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Antibody Profiles Characteristic of *Mycobacterium tuberculosis* Infection State

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The relationship between specific antibody profiles and tuberculosis (TB) state was investigated by measuring serum antibody levels to six *Mycobacterium tuberculosis* antigens in human subjects grouped into four diagnostic categories: active disease, inactive (past) tuberculosis, latent infection without radiographic chest abnormalities, and infection free. Statistical data analyses showed that the latter two groups were serologically indistinguishable and that active tuberculosis and inactive tuberculosis were characterized by different antibody profiles. Antibodies to the 38-kDa antigen, alanine dehydrogenase, and Rv2626c were associated with active TB, while antibodies to the 16-kDa antigen, ferredoxin A, and ESAT-6 were associated with inactive TB. Thus, the targets of the immune response vary with tuberculosis state. The correlation between bacterial antigen production and infection stage was investigated in mice infected with *M. tuberculosis* by bacterial transcription profiling. It was found that levels of transcripts encoding the six *M. tuberculosis* antigens varied during infection. Together, the data indicate that antigen composition of tubercle bacilli varies with stage of infection and that immunoprofiling can distinguish between tuberculosis states.

Infection with *Mycobacterium tuberculosis* passes through several stages. In most cases, host defenses either clear infection or drive it into a chronic, latent state that is asymptomatic and potentially long lasting. Subsequent weakening of host immunity allows reactivation of disease, which typically localizes in the lung. Resolution of lung disease, which may occur either spontaneously or as a result of antibiotic treatment, leads to inactive tuberculosis (TB) (1), a state associated with a greater risk (up to 20-fold) of reactivating disease than latent infection (6, 7, 16). It has been suggested that the physiological state of *M. tuberculosis* varies during the course of infection (10, 15, 17, 23, 26). Work with mouse models has supported this idea by showing that *M. tuberculosis* adaptation to host immunity involves changes in bacterial metabolism (for examples, see references 14 and 27) and in bacterial transcription profiles (19). The latter includes genes encoding immunodominant antigens (Ags) of *M. tuberculosis* (20), which is suggestive of changes in bacterial antigen composition over the course of infection. However, little is known about the metabolic and antigenic changes of tubercle bacilli during human infection.

The metabolic state of tubercle bacilli in the human lung must be investigated by indirect methods, because gaining access to tubercle bacilli in the human lung is exceedingly difficult. An extensive body of literature suggests the possibility that antigen-specific immune responses can provide an indirect readout of bacterial metabolic changes during infection. For example, the antibody against the secreted 38-kDa antigen of *M. tuberculosis* best correlates with advanced, multibacillary disease, while the antibody against the cell-associated 16-kDa antigen (α-crystallin) is detected preferentially in asymptomatic, infected individuals (3, 4, 21, 28). Thus, tuberculosis states can be characterized by particular antibody profiles.

The purpose of the present study was to characterize antibody profiles for six *M. tuberculosis* antigens in four tuberculosis states: active tuberculosis, inactive (past) tuberculosis, latent infection (without radiographic abnormalities), and infection free. We found that active tuberculosis and inactive tuberculosis were associated with serological reactivity to different antigen sets. In follow-up experiments, we found that levels of *M. tuberculosis* transcripts encoding the six antigens of *M. tuberculosis* varied in the lung of mice during the course of *M. tuberculosis* infection. Collectively, these data suggest that the antigen composition of tubercle bacilli changes over the course of infection and that antibody profiles reflect those changes.

MATERIALS AND METHODS

Study population. The study was conducted with stored serum samples obtained between 1995 and 1998 from immigrants referred to the Montreal Chest Institute, Montreal, Canada, as TB suspects and from Canadian-born persons with pulmonary TB. Informed consent was obtained from patients; human experimentation guidelines of the U.S. Department of Health and Human Services and/or those of the authors' institutions (Montreal Chest Institute Research Ethics Board and New York University Institutional Review Board) were followed in the conduct of this work.

Sera were collected from four groups prior to diagnosis.

(i) Active tuberculosis. A total of 53 persons were diagnosed as having active pulmonary TB based on microbiological data and clinical evaluation. Seven were culture and smear positive, 31 were culture positive and smear negative, and the remaining 15 were negative according to both tests. Diagnosis of active TB in the latter group was based on response to anti-TB treatment, as assessed by evaluation of paired chest X-ray (CXR) films by two independent reviewers who were blinded to the identity of patients, diagnosis, and chronological order of films.

