (Dionex Corporation, Sunnyvale, CA) and microsequencing analysis (Nottingham University Biopolymer Analysis Unit, Nottingham, UK).

Isolation of the 16K antigen from M. tuberculosis

Mycobacterium tuberculosis was cultured in Sauton medium and harvested as previously described [20]. A freeze-dried 100 000 *g* supernatant of sonicated *M. tuberculosis* was solubilized to a concentration of 20 mg dry weight per ml in 2·8% (w/v) SDS and 0·2 M sodium phosphate buffer, pH 6·8. The solution was heated for 5 min at 100°C and centrifuged at 30 000 *g*. The supernatant was passed through a 0·45 μm filter and applied in portions of 100 μl to two gel filtration columns (9·4 × 250 mm, Du Pont) used in series. An LKB liquid chromatography system was used. The columns were eluted with 0·1% SDS in 0·2 M sodium phosphate buffer, pH 6·8, at a flow rate of 0·5 ml/min. Fractions containing the 16K antigen were applied to the gel permeation columns once again and the fractions containing the 16K protein were pooled.

The purity of the 16K antigen was assessed by SDS–PAGE followed by staining with coomassie brilliant blue (CBB) and Western blot analysis.

Production of recombinant 16K protein

Expression of the *cro-lacZ-16K* hybrid genes from plasmids pPH6001, pTHL1060 and pTHT1031 was done as described by Zabeau & Stanley [25]. The expression of *lacZ-16K* hybrid gene from phage 60.1.1 was done using the method of Young *et al.* [26].

SDS–PAGE

This was done as described by Laemmli [27] on 13% (w/v) slab gels.

Western blot analysis

Western blot analysis was performed as described previously [28].

ELISA with peptides

Peptides were coated onto polystyrene microtitre plates with high binding capacity (Greiner, Nürtingen, Germany). Concentrations of 10^{-4} M, 10^{-5} M and 10^{-6} M (50 μl/well) in 0·05 M sodium bicarbonate buffer, pH 9·6 were used. Plates were incubated at 37°C for 18 h. Plates were blocked for 1 h at 37°C with 150 μl/well of either 10% (v/v) normal sheep (NSS) or normal goat serum (NGS) in sodium bicarbonate buffer, pH 9·6. MoAb TB68 (partially purified by sodium sulphate precipitation) was used at a dilution of 1:100, the other MoAbs were used at a dilution of 1:1000 in 10% NSS in phosphate buffered saline, pH 7·2, with 0·05% Tween 20 (PBST). Patient and control sera, diluted 1:100 in 10% NGS in PBST, were incubated at 37°C for 1 h and washed four times with PBST. Peroxidase labelled conjugate (sheep anti-mouse and goat anti-human conjugate for MoAbs and human sera, respectively) was added at a dilution of 1:1000 in PBST containing 10% NSS or NGS. After incubation at 37°C for 1 h, the plates were washed four times with PBST. Substrate was added and the absorbance was measured at 405 nm with an ELISA plate reader (Titertek Multiscan).

Reactivity of the MoAbs with the peptides was considered positive when the absorbance was higher than 50% of the maximum binding. The cutoff value for human sera was defined as the mean value of the control group plus twice the standard deviation.

Inhibition of binding by peptides

Polystyrene microtitre plates were incubated for 1 h with 50 μl/well of a solution containing 20 μg/ml of *M. tuberculosis* sonicate in 0·05 M bicarbonate buffer, pH 9·6. Non-specific binding was blocked with 200 μl/well of 1% (w/v) bovine serum albumin (BSA) and 10% NSS in PBS for 1 h at 37°C. Each MoAb was diluted to the concentration that gave 70% of the maximum binding. Six concentrations of the peptides (10^{-3} M, 4×10^{-4} M, 10^{-4} M, 10^{-5} M, 10^{-6} M and 10^{-7} M) were added to the solution containing the MoAb. The plates were then incubated with 50 μl/well of the MoAb-peptide dilutions for 1 h at 37°C and overnight at 4°C. ELISA was further performed as described in the ELISA with peptides subsection above.

