Homogeneity of Antibody Responses in Tuberculosis Patients

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The global resurgence of tuberculosis (TB) has made it imperative that improved diagnostics, therapeutics, and vaccines be devised for the control of this epidemic (21). A vast majority of TB cases occur in developing countries with limited resources where rapid, inexpensive diagnostic tests would aid in limiting the spread of infection in the community. Interest in the development of antibody-based diagnosis has been rekindled in recent years, and several companies and laboratories are currently involved in this venture (12, 17, 19, 27). A majority of currently available tests are based on a 38-kDa (PhoS1) antigen, alone or in combination with other proteins, but recent studies with several formats have reported sensitivities from only 41 to 55% (19). Although the 38-kDa antigen provides high specificity, the presence of anti-38-kDa antigen antibodies, primarily in individuals with chronic, cavitary disease, limits its utility in a diagnostic assay (3, 7, 16). The search for antigens that can provide more sensitive and specific diagnosis is therefore continuing (12, 17, 27).

In recent years, most studies have focused on the culture filtrate proteins (CFP) of in vitro-grown Mycobacterium tuberculosis, and several proteins have been cloned and evaluated for their serodiagnostic potential (12, 17, 27, 32). Studies performed with several different recombinant M. tuberculosis culture filtrate antigens suggest that immune recognition varies randomly from patient to patient and there is no definitive antigen or set of antigens that is recognized by all or a majority of patients (17). Based on these results, it was suggested that antibody responses of TB patients are heterogeneous and that a cocktail of a large number of antigens would be required to devise a serodiagnostic test for TB. Results with cocktails of as many as 10 to 12 recombinant antigens, including the 38-kDa antigen, have been used to achieve sensitivities ranging from 46 to 80% in different cohorts of TB patients (17; S. Perry, A. Cantararo, K. P. Lyashchenko, P. A. LoBue, A. Rendon, and M. L. Gennaro, Tuberculosis: Past, Present and Future, p. 44, 2000).

Recent studies from different laboratories have also shown that several proteins of M. tuberculosis that were expressed in Escherichia coli were unable to completely mimic their native counterparts in structure and function. Thus, the enzymatic activity of M. tuberculosis superoxide dismutase was retained by the recombinant form expressed in Mycobacterium smegmatis but not in the molecule expressed in E. coli (33). Antibodies to the culture-filtrate-derived 38-kDa protein are present in ~50 to 80% of smear-positive TB patients, but the recombinant 38-kDa protein provides sensitivities of 0 to 25% in similar cohorts (3, 7, 19, 31). Experiments have recently been reported wherein the reactivity of sera from cohorts of smear-positive and smear-negative TB patients with native Ag 85C and recombinant Ag 85C expressed in E. coli was evaluated under similar conditions. Results showed that although the native molecule was recognized by ~80% of the smear-positive and ~33% of the smear-negative sera, Ag 85C expressed in E. coli was recognized by only ~10% of the former and none of the latter sera (27). Similarly, sera from significantly fewer patients recognized recombinant MPT 32 expressed in E. coli when the reactivity of sera from the same TB patients with native and recombinant antigens was compared (27). Differences between native and recombinant M. tuberculosis proteins in the ability to elicit cellular responses have also been reported. Thus, in contrast to native MPT 64, the recombinant form expressed in E. coli was unable to elicit delayed-type hypersensitivity responses in sensitized animals (22), and comparison of native and recombinant heparin-binding hemagglutinin expressed in
E. coli showed that the recombinant form was unable to elicit gamma interferon production from peripheral blood mononuclear cells of purified protein derivative (PPD) skin test-positive individuals who responded strongly to the native heparin-binding hemagglutinin (F. Mascart, C. Masungi, J. P. Van Vooren, A. Drowart, K. Pethe, F. Menozzi, and C. Locht, Tuberculosis: Past, Present and Future, p. 94, 2000). These studies suggest that the lack of posttranslational modifications and alterations in protein conformation in recombinant molecules may lead to significant structural, functional, and immunological differences between the recombinant and the native proteins of M. tuberculosis. This concept is further strengthened by reports that native, deglycosylated MPT 32 had a significantly lower capacity to elicit delayed-type hypersensitivity reactivity in vivo and to activate T cells in vitro (23).