(ii) Inactive tuberculosis. The inactive tuberculosis category was defined by a positive response to the tuberculin skin test (TST) (>10 mm), the absence of clinical, bacteriological, or radiographic evidence of current disease, and abnor-

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mal but stable CXR findings consistent with past TB (1). Evaluation of response to anti-TB therapy was conducted on paired chest X-ray films as described above; patients who showed no chest X-ray improvement with anti-TB chemotherapy were classified as inactive TB cases. Inactive TB was diagnosed in 218 persons, none of whom had a history of treated TB.

(iii) TST positive. A total of 32 subjects were positive by TST (>10 mm) and had a normal chest X ray.

(iv) TST negative. A total of 50 study subjects were TST negative.

Sera from all subjects in the study were tested by enzyme-linked immunosorbent assay (ELISA) for antibodies to *M. tuberculosis* antigens, as described below.

Antigens. Proteins of *M. tuberculosis* were selected either because they were known to elicit antibody responses, e.g., the 38-kDa Ag (8), ESAT-6 (22), glutamine synthase (GluS) (9), alanine dehydrogenase (AlaDH) (11), superoxide dismutase A (SodA) (30), and the 16-kDa Ag (29), or because they were expected to be preferentially expressed in nonreplicating bacilli. For example, the 16-kDa Ag (α-crystallin), ferredoxin A (FdxA), and Rv2626c are all encoded by genes found in the so-called dormancy (*dosR*) regulon (18, 25). *M. tuberculosis* proteins were expressed as recombinant products in *Escherichia coli* and purified to near homogeneity by sequential column chromatography, as described previously (5).

ELISA. Polystyrene 96-well microtiter plates (Bio-Rad Laboratories, Hercules, CA) were coated at 4°C overnight with 2.0 μg/ml (0.2 ml/well) purified antigen in carbonate-bicarbonate buffer (pH 9.6). Plates were blocked with 1% nonfat skim milk in phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20 (PBS-T) for 3 h at 37°C and washed twice with PBS-T. Serum was diluted 1:50 in PBS-T containing 1% skim milk, and 0.2 ml of diluted serum was added to antigen-coated wells in duplicate and incubated for 30 min at 37°C. Positive and negative control sera were included in duplicate to control for inter- and intrarun variations. After washing with PBS-T, plates were incubated with 0.2 ml/well goat anti-human immunoglobulin G conjugated with horseradish peroxidase (Dako, Glostrup, Denmark) diluted 1:20,000 in PBS-T plus 1% skim milk for 30 min at 37°C. Plates were washed with PBS-T, and enzyme activity was assayed by incubation for 30 min at room temperature with 0.2 ml/well TMB peroxidase substrate kit (Bio-Rad Laboratories, Hercules, CA). Reactions were stopped by adding 0.05 ml of 1N H_2SO_4. Optical density at 450 nm (OD_{450}) was measured with an automatic microplate reader (Spectra Shell; Tecan Systems Inc., San Jose, CA).

Serologic data analysis. Chi-square tests were used to assess associations between demographic and diagnostic categories. Comparisons of antibody responses by tuberculosis state were conducted using nonparametric tests, such as the Wilcoxon rank sum test for two independent variables and the Kruskal-Wallis test for three or more independent variables.

A logistic regression model was estimated separately for active TB versus inactive TB cases and for "CXR-normal" (i.e., subjects who had no radiographic signs of active or inactive TB) versus inactive TB cases via backward elimination from a full model containing antibody responses identified as statistically significant by the analysis described above, adjusting for BCG vaccination and world region of origin. Antibody results were dichotomized into the categories "low" and "high" according to being higher or lower than the median or were trichotomized into "low," "medium," and "high" according to the tertiles of the antibody distribution. The initial modeling approach utilized a trichotomous parameterization of the antibodies; when differences in risk between two contiguous antibody categories were small, a dichotomous parameterization was selected.