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MATTLPVQRHPRSLFPEFSELFAAFPSFAG   30
         1        <----------------->
                     2    <----------->
    <-----3A----->            3
LRPTFDTRLMRLEDEMKEGRYEVRAELPGV   60
 <----------->
         4        <----------------->
                     5    <----------->
    <-----6A----->            6
DPDKDVDIMVRDGQLTIKAERTEQKDFDGR   90
 <----------->
         7        <----------------->
                     8    <----------->
                             9
SEFAYGSFVRTVSLPVGADEDDIKATYDKG   120
 <----------->
        10        <----------------->
                     11   <----------->
                             12
ILTVSVAVSEGKPTEKHIQIRSTN   144
 <----------->
        13        <----------->
             14
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Fig. 1. Peptides of 20 amino acids in length with an overlap of 10 amino acids (peptides 1–14) and two decapeptides (3A and 6A) representing the 16K protein of *Mycobacterium tuberculosis*.

Inhibition of binding was defined as a greater than 20% decrease of binding at a peptide concentration of 10^{-5} M or less. Decrease of binding was defined as a decrease of binding of at least 20% at a concentration of 10^{-4} M or higher.

ELISA with the purified 16K protein
Polystyrene microtitre plates were coated with 0·2 μg/well (2·5 × 10^{-7} M) of purified 16K protein diluted in 0·05 M sodium bicarbonate buffer, pH 9·6 and incubated for 1 h at 37°C and ELISA was performed as described previously [20]. Patient and control sera were diluted 1:400 in 10% NGS in PBST. Results are given as the 'absorbance index', calculated by expressing the result for the test serum as a fraction of the binding of a high positive reference serum, after correcting for reaction in control wells coated only with polyvinylpyrrolidone. The cutoff value of a positive reaction was defined as the mean value of the control group plus twice the standard deviation.

RESULTS

Murine B cell epitope mapping of the 16K antigen
Overlapping peptides (Fig. 1) based on the amino acid sequence of the 16K protein were synthesized and tested in ELISA with eight murine MoAbs directed against the 16K protein. MoAbs F67-8 and F67-16 both reacted with peptides 3 and 4 (Table 1). This finding indicated that their common recognition site was LRPTFDTRLM (amino acids 31–40). However, no reaction was found with peptide 3A consisting of these 10 amino acids. MoAbs F67-8 and F67-16 also reacted with peptide 8, but only when the coating concentration was 10^{-4} M. Examination of the amino acid sequences revealed homology in a four amino acid region between peptide 8 (FDGR) and peptides 3 and 4 (FDTR). Glycine is a conserved amino acid replacement for threonine. This suggests that the binding to peptide 8 of MoAbs F67-8 and F67-16 may be due to cross-reaction, only occurring at a high coating concentration.

MoAbs F159-1 and F159-11 reacted with peptides 6 and 7, suggesting that their common recognition site is DPDKDVDIMV (amino acids 61–70). The MoAbs did not react with peptide 6A, which consists of the ten overlapping residues (Table 1).

The four MoAbs F23-41, F23-49, F24-2 and TB68 showed no reaction with any of the peptides except for peptide 13 with which all eight MoAbs reacted weakly (Table 1). Other MoAbs, not directed against the 16K protein, such as SA-12, F67-2 and F116-5 also reacted with peptide 13, suggesting that this weak reaction is non-specific (results not shown).

Inhibition of binding by peptides
Inhibition by the peptides of binding of the MoAbs to the 16K protein was studied using six peptide concentrations (10^{-3} M, 4×10^{-4} M, 10^{-4} M, 10^{-5} M, 10^{-6} M and 10^{-7} M). In Figs 2 and 3 inhibitions by the peptides in two concentrations, i.e. 10^{-3} M and 10^{-5} M, are shown. Peptides 3 and 4 inhibited binding of MoAbs F67-8 and F67-16 at concentrations as low as 10^{-7} M (data not shown). Peptides 3A and 8 decreased binding of both MoAbs at concentrations of 10^{-3} M and 10^{-4} M (Fig. 2). This suggests that the MoAbs have a lower binding affinity to peptides 3A and 8 than to peptides 3 and 4. Binding of MoAbs F159-1 and F159-11 was inhibited by peptides 6 and 7 even at a concentration of 10^{-7} M. Peptide 6A inhibited binding at a concentration 10^{-5} M (Fig. 2).