Our laboratories have performed a systematic analysis of the humoral immune responses of TB patients (16, 26, 27). Based on two-dimensional (2-D) fractionation of native culture filtrate antigens of M. tuberculosis and probing with pools of sera from PPD-positive healthy individuals and TB patients, it was demonstrated that only about a quarter of the more than 100 proteins present in culture filtrates of M. tuberculosis are strongly reactive with serum antibodies from TB patients, suggesting either that many of the proteins expressed during growth in bacteriological media may not be well expressed in vivo during active infection or that these proteins are poorly immunogenic (26). Moreover, the results suggest that the profile of antigens recognized by antibodies may be affected by disease progression (26). The goals of the present study were twofold: (i) to compare the profiles of culture filtrate antigens recognized by antibodies from cavitary and noncavitary TB patients and (ii) to determine the extent of variation in recognition of antigens that exists between individual TB patients. In contrast to earlier studies based on recombinant antigens, our results with native antigens of M. tuberculosis demonstrate a remarkable homogeneity in antigen profiles recognized by antibodies in TB patients, with little patient-to-patient variation. We provide evidence that there is a defined subset of culture filtrate antigens that is recognized by antibodies from both cavitary and noncavitary TB patients and an additional subset that is recognized only by patients with cavitary disease.

**MATERIALS AND METHODS**

**Antigen.** M. tuberculosis H37Rv (ATCC 27294) was grown in glycerol-alanine salt media for 14 days at 37°C with gentle agitation, the culture supernatant was removed from the cells by filtration, and the CFP were processed as described previously (16). This preparation contains more than 100 different proteins, and a 2-D map of the total protein profile, as well as a 2-D map of the antigens recognized by pooled sera from TB patients, has been described previously (26, 28).

**Subjects.** Serum samples from the following groups of individuals were included in the study:

(i) **Six PPD-negative healthy individuals.** All were U.S. citizens working in the human immunodeficiency virus (HIV) laboratory at the Veterans Affairs Medical Center, New York, N.Y.

(ii) **Twelve PPD-positive healthy controls.** These controls were either individuals who were recent immigrants from countries where M. tuberculosis is endemic, many of whom had been vaccinated with Mycobacterium bovis BCG or were individuals who are involved with patient care at the Veterans Affairs Medical Center, New York, N.Y.

(iii) **Thirteen noncavitary TB patients with no recognizable cavitary lesions on chest X rays.** Seven of these patients were sputum smear-negative for acid-fast bacilli (AFB); the remaining six were AFB positive. All patients were sputum culture AFB positive. None of the patients were HIV infected. These individuals were bled either prior to or within 2 weeks of the initiation of therapy for TB.

(iv) **Nineteen cavitary TB patients, with moderate-to-advanced cavitary lesions as determined by chest X rays.** All patients were sputum smear AFB positive. None of these patients were HIV infected. These patients were bled within 4 to 24 weeks after the initiation of therapy.

(v) **Four HIV-positive TB patients, two of whom were sputum smear AFB negative and two of whom were positive.** All four patients were sputum culture AFB positive. None of the patients had any radiological evidence of cavitary lesions. Chest X rays of one patient showed pleural effusion, a second patient had pulmonary infiltration, and the remaining two patients showed no visible pulmonary changes. All four patients were known to possess antibodies to the CFP of M. tuberculosis when tested by enzyme-linked immunosorbent assay in earlier studies (15, 27). These patients were bled either prior to or within 2 weeks of the initiation of therapy for TB.

**Adsorption of sera.** All sera were preadsorbed with E. coli lysates to reduce levels of cross-reactive antibodies as described earlier (26). Briefly, overnight cultures of E. coli grown in Luria-Bertani medium were centrifuged, and the bacterial pellets were resuspended in phosphate-buffered saline (PBS) containing protease inhibitors (1 mM each dithiothreitol, phenylmethylsulfonyl fluoride, and EDTA) and sonicated for 30 s. The lysates were suspended at 500 μg/ml, and the E. coli proteins were allowed to bind to nitrocellulose disks (90 mm) overnight by soaking the disks in volumes sufficient to cover them. The E. coli-coated disks were washed three times with PBS-Tween 20 (PBST), blocked with PBST-bovine serum albumin (5%), and washed again, and the individual sera diluted 1:10 with PBST were exposed to the immobilized proteins for 1.5 h. Each serum was exposed to eight cycles of depletion, filter sterilized, aliquoted, and stored frozen at −70°C.