Mouse infection and bacterial RNA measurements. Infection of B6D2/F1 mice (Charles River Breeding Laboratories) with ~100 CFU of *M. tuberculosis* strain HN878 cultivated to mid-log-phase growth in Proskauer and Beck medium containing 0.01% Tween 80 was carried out as described previously (13). Guidelines of the U.S. Department of Health and Human Services and those of the authors' institutions were followed in the conduct of animal experiments in the present study. At selected times, lungs were harvested from three to four mice per time point. The number of CFU was determined by plating 10-fold serial dilutions of homogenates from half of the lung (attached to the left bronchus) on plates containing Middlebrook 7H11 agar enriched with oleic acid, albumin, dextrose, and catalase, followed by bacterial colony counting after 3 weeks of incubation at 37°C. The half-lung attached to the right bronchus was used to measure selected mRNAs and 16S rRNA of *M. tuberculosis*. Methods used for lung RNA extraction and quantification of bacterial mRNA by real-time reverse transcription (RT)-PCR were described previously (19; http://www.phri.org/research/res_pigennaro.asp). Briefly, total RNA was extracted from lung tissue by combined use of a guanidinium thiocyanate-based buffer and rapid mechanical lysis of *M. tuberculosis*. Reverse transcription and quantification of *M. tuberculosis* mRNAs by real-time RT-PCR were carried out using gene-specific primers and molecular beacons. Nucleotide sequences of the oligonucleotide primers and molecular beacons used for enumeration of Rv2626c, *acr*, *esat-6*, and *pstS-1* bacterial transcripts were published previously (19, 20).

For *ald* and *fdxA*, oligonucleotide sequences were as follows: RT primers were 5′-GTCGAAAGACCTTTGGCTAGTGC-3′ (*ald*) and 5′-CTGGTGTTGATC GTCGGGTAGAT-3′ (*fdxA*); PCR primer pairs were 5′-GCCGGATCCACAC TCGCTACT-3′ (forward) and 5′-CTGGTTTCATATGCGCGACAAGT-3′ (reverse) for *ald* and 5′-CAGGAGTGTCCGGTCGACTGTA-3′ (forward) and 5′-TGCACGCACCACAATCCAC-3′ (reverse) for *fdxA*; molecular beacons were 5′–6-carboxyfluorescein–CGCCCGCCGACCTGGTGATTGGGGCCCG GGCG-Dabcyl-3′ (*ald*) and 5′–6-carboxyfluorescein–ACGGGGCCGAATGCT CTACATCAACCCCGT-Dabcyl-3′ (*fdxA*). 16S rRNA of *M. tuberculosis* was used as a normalization factor to enumerate bacterial transcripts per cell because 16S rRNA levels correlate well with CFU during the course of lung infection regardless of growth stage ($Y = 1.0457X + 3.1093$ [$R^2 = 0.954$]) (19).

TABLE 1. Diagnosis with respect to TB state[a]

Diagnosis	Frequency (no. of cases)	% of cases
Active TB	53	15.0
Inactive TB	218	61.8
TST positive	32	9.1
TST negative	50	14.2
Total	353	100.0

[a] Diagnostic definitions are provided in Materials and Methods.

RESULTS

Characteristics of the study population. The 353 subjects included in the study were divided in four categories: active TB, inactive TB, latent *M. tuberculosis* infection (TST positive), and free of *M. tuberculosis* infection (TST negative) (Table 1). The demographic characteristics of the study population are described in Table 2. No statistically significant difference was found by chi-square test among the four diagnostic categories for factors associated with tuberculosis risk (1), such as age group ($P = 0.29$), gender ($P = 0.07$), country of origin ($P = 0.12$), status of vaccination with *M. bovis* BCG ($P = 0.19$), or years in Canada (less than 1 or greater than 1 [$P = 0.53$]).

Distribution of antibody levels. Serum levels of specific immunoglobulin G antibodies were measured by ELISA and expressed as OD_{450}. Only the Rv2626c antibody was approximately normal after log transformation (data not shown). Therefore, ELISA measurements of serum antibody levels for all 353 subjects are presented as median and range (minimum and maximum) (Table 3).

Comparisons of antibody distributions were conducted by nonparametric statistical methods, such as the Wilcoxon rank sum test and the Kruskal-Wallis test, rather than by one-way analysis of variance, which requires an assumption of normality. No statistically significant difference was found between the TST-positive and the TST-negative groups for any of the antibodies considered ($P > 0.50$ for all) (data not shown). This result agrees with the notion that latent infection per se fails to provide sufficient antigenic stimulus to elicit a strong antibody response (3, 12, 24). Thus, these two categories were combined for subsequent analysis into a single, radiographically normal group ("CXR-normal"), regardless of *M. tuberculosis* infection.