Binding of MoAb F23-41 was decreased by peptides 3, 5 and 8, binding of F23-49 by peptides 4 and 8, of F24-2 by peptides 1, 3 and 3A, and of TB68 by peptides 1, 3 and 8 at a concentration of 10^{-3} M (Fig. 3). Different peptides decreased binding of different MoAbs, e.g. peptides 1 and 3 decreased binding of MoAb F24-2, but not of F23-49, and thus aspecific reactivity seems unlikely. No inhibition of binding of MoAbs F24-2, F23-49, F23-41 and TB68 was detected with peptide concentrations lower than 10^{-4} M (data not shown).

Only a limited amount of peptide 6 was available, therefore inhibition assays with this peptide were only done with MoAbs F67-8, F159-1 and F159-11. Since the other MoAbs showed no reaction with peptide 6 coated onto the plate and were not inhibited by peptide 5, 7 or 6A, it is unlikely that peptide 6 comprises a recognition site for these MoAbs.

Binding of the MoAbs to β-galactosidase fusion proteins comprising parts of the 16K protein
The reactivity of the anti-16K MoAbs with the β-galactosidase 16K fusion protein comprising amino acids 92–144 expressed by the plasmid pTHT1031 was analysed in addition to the reactions with other fusion proteins [4]. The results are summarized in Table 2. As expected, MoAbs F67-8 and F67-16 reacted with the fusion proteins that contained the common recognition site LRPTFDTRLM (amino acids 31–40) and MoAbs F159-1 and F159-11 with the fusion proteins that contained their common recognition site DPDKDVDIMV (amino acids 61–70). None of the MoAbs reacted with the fusion protein of pTHT1031. SDS-PAGE of this fusion protein revealed that the plasmid produced a clearly visible protein band of the expected size (data not shown). Thus, absence of reaction with the MoAbs is due to absence of binding rather than to instability of the fusion protein.

Human antibody response to the 16K antigen and peptides
The purity of the 16K protein isolated from *M. tuberculosis* was assessed by SDS-PAGE. SDS-PAGE showed a single band of 16K and Western blot analysis revealed that all MoAbs directed against the 16K antigen reacted with the purified antigen (results not shown). The purified 16K antigen was tested with a panel of

Table 1. Reactivity of 16K peptides in Western blot with eight MoAbs directed against the 16K protein

Peptide	Amino acid	F67-8	F67-16	F159-1	F159-11	F23-41	F23-49	F24-2	TB68
1	1–20	—	—	—	—	—	—	—	—
2	11–30	—	—	—	—	—	—	—	—
3	21–40	10^{-6}	10^{-6}	—	—	—	—	—	—
4	31–51	10^{-6}	10^{-6}	—	—	—	—	—	—
5	41–60	—	—	—	—	—	—	—	—
6	51–70	—	—	10^{-6}	10^{-6}	—	—	—	—
8	71–91	10^{-4}	10^{-4}	—	—	—	—	—	—
9	81–100	—	—	—	—	—	—	—	—
10	91–110	—	—	—	—	—	—	—	—
11	101–120	—	—	—	—	—	—	—	—
12	111–130	—	—	—	—	—	—	—	—
13	121–140	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}
14	131–144	—	—	—	—	—	—	—	—
3A	31–40	—	—	—	—	—	—	—	—
6A	61–70	—	—	—	—	—	—	—	—