**SDS-polyacrylamide gel electrophoresis and Western blotting.** 1- and 2-D polyacrylamide gel electrophoresis and immunoblotting were performed as described previously (16, 26). Briefly, 10 μg of CFP was fractionated on sodium dodecyl sulfate (SDS)–10% polyacrylamide gels for the 1-D separation, and after transfer of the fractionated proteins to nitrocellulose membranes, individual lanes were probed with a 1:100 dilution of individual sera. For 2-D fractionation, 30 μg of CFP suspended in isoelectric focusing buffer was applied to a 6% polyacrylamide isoelectric focusing tube gel containing 5% pharmalytes, pH 3 to 10 and 4 to 6.5, at a 1:4 ratio and focused for 3 h at 1 kV. The tube gels were electrophoresed on SDS–15% polyacrylamide gels, and Western blots were prepared from the fractionated proteins. Both 1- and 2-D blots were blocked with 3% bovine serum albumin in PBS, washed with PBST, and probed with individual sera. Alkaline- phosphatase-conjugated anti-human immunoglobulin G was used at a dilution of 1:2,000 with BCP (5-bromo-4-chloro-3-indolylphosphate)–nitroblue tetrazolium substrate (Kirkegaard & Perry Laboratories, Gaithersburg, Md.). 1-D blotting was performed over an ~2-year time period; however, each batch of blots included sera from both patients and controls so that all patient sera were evaluated in the context of control sera under exactly the same conditions in each individual experiment. The color development of all blots in a given batch was stopped when reactivity between control sera and any fractionated proteins on the blots was observed.

**RESULTS**

Several studies have demonstrated that M. tuberculosis possesses many proteins that have significant homology with analogous proteins in other mycobacterial and nonmycobacterial prokaryotes (reviewed in reference 14). Studies have also shown that almost all individuals (healthy or diseased) possess antibodies, elicited by exposure to commensal bacteria, environmental bacteria, and vaccinations, that cross-react with several M. tuberculosis antigens (1, 8, 11, 16). Previous studies from our lab have provided evidence that adsorption of sera with lysates of E. coli results in significant reduction in the levels of cross-reactive antibodies in sera from both healthy individuals and TB patients, without affecting the detection of antibodies against antigens/epitopes that are specific to mycobacteria (16). Using cross-reactive antibody-depleted sera, we have reported the identification of an immunodominant 88-kDa antigen in the culture filtrates of M. tuberculosis which is recognized by serum antibodies from ~75% of cavitary TB patients...
patients, ~35% of noncavitary TB patients, and ~70% of HIV-infected TB patients (16, 27). This 88-kDa antigen has now been identified as the 81-kDa malate synthase of *M. tuberculosis* (GlcB) (12; unpublished data) and is referred to here as the 81(88)-kDa antigen.

**Antigen profiles recognized by TB patients on 1-D Western blots.** When resolved on 1-D gels, the CFP preparation displayed a broad range of protein bands, from ~14 to 112 kDa, as seen by silver staining (data not shown). 1-D fractionated CFP were probed with cross-reactive antibody-depleted sera from 18 healthy individuals (6 were PPD negative and 12 were PPD positive) and 32 TB patients (13 noncavitary TB and 19 cavitary TB). The profile of antigens recognized by these sera is shown in Fig. 1.

Since different sera were tested on antigens fractionated on different gels, all fractionated proteins cannot be aligned for all lanes; however, each gel included its own molecular mass markers. There were no differences in the reactivities of sera from PPD-negative (Fig. 1, lanes 1 to 6) and PPD-positive (Fig. 1, lanes 7 to 18) healthy controls with the fractionated proteins. Despite adsorption with *E. coli* lysates, all 18 sera showed cross-reactivity with a doublet of protein bands at ~30 to 32 kDa and with a protein band at ~65 kDa, and the latter band was used to align the different blots. In addition to the reactivities with the ~30 to 32-kDa doublet and the ~65-kDa band, eight sera also recognized a 26-kDa band and two sera recognized an additional ~68-kDa band (Fig. 1).

