Antibodies against Mycobacterial Proteins as Biomarkers for HIV-Associated Smear-Negative Tuberculosis

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Serology data are limited for patients with sputum smear-negative HIV-associated active tuberculosis (TB). We evaluated the serum antibody responses against the mycobacterial proteins MPT51, MS, and echA1 and the 38-kDa protein via enzyme-linked immunosorbent assay (ELISA) in South African (S.A.) HIV-positive (HIV⁺) smear-negative TB patients (n = 56), U.S. HIV⁺ controls with a positive tuberculin skin test (TST⁺; n = 21), and S.A. HIV-negative (HIV⁻) (n = 18) and HIV⁺ (n = 24) controls. TB patients had positive antibody reactivity against MPT51 (73%), echA1 (59%), MS (36%), and the 38-kDa protein (11%). Little reactivity against MPT51 and echA1 was observed in control groups at low risk for TB, i.e., S.A. HIV⁻ (0% and 6%, respectively), and at moderate risk for TB development, i.e., U.S. HIV⁺ TST⁺ controls (14% and 10%, respectively). By contrast, more reactivity was detected in the S.A. HIV⁺ control group at higher risk for TB (25% and 45%, respectively). Our data hold promise that antibody detection against MPT51 and echA1 might have adjunctive value in the detection of HIV⁺ smear-negative TB and might reflect increasing *Mycobacterium tuberculosis* infection activity in asymptomatic HIV⁺ individuals.

Addtional diagnostics for HIV-associated active tuberculosis (TB) are urgently needed (1, 2). TB remains one of the deadliest infectious diseases worldwide and is the leading cause of death among HIV-positive (HIV⁺) individuals (3, 4). HIV infection is a major risk factor for progression from latent TB infection (LTBI) to TB, and coinfection leads to the acceleration of both diseases (2, 4). Hence, the TB and HIV epidemics are fueling one another, particularly in sub-Saharan Africa, resulting in about 1.3 million cases of HIV-associated TB, with about 320,000 deaths in 2012 (4). Early TB diagnosis in HIV⁺ individuals could reduce transmission, morbidity, and mortality but is especially challenging because HIV⁺ TB patients often present with atypical signs and symptoms. The most commonly used rapid diagnostic test for TB, sputum microscopy, is frequently negative in HIV-associated TB, and more-sensitive diagnostics, such as sputum culture or nucleic acid detection assays, are often unavailable in resource-limited settings, thereby resulting in TB case detection rates as low as 20 to 35% (5, 6). The occurrence of exclusively extrapulmonary disease in up to 50% of HIV⁺ TB patients (4) complicates matters further, since diagnosis typically requires an invasive procedure to obtain a specimen from the site of disease for microbiologic or histological confirmation. Thus, diagnosis of HIV⁺ TB is often delayed, resulting in up to 50% of HIV⁺ Africans having undiagnosed TB at the time of death (7, 8).

Despite the urgent need, a simple, inexpensive, rapid, point-of-care (POC) test for TB is still not available (1). The recently developed POC format for the detection of the mycobacterial cell wall glycolipid lipoarabinomannan (LAM) in urine has high sensitivities in HIV-associated TB at very low CD4 counts but limited sensitivity at higher CD4 counts and in HIV⁺ TB (reviewed in reference 56). Tests independent of the site of disease will likely have to be based on the detection of a host response to TB in an easily accessible body fluid, such as blood. Due to the considerable influence of HIV on the host immune response to TB (reviewed in reference 9), it can be anticipated that different tests will be required to detect TB in HIV⁺ versus HIV-negative (HIV⁻) individuals. Serum antibodies (Abs) can be detected by rapid dipstick formats suitable for POC testing (10–12). However, no accurate serodiagnostic test for TB has been developed to date, and sensitivities have been especially poor for HIV-associated TB (reviewed in references 13 to 15). Despite the WHO’s caution against the use of serodiagnostic assays for TB, further research in this area is encouraged given the limitations of current diagnostic tests (16). We have previously investigated Ab responses to a variety of mycobacterial antigens according to HIV status. We found that IgG responses against polysaccharide antigens were significantly lower in HIV⁺ than in HIV⁻ TB patients (17). In contrast, IgG reactivity against certain mycobacterial proteins, specifically, MPT51 and malate synthase (MS), were significantly higher in HIV⁺ than in HIV⁻ TB patients, and reactivity against MPT51 could distinguish between HIV⁺ TB and other respiratory diseases (18).