Antibody responses were analyzed by state, i.e., active TB, inactive TB, and CXR-normal; differences among the states were found to be statistically significant at a P value of <0.05

TABLE 2. Demographics

Characteristic	Frequency (no. of cases)	% of cases
Age (yr)		
25 and under	29	8.2
25 to 34	106	30.0
35 to 44	88	24.9
45 to 54	40	11.3
55 and over	90	25.5
Gender		
Female	126	35.7
Male	227	64.3
World region of birth		
Canada and Western Europe	31	8.8
Eastern Europe	30	8.5
Africa and Middle East	67	19.0
South Asia	83	23.5
Southeast Asia	62	17.6
Caribbean and Latin America	80	22.7
Yrs in Canada[a]		
Less than 1	233	66.0
More than 1	76	21.5
NA or unknown	44	12.5
BCG vaccination		
No	169	47.9
Unknown	76	21.5
Yes	108	30.6
Total	353	100.0

[a] A 1-year mark was selected because there is substantial evidence that a large proportion of TB among immigrants occurs soon after arrival in the new country (31). NA, not applicable.

TABLE 3. Unadjusted median antibody responses by disease state[a]

Antigen	Disease state	Median	Range		*P* value
			Min	Max	
Rv2626c	Active	0.245	0.09	1.09	<0.001**
	Inactive	0.178	0.02	1.38	NA
	CXR-normal	0.185	0.02	1.15	0.356
FdxA	Active	0.176	0.06	1.76	0.284
	Inactive	0.164	0.01	1.95	NA
	CXR-normal	0.107	0.02	0.81	<0.001**
AlaDH	Active	0.199	0.07	1.23	0.005*
	Inactive	0.140	0.01	3.56	NA
	CXR-normal	0.106	0.01	1.08	0.001**
38-kDa Ag	Active	0.510	0.01	3.90	0.020*
	Inactive	0.260	0.01	3.99	NA
	CXR-normal	0.155	0.01	2.01	0.002**
ESAT-6	Active	0.090	0.01	3.86	<0.001**
	Inactive	0.210	0.02	3.98	NA
	CXR-normal	0.065	0.01	2.26	<0.001**
16-kDa Ag	Active	0.050	0.01	3.81	<0.001**
	Inactive	0.140	0.01	3.60	NA
	CXR-normal	0.050	0.01	0.58	<0.001**

[a] In this analysis, no attempt was made to separately evaluate active TB patients according to different culture and sputum smear results, given the small sizes of the resulting subgroups (7 active TB patients were culture and smear positive, 31 were culture positive and smear negative, and the remaining 15 were negative by both tests). Values of median and range (minimum and maximum) are shown since the antibody distributions were not normally distributed. The Kruskal-Wallis test was used to calculate *P* values for comparisons of inactive TB with active TB and of inactive TB with CXR-normal. NA, not applicable (inactive TB was the reference state in this analysis). * denotes differences that are statistically significant at an alpha of 0.05; ** denotes differences that are statistically significant at an alpha of 0.003. Alpha was selected according to the Bonferroni criterion, since the analysis included eight antibodies and two comparisons: inactive versus active and inactive versus CXR-normal [$0.003 \approx 0.05/(2 \times 8)$].

for all antibodies. The finding that the inactive TB and CXR-normal groups were serologically distinguishable strongly implies that persons having inactive tuberculosis are more likely to bear a higher antigen burden than those having latent infection without CXR abnormalities. This interpretation is consistent with the greater risk of disease reactivation associated with inactive TB than with latent infection with normal CXR (6, 7, 16).

In post-hoc comparisons, statistical significance was declared at an alpha of 0.003, thereby controlling for multiple comparisons with the Bonferroni approach [eight antigens and two comparisons between disease states implies the following: alpha = $0.003 \approx 0.05/(8 \times 2)$]. Inactive tuberculosis was arbitrarily taken as the reference state for this analysis (Table 3). Antibodies against AlaDH, the 38-kDa Ag, ESAT-6, and the 16-kDa Ag distinguished inactive TB from both active TB and the CXR-normal state. The antibody against Rv2626c distinguished inactive TB from active TB, while the antibody against FdxA distinguished inactive TB from the CXR-normal state. No difference was found in the levels of antibodies against SodA and GluS in the three states (data not shown). Thus, these two antibodies were excluded from further analyses.