Sera from all TB patients also reacted with the ~30 to 32- and ~65-kDa antigens that were reactive with the control sera, albeit more strongly. In addition, sera from several noncavitary TB patients showed weak reactivities with protein bands ranging from 26 to 120 kDa. In general, sera from smear-negative, noncavitary TB patients (Fig. 1, lanes 19 to 25) showed very poor reactivities compared to sera from smear-positive, noncavitary TB patients (Fig. 1, lanes 26 to 31). Sera from the six smear-positive patients were reactive with an 81(88)-kDa protein, although only one patient had strong reactivity. None of the 13 noncavitary TB patient sera showed any significant reactivity with any protein band at 38 kDa.

Of the 19 cavitary TB patients, sera from 3 patients showed no discernible reactivity with the fractionated CFP (Fig. 1, lanes 32 to 34), and one patient’s serum recognized only two major bands (Fig. 1, lane 35). Sera from the remaining 15 patients showed significantly stronger reactivity (Fig. 1, lanes 36–50). The antigen bands recognized by the sera from cavitary patients also ranged from ~26 to 120 kDa, and the patterns of the antigenic proteins were similar to those recognized by the sera from noncavitary TB patients. Sera from several cavitary patients also recognized some additional protein bands. The most prominent of these additional bands was the 38-kDa protein, which was identified as the PhoS1 protein on the basis of reactivity with monoclonal antibody IT23 (Fig. 1, lane 51). The 81(88)-kDa protein band was strongly recognized by sera from 15 of 19 patients, including all 11 patients who possessed anti-38-kDa protein antibodies.

**Antigen profiles recognized on 2-D Western blots.** Since 1-D electrophoresis provides limited resolution, further analysis of the antigen repertoires recognized by sera from individual noncavitary and cavitary TB patients was performed by 2-D electrophoresis and Western blotting. The reactivity of sera from individual smear-negative, noncavitary TB patients was compared with the reactivity of individual smear-positive, cavitary TB patients. A map of 2-D fractionated CFP, with identities of
several proteins, was generated recently (28). Mapping of the seroreactive culture filtrate antigens by probing 2-D fractionated proteins with a pool of sera from TB patients has also been published (26). In the present study, major antigens whose identities are known have been indicated and the remaining antigens are referred to as protein spots. Since many of the CFP occur in multiple isomeric forms, appearing as protein clusters, an exact determination of the number of reactive proteins is not possible and closely separating protein clusters have arbitrarily been considered single proteins (28).

The reactivities of sera from four PPD-positive healthy controls with the CFP on 2-D blots are shown in Fig. 2. All four individuals showed some reactivity with the individual components of the Ag 85 complex, at about 30 to 32 kDa. The same pattern of reactivity was observed with sera from healthy individuals whose PPD status was not known (data not shown). In contrast to the reactivity observed on the 1-D blots, on 2-D blots no reactivity with any antigens at $\approx 65$ kDa was discernible. Previous studies from our lab in which pooled sera (six individuals in each pool) were used to probe similar 2-D blots had shown that there are three proteins of $\approx 65$ kDa which are recognized by sera from controls and TB patients (26). Possibly, in individual sera, the titers of antibodies to the three proteins are too low to detect the fractionated proteins, although when the three overlap in the 1-D blots, a band at $\approx 65$ kDa is discernible.

The reactivities of sera from four HIV-negative, smear-negative, noncavitary TB patients with fractionated CFP are shown in Fig. 3. Since the sera from smear-negative, noncavitary TB patients had shown poor reactivity on the 1-D blots which were probed with a 1:100 serum dilution (Fig. 1, lanes 19 to 25), these sera were tested at a dilution of 1:50 on the 2-D blots. Control sera for these experiments were also used at a 1:50 dilution. Sera from all four noncavitary TB patients showed strong reactivity with the Ag 85 complex proteins and poorer reactivity with a subset of $\approx 12$ culture filtrate antigens. The subset of antigens recognized by the four individual patients showed a significant overlap in that each individual patient serum reacted with at least 10 of the 12 antigens. All patients had detectable antibodies against the 81(88)-kDa protein, although except for one patient, the reactivity was weak. Sera from three of the four patients had antibodies directed...
against MPT 51. Sera from two patients showed faint reactivity with two isomers of the 38-kDa PhoS protein, and sera from one patient showed faint reactivity with two isomers of MPT 32. Three of the four sera were also tested at a dilution of 1:200, and while the antigen profiles recognized at the two dilutions were similar, the reactivity at the latter dilution was significantly poorer. Reactivity with sera from the remaining three smear-negative, noncavitary TB patients was too weak to provide clear results even when tested at a 1:50 dilution (not shown).