—, No reaction; 10^{-4}, 10^{-5}, 10^{-6}, lowest coating concentration of the peptides in moles/l at which reactivity was still higher than 50% of the maximum binding.
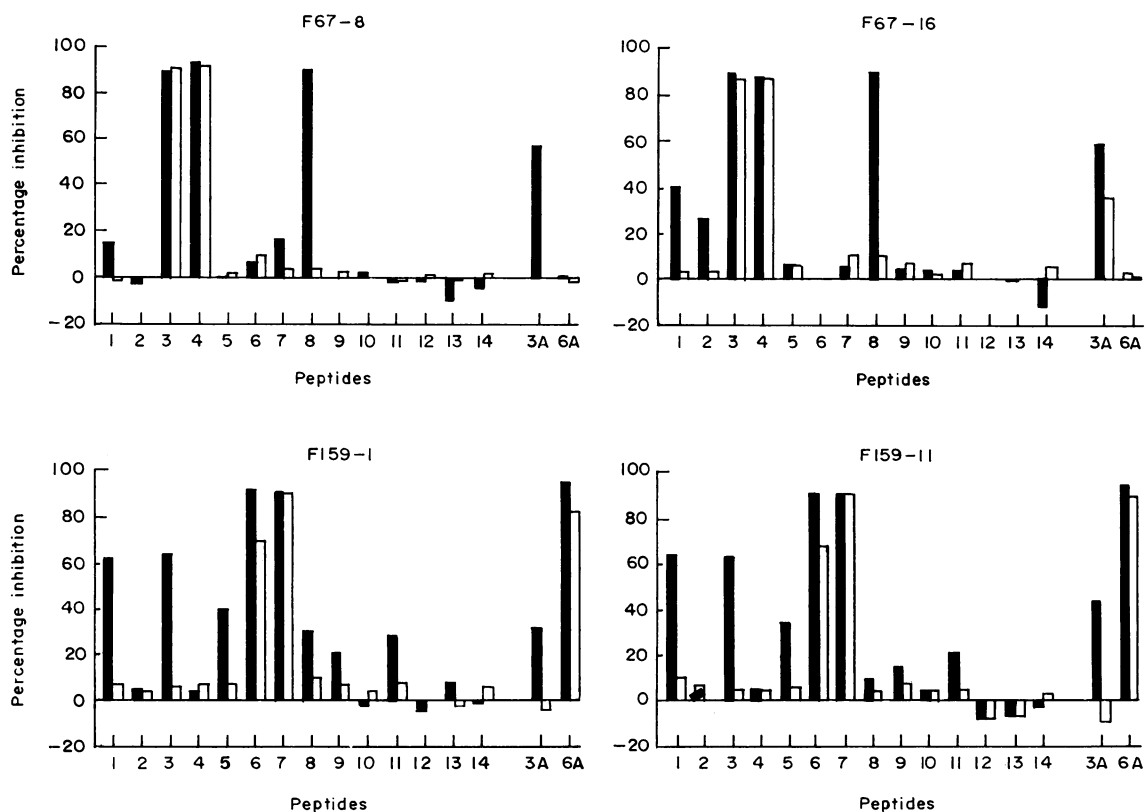

Fig. 2. Inhibition of binding to *Mycobacterium tuberculosis* sonicate by synthetic peptides at a dilution of 10^{-3} M (■) and at a dilution of 10^{-5} M (□) of MoAbs F67-8, F67-16, F159-1 and F159-11. The amino acid sequence and numbering of the peptides is as presented in Fig. 1.