Logistic regression results. Since antibody profiles differed among the three TB states (active TB, inactive TB, and CXR-normal), we estimated logistic regression models to predict TB state as a function of the antibodies identified as statistically significant in the analysis presented in Table 3. Backward elimination was used for all models. Two models of inactive TB versus active TB were estimated: model 1 was based on antibodies identified as being statistically significant at an alpha of 0.003, and model 2 was based on antibodies identified as statistically significant at an alpha of 0.05 (Table 4). According to model 1, high levels of antibodies against the 16-kDa Ag and ESAT-6 and low levels of antibodies against Rv2626c increased the odds of inactive TB compared with active TB. Model 2 additionally indicated that low levels of antibodies against AlaDH and the 38-kDa Ag increased the odds of inactive TB over active TB. With the latter model, the contribution of the antibody against Rv2626c lost statistical significance (the confidence interval for this antigen in model 2 includes the value 1).

Only one model was estimated for inactive TB versus CXR-normal (model 3), because for that comparison, all antibodies were statistically significant both at an alpha of 0.003 and at an alpha of 0.05 (Table 5). According to model 3, high levels of antibodies against the 16-kDa Ag, ESAT-6, and FdxA increased the odds of inactive TB versus CXR-normal. A trend of increasing odds was detected in the three categories of antibody level, further strengthening the results of this comparison.

TABLE 4. Models of inactive TB versus active TB adjusted for BCG vaccination status and place of birth[a]

Antibody to	Category	Range	OR (CI), model 1	OR (CI), model 2
16-kDa Ag	Low	≤0.11	1.0 (Ref.)	1.0 (Ref.)
	High	0.12–3.81	5.5 (2.4, 12.5)	7.3 (3.0, 17.9)
Rv2626c	Low	≤0.19	1.0 (Ref.)	1.0 (Ref.)
	High	0.19–1.38	0.4 (0.2, 0.8)	0.6 (0.2, 1.4)
ESAT-6	Low	≤0.15	1.0 (Ref.)	1.0 (Ref.)
	High	0.15, 3.98	3.0 (1.4, 6.6)	2.7 (1.2, 6.1)
AlaDH	Low	≤0.15	NA	1.0 (Ref.)
	High	0.15–3.56	NA	0.3 (0.1, 0.7)
38-kDa Ag	Low	≤0.14	NA	1.0 (Ref.)
	Medium	0.14–0.49	NA	0.6 (0.2, 1.6)
	High	0.50–3.99	NA	0.3 (0.1, 0.7)

[a] ELISA results were dichotomized into categories "low"and "high," according to being higher or lower than the median, or trichotomized into "low," "medium," and "high," according to the tertiles of the antibody distribution. Model 1 is based on a backward elimination from a model using only antibodies significant at an alpha of 0.003 (Table 3); model 2 is derived from a backward elimination from a model using antibodies significant at an alpha of 0.05 (Table 3). OR, odds ratio; CI, confidence interval; Ref., reference range; NA, not applicable.

TABLE 5. Models of inactive TB versus CXR-normal adjusted for BCG vaccination status and place of birth[a]

Antibody to	Category	Range	OR (CI), model 3	*P* (test for trend)
16-kDa Ag	Low	≤0.06	1.0 (Ref.)	<0.001
	Medium	0.07, 0.14	4.0 (1.9, 8.5)	
	High	0.15, 3.60	11.7 (4.9, 34.0)	
ESAT-6	Low	≤0.09	1.0 (Ref.)	0.001
	Medium	0.09–0.2	1.9 (0.8, 4.1)	
	High	0.2–4.0	5.9 (2.0, 17.6)	
FdxA	Low	≤0.11	1.0 (Ref.)	0.016
	Medium	0.11–0.19	1.5 (0.7, 3.2)	
	High	0.19–1.95	2.8 (1.2, 6.4)	

[a] ELISA results were trichotomized into the categories "low," "medium," and "high" according to the tertiles of the overall antibody distribution. Model 3 is based on backward elimination from antibodies found to be significant at an alpha of 0.003 (Table 3). OR, odds ratio; CI, confidence interval; Ref., reference range.