The reactivities of sera from five smear-positive, cavitary TB patients tested at a dilution of 1:200, are shown in Fig. 4. Compared to the noncavitary TB patients, sera from all five cavitary TB patients showed intense reactivity. All five cavitary TB patients possessed antibodies against 9 or more antigens from the subset of 12 antigens that was recognized by the sera from noncavitary TB patients. All five sera showed strong reactivity with the 81(88)-kDa antigen and the 38-kDa antigen, and three of five patients’ sera reacted with MPT 32. In contrast to the noncavitary TB patient sera, only one of five cavitary TB patient sera reacted with MPT 51. In addition to the antigens that were reactive with sera from both cavitary and noncavitary TB patients, there were some additional antigens that were recognized only by the cavitary TB patient sera. These include an ~50-kDa protein and another 38-kDa protein that was recognized by all five patient sera tested.

In order to further confirm the association between the subset of culture filtrate antigens recognized during noncavitary TB, sera from four HIV-infected TB patients, all of whom had no recognizable cavitary lesions, were tested for reactivity with the 2-D fractionated CFP. The sera from all four HIV-positive TB patients (diluted 1:200) showed strong reactivity with the Ag 85 complex proteins and with several discrete proteins (~14) on the 2-D blots (Fig. 5). The repertoires of proteins recognized by the individual patient sera were remarkably consistent between the four patients (Fig. 5) and showed significant overlap with the antigens that were recognized by sera from non-HIV, noncavitary TB patients. All four patient sera showed reactivity with the 81(88)-kDa antigen and
FIG. 4. Reactivities of sera from five non-HIV-infected, smear-positive, cavitary TB patients (diluted 1:200) with 2-D polyacrylamide gel-fractionated, lipoarabinomannan-free CFP of *M. tuberculosis*. Thirty micrograms of antigen was fractionated on each blot. Antigens recognized by sera from controls also are circled in blue. Antigens recognized by sera from noncavitary and cavitary TB patients but not from control individuals are circled in green. Antigens recognized primarily, and strongly, by sera from cavitary TB patients are circled in red. 2-D blots and lanes corresponding to Fig. 1: panel A, lane 47; panel B, not known; panel C, lane 48, panel D, lane 41; panel E, not known. Molecular masses (in kilodaltons) are indicated on the left of each panel.
with MPT 51; one of the four patients showed reactivity with MPT 32 and with two isomers of the 38-kDa protein.

Thus, these 1- and 2-D blots show that there is a set of ~12 to 15 culture filtrate antigens that are recognized by antibodies from both noncavitary and cavitary TB patients. One antigen from this set has been identified as the 81(88)-kDa protein in earlier studies (26), and another antigen is MPT 51. The identities of the remaining antigens have yet to be determined. In addition, there is another set of antigens that is recognized primarily by antibodies from patients who have cavitary lesions. MPT 32 and the 38-kDa PhoS1 protein belong to this category of antigens.