sera from 19 patients with tuberculosis and from 19 control subjects. The mean value of sera from patients with tuberculosis tested with the 16K protein was significantly higher than that of control subjects (Fig. 4). Using as cutoff value the mean of the control group plus twice the standard deviation, sera from 14 patients with tuberculosis were positive and none of the control subjects. To map the human B cell epitopes, all sera were tested with peptides of the 16K protein (Fig. 1) coated on a polystyrene plate. Marked differences in the mean values of sera from patients with tuberculosis and from control subjects were found
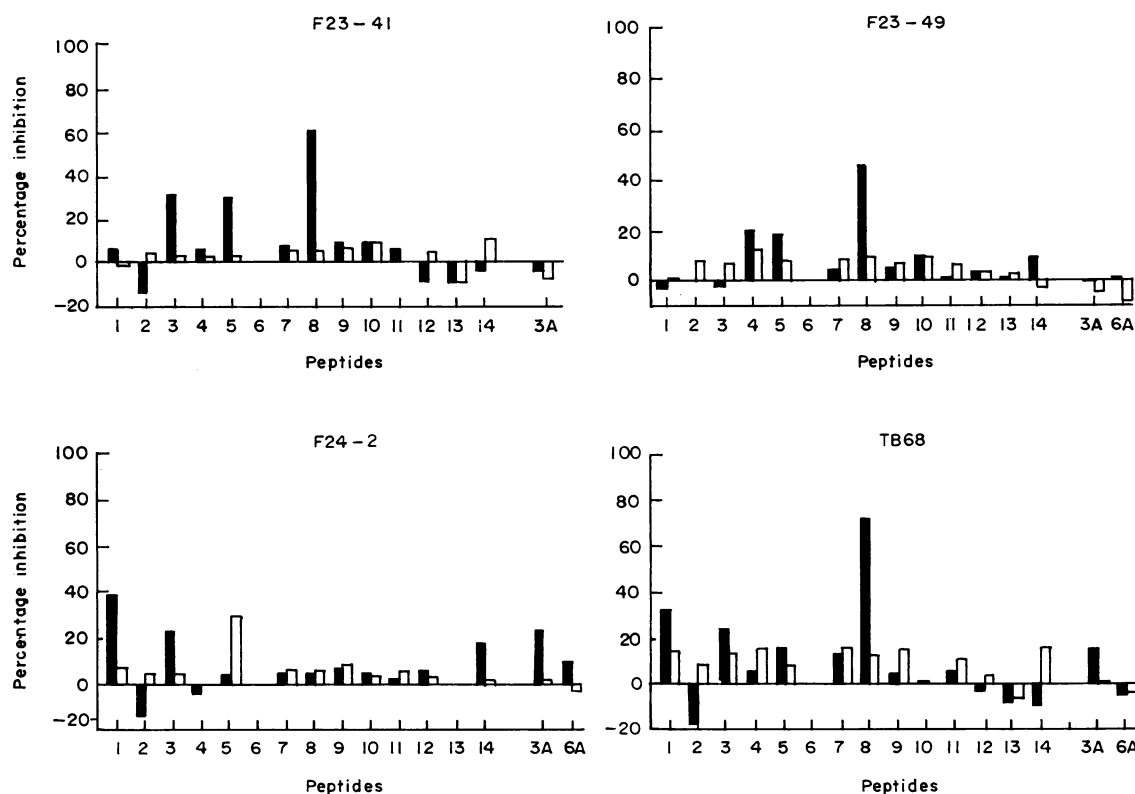
Fig. 3. Inhibition of binding to *Mycobacterium tuberculosis* sonicate by synthetic peptides at a dilution of 10^{-3} M (■) and at a dilution of 10^{-5} M (□) of MoAbs F23-41, F23-49, F24-2 and TB68. The amino acid sequence and numbering of the peptides is as presented in Fig 1.

Table 2. Summary of the reactivity in Western blot of MoAbs with β-galactosidase fusion proteins comprising part of the 16K protein

Plasmid or phage expressing (amino acids)	Monoclonal antibodies							
	F67-8	F67-16	F159-1	F159-11	F23-41	F23-49	F24-2	TB68
pPH6001 (10-92)	+	+	+	+	−	−	−	−
60.1.1 (10-144)	+	+	+	+	+	+	+	+
pTHL1060 (41-144)	−	−	+	+	+	+	+	+
pTHT1031 (92-144)	−	−	−	−	−	−	−	−

+, Reaction with the appropriate protein band; −, no reaction.

with three peptides, i.e. 8, 9 and 11 (Fig. 4). Using as a cutoff value the mean of the control group plus twice the standard deviation, four sera from patients with tuberculosis, positive with the purified 16K protein, were also positive with the overlapping peptides 8 and 9. None of the sera from control subjects was positive with these two peptides. No cutoff value could be established that discriminated between patients with tuberculosis and control subjects for peptide 11 or for any of the other peptides.