Transcription profiling of *M. tuberculosis* during the course of mouse lung infection. The finding that antibody profiles detected in the inactive TB group differed from those found in active TB patients suggested that antigen expression changes during the course of infection. This idea can be tested in an animal model of tuberculosis but not in humans, primarily because bacteria harbored by latently infected persons are inaccessible to investigation. During infection of mice, expression of adaptive immunity in the lung blocks further *M. tuberculosis* growth after 2 to 3 weeks of bacterial multiplication (Fig. 1A; 19). By using real-time RT-PCR, we measured levels of transcripts encoding the six antigens listed in Table 3 in the lung of B6D2/F1 mice infected with a strain of *M. tuberculosis* that belongs to the W-Beijing family (2, 13). This *M. tuberculosis* strain was selected because of its clinical relevance for human tuberculosis. Normalized copy numbers of the *pstS1*

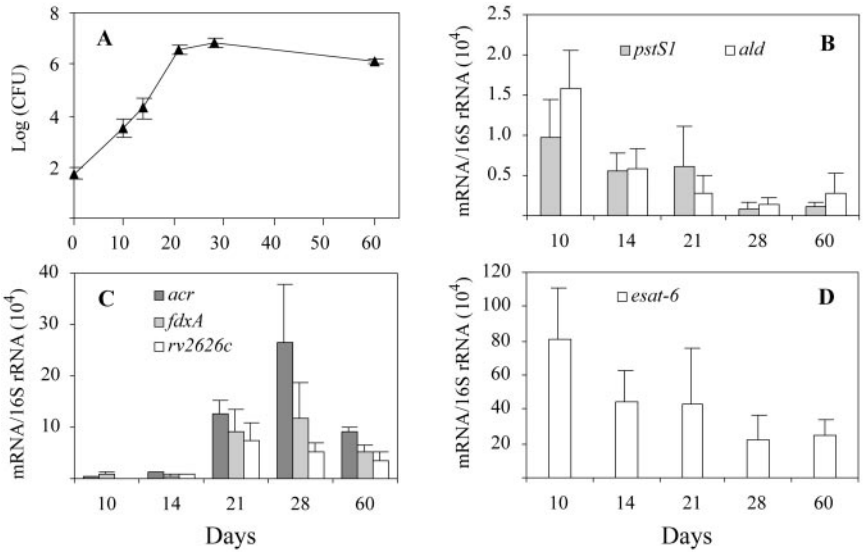

FIG. 1. Growth of *M. tuberculosis* HN878 in lungs of B6D2/F_1 mice in a low-dose aerosol model and transcript levels of selected bacterial antigen genes during infection. (A) Course of *M. tuberculosis* infection in the lung of mice. B6D2/F_1 mice were infected with 100 CFU of *M. tuberculosis* HN878 via the respiratory route. At the times indicated, half of the lung was used to determine the number of CFU. Data points represent means (± standard deviations) (in log units) of CFU per lung obtained with three to four animals per time point during the first 60 days of infection. Additional data on course of lung infection and mouse survival with this mouse strain and bacterial strain have been published previously (13). (B to D) Measurements of bacterial mRNA levels in mouse lung by real-time RT-PCR. Half of the infected lung was used for extraction of total RNA. Copy numbers of selected bacterial mRNAs, as indicated, were measured by real-time RT-PCR with molecular beacons. Levels of bacterial transcript per cell were obtained by normalizing mRNA copy numbers against 16S rRNA copy number, as previously described (19). Data were expressed as means ± standard deviations of measurements from lungs of three to four mice per time point. Statistically significant differences were found by the Student's *t* test between transcript levels during the acute phase (day 10) and the chronic phase (day 28) of infection for all genes ($P \leq 0.01$) except for *esat-6*.

mRNA (encoding the 38-kDa Ag) and of the *ald* transcript (encoding AlaDH) decreased with bacterial growth arrest (~10-fold decrease on day 28 relative to day 10 of lung infection) (Fig. 1B). In contrast, normalized copy numbers of the transcripts encoding *acr* (encoding the 16-kDa Ag), *fdxA*, and Rv2626c increased >10-fold in growth-arrested bacilli relative to multiplying cells (Fig. 1C). Expression levels of *acr*, *fdxA*, and Rv2626c in growth-arrested cells were similar (*acr-fdxA*-Rv2626c, 3:1.7:1). The *esat-6* mRNA copy numbers (Fig. 1D) showed only a 3.5-fold decrease on day 28 relative to day 10 of lung infection. These changes in mRNA copy numbers indicate that the antigen composition of tubercle bacilli changes in parallel with immunity-induced bacterial growth arrest.