**DISCUSSION**

The 1- and 2-D immunoblots with individual patient sera provide evidence that there is a remarkable consistency in the repertoire of culture filtrate antigens of *M. tuberculosis* that are recognized by antibodies from TB patients. There is a subset of 12 to 15 culture filtrate antigens, a majority of which are recognized by both noncavitary and cavitary TB patients. The recognition of the same defined subset of proteins in noncavitary TB patients, regardless of HIV infection, further emphasizes the extent of homogeneity that prevails. Moreover, although the cavitary TB patients had antibodies directed against some additional antigens, the set of antigens that is associated with cavitary disease is also well conserved in that sera from individual patients recognized an overlapping subset of proteins. Some differences in the antigen recognition profile between individual patients are expected because antibody titers against individual antigens differ among individuals. Moreover, low titers of antibodies can be bound in immune complexes (4, 20). In addition, there may be some differences in the mycobacterial strains that infect different individuals. In view of these possible variables, studies based on native culture filtrate antigens of *M. tuberculosis* provide evidence that the antibody responses of TB patients are well conserved, with little patient-to-patient variation. These observations are in direct contrast to earlier reports based on studies with recombinant culture
filtrate antigens in which antibodies from individual TB patients, regardless of the stage of the disease, were found to randomly recognize different cloned proteins (17). Many recombinant proteins of M. tuberculosis have been shown to be poorly recognized by the immune responses initially elicited by the native antigens (22, 27, 31; Mascart et al., Tuberculosis), and the limited recognition of individual cloned proteins by patient antibodies may be a consequence of their lack of important antigenic determinants (17). This was also observed in earlier studies wherein reactivities of the same cohort of TB and control sera with native and recombinant Ag 85C and MPT 32 were compared under identical conditions, and the reactivity with the recombinant forms was found to be significantly compromised (27). In view of our present results and the increasing number of reports about differences between some native and recombinant mycobacterial antigens, studies based entirely on cloned molecules need to be interpreted with caution.

The remarkable similarities in the profiles of antigens recognized by smear-negative (Fig. 3 and 5C and D) and smear-positive (Fig. 5A and B) noncavitary TB patients suggest that higher bacterial loads do not significantly affect the profile of antigens expressed by the in vivo bacteria. In contrast, while the sera of smear-positive, noncavitary TB patients (Fig. 5A and B) recognize the same antigens as the sera of smear-negative, noncavitary TB patients, the sera of smear-positive, cavitary TB patients have antibodies against additional antigens (Fig. 4). These comparisons show that the presence or absence of cavitary lesions has a significant, reproducible effect on the profile of antigens that are recognized by the antibodies.

The presence of antibodies against some antigens primarily in cavitary TB patients suggests that in vivo either some of the antigens found in culture filtrates are expressed primarily during extracellular replication of the bacteria in liquefied caseous material or these antigens are accessible to the immune response only during this stage of the disease. Interestingly, although the cavitary TB patients were clinically similar and all patients had antibodies to the subset of antigens recognized by the noncavitary TB patients, the additional antigens recognized by the sera of individual cavitary TB patients showed some variation. Thus, the 1-D blots showed negligible reactivity with sera from three patients. Whether this was due to a real absence of antibodies or to a mop-up of antibodies in immune complexes needs to be investigated (4, 20). Moreover, anti-38-kDa antibodies were seen in 11 of 19 (58%) cavitary TB patients. Among the five patient sera tested on the 2-D blots, all of whom had anti-38-kDa antibodies, all five also recognized another 38-kDa protein and a 50-kDa protein, but anti-MPT 32 antibodies were present only in three of five sera. It may be argued that differences in the infecting clinical isolates or in the immune responses generated in different genetic backgrounds are responsible, although the homogeneity of responses to the subset of antigens recognized by both cavitary and noncavitary TB patients would suggest that the latter may not have a major influence. Humans with pulmonary TB often have lesions at different stages of liquefaction, and it is possible that the differences between individual cavitary TB patients reflect differences in the extent of cavitation, liquefaction, bacterial replication, and cavitary bacterial loads or other differences in the environments in cavities in different individuals. Studies in which open, closed, and end-stage cavitary lesions from the same TB patients were studied showed that the bacterial loads and the metabolic state of the in vivo bacteria varied in different types of cavities (18). More recently, studies of cavitary lesions in aerosol-infected rabbits have also shown that multiplication of M. tuberculosis in liquefied caseous material varies from cavity to cavity, and it was suggested that in vivo extracellular bacillary growth may require specific environmental conditions in the cavity (5).

Despite extensive adsorption with E. coli lysates, the control sera showed cross-reactivity with members of the Ag 85 complex (26). Studies from other labs have reported similar results (30). Homologues of Ag 85 have been reported to be present in nonpathogenic mycobacteria and in corynebacteria (9), and possibly, environmental exposure to these organisms is responsible for eliciting the cross-reactive antibodies. However, the stronger reactivity of sera from TB patients with these antigens suggests that the mycobacterial proteins also possess specific seroaggressive epitopes.