DISCUSSION

In this study overlapping peptides of 20 amino acids, synthesized according to the amino acid sequence of the 16K protein

[4] were used to characterize the binding sites of eight murine MoAbs and human sera. Binding sites of four MoAbs, i.e. F67-8, F67-16, F159-1 and F159-11, could be identified, indicating that their epitopes are at least partly linear. The binding sites of MoAbs F23-41, F23-49, F24-2 and TB68 could not be mapped, suggesting their epitopes are mainly conformational. The selection procedure of antibody-producing clones seems to have some influence; MoAbs F67-8, F67-16, F159-1 and F159-11 that recognize linear epitopes were selected by Western blotting [4]. MoAbs that recognize conformational epitopes were selected by ELISA, F23-41, F23-49 and F24-2 [6] or by radioimmunoassay (RIA), TB68 [17]. No association was found between immunization with live *M. tuberculosis*, sonicated bacteria, or supernatant of sonicated bacteria and the kind of epitopes recognized.
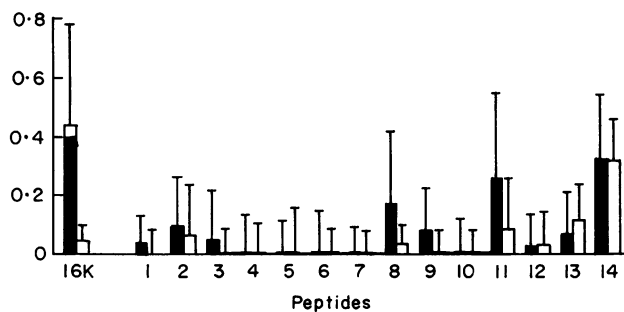
Fig. 4. Reactivity of human sera with the purified 16K protein and overlapping peptides representing the 16K protein. Each bar represents the mean of the sera tested and the standard deviation is indicated on top of the bars. ■, Sera from 19 patients with tuberculosis; □, sera from 19 control subjects. First bar on the left represents the reaction with the complete 16K protein expressed as absorbance index (described in Materials and Methods). The reactions with the peptides are expressed as absorbance at 405 nm. The amino acid sequence and numbering of the peptides is as presented in Fig. 1.

In an ELISA with the peptides coated on plates, MoAbs F67-8 and F67-16 bound to peptides 3 and 4, sharing the 10 amino acids region LRPTFDTRLM, but no reaction was found with peptide 3A, consisting of these 10 overlapping amino acids. Although it cannot be excluded that this test system is less suitable for shorter peptides, it is also possible that LRPTFDTRLM (amino acids 31–40) is essential for recognition and that additional amino acids are needed for binding of the antibodies. Similar findings were obtained with MoAbs F159-1 and F159-11, which bound to peptides 6 and 7, but not with peptide 6A consisting of the 10 overlapping amino acids, DPDKDVDIMV. MoAbs F23-41, F23-49, F24-2 and TB68 did not bind to any of the peptides in ELISA. These four MoAbs reacted strongly with the 16K protein isolated from a mycobacterial culture in ELISA (results not shown) and in Western blot [4]. Our results suggest that these four MoAbs require epitopes in a three-dimensional configuration for binding, and bind only marginally to peptides which may contain linear parts of these epitopes.