DISCUSSION

We found that antibody profiles associated with inactive TB differed from those associated with active TB, strongly suggesting that the targets of the antibody response during latent infection differ from those occurring during active disease. This possibility is supported by the observation that tubercle bacilli at different growth states during murine lung infection express different immunodominant antigens (Fig. 1; 20). We infer from these data that in humans, each tuberculosis state is characterized by bacterial antigen signatures. These signatures resemble "bar codes," i.e., particular combinations of *presence* and *absence* of antigen-specific markers. The bar code idea reveals a flaw in current strategies of TB immunodiagnostics development, which have been based solely on identifying markers *positively* associated with a particular state.

A clear, direct correlation exists between production of particular *M. tuberculosis* antigens in a mouse infection model and the corresponding human antibody profiles. First, preferential expression of the *pstS1* and *ald* genes by multiplying tubercle bacilli in the mouse lung fits well with the correlation we observe in humans between the corresponding antibodies (anti-38-kDa Ag and anti-AlaDH) and active disease, which is presumably associated with growing tubercle bacilli. Additionally, a gradual increase was seen in levels of the antibody to the 38-kDa Ag from CXR-normal through inactive TB to active TB (Table 3), suggesting that the level of this antibody is proportional to bacterial burden. These data are consistent with observations that the antibody response to the 38-kDa Ag is associated with multibacillary or advanced pulmonary TB (3, 28). Second, a parallel exists between preferential expression of *acr* and *fdxA* by growth-arrested bacteria and the correlation of the corresponding antibodies (anti-16-kDa Ag and anti-FdxA) with inactive tuberculosis: as a form of latent infection, inactive TB is likely associated with a predominantly "dormant" bacterial population. This view is consistent with previously described evidence of an association of the anti-16-kDa antibody with asymptomatic contacts of active tuberculosis cases rather than with active disease cases (4).

Other aspects of the antibody profiles generated in the study are less straightforward. We found that the antibody profiles for the 16-kDa Ag, FdxA, and Rv2626c are specific for different tuberculosis states. However, these three antigens are encoded by genes (*acr*, *fdxA*, and Rv2626c) that are members of the same "dormancy" regulon (18, 25). Moreover, all three genes are induced in growth-arrested bacilli in mouse lung at comparable levels (Fig. 1C). Different antibody profiles for these antigens are therefore suggestive of differential regulation of these bacterial genes in humans or of differences in relative immunodominance, antibody affinity, or immune regulation. For example, unlike FdxA and the 16-kDa Ag, Rv2626c may not achieve threshold levels for antibody production in inactive TB, thus becoming detectable only in active TB, which is associated with a higher bacterial burden. More intriguingly, the detection of antibodies against FdxA and Rv2626c in active TB indicates sufficient antigenic stimulus, suggesting that the concurrent lack of antibody responses to the 16-kDa Ag is likely due either to a selective down-regulation of *acr* in particular human host microenvironments or to a failure of this antigen to elicit antibody production in forms of disease characterized by tubercle bacilli growing at low multiplicity (most active TB cases in the present study had smear-negative pulmonary disease). A similar interpretation can be given to the data on the anti-ESAT-6 antibody, which strongly correlates with inactive TB, even though production of ESAT-6 is high in both multiplying and growth-arrested bacilli in mouse lung. Indeed, the anti-16-kDa Ag and anti-ESAT-6 antibodies correlate with each other (data not shown). We are now examining correlations between antigen expression and antigen-specific immune responses in animal models.

The present study has some limitations. One lies in the composition of the serum bank, which was characterized by highly diverse demographics and by a vast predominance of inactive TB cases. A second limitation is the comparison between antibody profiles in humans and bacterial antigen profiles in mice, given that gene expression patterns of *M. tuberculosis* may differ in the two host species, due to well-recognized differences in the human and murine lung microenvironments. Third, the current analysis was limited to only eight antibody profiles. However, statistically filtering the serological data with the Kruskal-Wallis test to select antibodies for use in subsequent logistic regression models utilizes a strategy that may be employed with very large numbers of antibodies, such as those that might be detected by use of *M. tuberculosis* protein microarrays. Moreover, correlations between antibody profiles and tuberculosis states measured in the present study will have to be validated in independent populations.

The identification of immune profiles characteristic of tuberculosis states suggests that progression from latent *M. tuberculosis* infection to active disease, which is presumably accompanied by resumed bacterial multiplication, may also be accompanied by changes of bacterial antigen composition. Thus, asymptomatic, infected individuals that are progressing to reactivation of disease may be serologically distinguishable from those that are not. Identification of "progressors" through immunological screens should greatly help target the treatment of latent tuberculosis.

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