Despite significant efforts by several laboratories, success in developing a serodiagnostic test for TB has been limited. Our studies provide explanations for several of the observations made and problems encountered: the native antigens that have been purified and used in serodiagnosis were chosen because they are major constituents of culture filtrates (6, 24, 25), whereas the 2-D immunoblots show that most of the commonly recognized seroreactive antigens, especially those that elicit antibodies in both cavitary and noncavitary TB patients, are minor constituents of the culture filtrates, making them unlikely candidates of choice for biochemical purification from culture filtrates. Also, many studies were based on sonicates of M. tuberculosis, which would contain several of the conserved ubiquitous prokaryotic proteins (heat shock proteins, enzymes of biosynthetic pathways, and structural proteins, etc.), many of which may react with cross-reacting antibodies in the sera (2). In contrast, studies from our laboratories are based on culture filtrates of bacteria at the late logarithmic phase of growth, which lack a vast majority of the cytoplasmic proteins (28), and the sera are adsorbed with lysates of E. coli to reduce the levels of cross-reactive antibodies (16).

It is becoming increasingly clear that the immunogenicity of many proteins is affected by posttranslational modifications, especially glycosylation and acylation (22, 23, 27, 31; Mascart et al., Tuberculosis). Indeed, even recombinant MPT 32 expressed in M. smegmatis was unable to mimic native MPT 32 and was poorly recognized by sera that recognized the native molecule (unpublished data). Thus, even when antigens that elicit antibodies during natural infection are cloned, the recombinant forms may fail to express all the epitopes presented by the native molecules. However, since recombinant 65- and 12-kDa antigens of M. tuberculosis were well recognized by patient antibodies (13, 31), as was the 81(88)-kDa antigen (12, 27), these results suggest that E. coli is an acceptable host for expression of some antigens but not all of them, especially not for those where posttranslational modifications or conformational epitopes may be involved in the immunological reactivity.

Our results with immunoblotting correlate well with the earlier observations from several investigators in that anti-38-kDa protein antibodies were present only in ~60% of the
cavitary TB patients, and they were rarely present in noncavitary TB patients (3, 7). The limited diagnostic capacity of the 38-kDa PhoS1 protein in TB patients reported by several laboratories is due to the absence of antibodies in patients who have not developed cavitary lesions. The absence of anti-38-kDa protein and anti-MPT 32 antibodies in most HIV-positive TB patients may be related to their inability to develop cavitary lesions (10, 15, 29) rather than to dysfunctional B-cell responses. It is encouraging that several antigens that are recognized by antibodies in the absence of extensive cavitary lesions, and in HIV-infected individuals, are present in culture filtrates since their identification and use are likely to provide improved diagnostic tests for cointected patients.

Although antibody titers were not determined, sera from the noncavitary, HIV-positive TB patients showed stronger reactivity on the 2-D immunoblots of the CFP than sera from the non-HIV-infected, noncavitary TB patients, suggesting that they had higher titers of antibodies. Better reactivity with the purified 81(88)-kDa antigen was also observed in enzyme-linked immunosorbent assay-based studies (27). Histopathological studies have shown that even in the absence of cavitary lesions, HIV-infected TB patients can have very high bacillary loads in their lungs, and high antigenic stimulation may be responsible for the higher titers of anticymocellular antibodies observed in these patients (10).

Antigens that present only conformational epitopes to the immune system were not recognized in our present study since fractionation on SDS gels destroys most conformational epitopes. Moreover, it is possible that there are antigens expressed by M. tuberculosis in vivo but that are absent in our culture filtrate preparation. Despite these limitations, our studies clearly show that the antibody responses in different TB patients show significant homogeneity in being directed against a well-defined subset of antigens. This homogeneity is borne out by previous studies from our and other laboratories which showed that the use of only one antigen of this subset, the 81(88)-kDa protein, enables recognition of antibodies in 70 to 90% of non-HIV- and HIV-infected TB patients (12, 27). The current mapping with individual patient sera suggests that inclusion of a small number of additional antigens from this subset will further enhance the sensitivity of antibody detection in TB patients, especially in difficult-to-diagnose, smear-negative, noncavitary TB patients.

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REFERENCES


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