Characterization of epitopes in a binding assay is likely to be influenced by the assay conditions which may cause, for instance, changes in the conformation of the peptides [29]. Therefore, we studied the inhibition of binding of the MoAbs by the peptides. Binding of MoAbs F67-8 and F67-16 was inhibited strongly by peptides 3 and 4 and only at high concentrations by peptides 3A and 8. Binding of F159-1 and F159-11 was inhibited by peptides 6 and 7 and by peptide 6A only at a higher concentration. It can not be excluded that the inhibition ELISA was more sensitive, especially for the shorter peptides, than the ELISA using direct coating of the peptides. However, our findings suggest that the conformation of the peptides used in the inhibition assay more closely resembled the conformation of the native protein than the peptides coated on the plate. The results of the inhibition assay are in accordance with the results obtained with the peptides coated onto the plate and again suggest that more than 10 amino acids are required for binding of MoAbs F67-8, F67-16, F159-1 and F159-11. It is possible that shortening of the peptide, even though the segment removed is not a part of the reactive region, may impair favourable folding of the peptide required for binding with

antibody [30]. For significant helix formation and for biologically meaningful binding of antibodies, a peptide length of 15–20 amino acids is required [16]. Accordingly, when the peptides were coated on the plate MoAbs F67-8 and F67-16 reacted more strongly with peptide 8 than with the decapeptide 3A (Table 1). Peptide 8 comprises only a four amino acid homologous region to peptides 3 and 4, but consists of 20 amino acids. Thus, although the 10 overlapping amino acids of peptides 3 and 4 (F67-8 and F67-16) or 6 and 7 (F159-1 and F159-11) are essential for binding, additional residues at the N- or C-terminus are needed for the peptide to fold in a conformation that is required for binding of the MoAbs.

Binding of MoAbs F23-41, F23-49, F24-2 and TB68 to the 16K protein was decreased by some peptides only at concentrations of 10^{-3} M and 10^{-4} M (Fig. 3). This concentration is similar to the concentration at which peptide 8 decreased binding of MoAbs F67-8 and F67-16. Peptide 8 contains only part of the putative epitope. Similarly, it is possible that peptides 3, 5 and 8 may comprise part of the epitope recognized by MoAb F23-41. Peptides 4 and 8 may be part of the epitope recognized by MoAb F23-49, peptides 1 and 3 of MoAb F24-2 and peptides 1, 3 and 8 may be part of the epitope recognized by MoAb TB68. MoAb TB68 inhibited binding of F23-49, but not of F24-2 [31], thus it seems likely that MoAbs F23-41, F23-49, F24-2 and TB68 recognize at least three different epitopes.

A reaction of all eight MoAbs in Western blot was found with different β-galactosidase fusion proteins (Table 2). Possibly, the fusion proteins in Western blot had a conformation required for binding. MoAbs F23-41, F23-49, F24-2 and TB68 reacted with a recombinant clone expressing a fusion protein comprising amino acids 41–144 of the 16K protein, but not with the recombinant clones expressing fusion proteins containing amino acids 10–92 or 92–144, nor with peptide 9 (amino acids 80–100). It is therefore likely that the fusion protein expressing amino acids 41–144 of the 16K protein presented the recognition site of the MoAbs in a way that is needed for binding. The shorter fusion proteins either missed a part of the epitope or did not have this kind of conformation. Our findings indicate that β-galactosidase fusion proteins can fold into a conformation recognized by antibodies.

In human sera, antibodies against the purified 16K protein were present in 14 of the 19 patients with tuberculosis and in none of the 19 control persons, suggesting that this antigen may be valuable in the development of a serological test. A positive reaction with the overlapping peptides 8 and 9 was detectable with only four sera from patients with tuberculosis and from none of the control subjects. This suggests that the majority of human antibodies directed against the 16K protein are reacting with conformational epitopes. Although serological assays using peptides were promising in viral infections [32,33], our results presented here and elsewhere [12] indicate that the use of synthetic peptides is not useful in serological diagnosis of tuberculosis. Since the purified 16K protein shows promising results and conformational epitopes seem to be present on the recombinant DNA clones expressing parts of the 16K protein as a fusion protein to β-galactosidase, the latter may be valuable in serodiagnostic assays. However, reactivity of sera from all individuals with β-galactosidase remains a disadvantage of β-galactosidase fusion proteins. The value of the 16K protein overproduced as native protein or fused to other proteins needs to be determined.

ACKNOWLEDGMENTS

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