Diagnosing smear-negative HIV⁺ TB presents a great clinical challenge, especially in resource-limited settings, but serologic studies of this patient population are limited (reviewed in reference 19). Up to 10% of HIV-associated TB cases can even present as subclinical TB with neither chest X-ray abnormalities nor TB-associated symptoms but with clinical deterioration if left un-
tuberculin skin test (TST) or the gamma interferon release assays (IGRA) may identify the presence of LTBI, no tests are currently capable of identifying the gradient of infection activity that could indicate an increased risk of progression to TB (1). Thus, the main objectives of this study were (i) to assess whether detection of Abs against certain mycobacterial proteins might have an adjunctive value in the identification of smear-negative HIV-associated TB in a setting of endemicity like South Africa and (ii) to assess whether Ab reactivity differs among control groups with different risks for the development of TB. The rationale for the selection of the mycobacterial proteins in this study was based on results from prior studies. The proteins MS and MPT51 were shown to induce strong Ab responses in U.S. and Indian HIV− and HIV+ TB patients (18, 20–22). The protein echA1, encoded by Rv0222, was shown to induce strong Ab responses in Ugandan HIV− and HIV+ TB patients (23). Rv0222 is part of the regions of difference (RD) in M. tuberculosis, which have been deleted from Mycobacterium bovis BCG (24, 25), and is thus particularly suited for TB serological studies. The 38-kDa protein was selected as a comparator protein, as it has been extensively evaluated in prior studies with HIV− and HIV+ TB patients and is also included in several commercially available serodiagnostic tests for TB (reviewed in reference 13 to 15). However, these proteins have not been evaluated in combination before.

MATERIALS AND METHODS

Study design, setting, and subjects. This was a case-control study comparing Ab reactivity to mycobacterial proteins in South African (S.A.) patients with confirmed smear-negative HIV-associated TB (Table 1) to those of 3 asymptomatic control groups comprised of subjects without TB: U.S. HIV+ TST− subjects, and S.A. HIV− and HIV+ subjects irrespective of TST results (Table 2). The samples from TB cases were obtained from 86 consecutively enrolled HIV+ adults for whom there was a high clinical suspicion of pulmonary TB but whose sputa were smear negative. Most of the subjects were also enrolled in a prospective study to determine the accuracy of a C-reactive protein (CRP) test for patients presumed to have HIV− TB (26). The subjects enrolled from August 2009 to December 2010 were referred from a primary care facility to Edendale Hospital in the uMgungundlovu District of KwaZulu-Natal, South Africa, and followed prospectively for 8 weeks. Inclusion criteria were (i) an age of ≥18 years, (ii) a positive test for HIV, (iii) a cough for ≥2 weeks, and (iv) two sputum smears negative for acid-fast bacilli (AFB) or a lack of sputum production. Exclusion criteria were (i) a Karnofsky performance score of ≥40, (ii) not completing 8 weeks of follow-up, and (iii) therapy with antituberculous drugs or a fluorquinolone within 3 months prior to enrollment. Subjects with TB that was ultimately confirmed either by a positive sputum culture for M. tuberculosis (n = 39) or, if culture negative, by a clinical response to antituberculous therapy (n = 17) were included in this study (Table 1), and their sera were tested for Ab responses to mycobacterial proteins. Only patients with a high clinical suspicion for culture-negative TB were started on empirical antituberculous therapy. Thirty patients with no culture or clinically confirmed TB diagnosis after 8 weeks of follow-up were not included in this study.

To reduce the likelihood of undetected TB in the HIV+ controls, especially those from South Africa, only individuals with absolute CD4 counts over 400 were enrolled. HIV+ controls from the United States were enrolled at Jacobi Hospital, Bronx, NY, and HIV− and HIV+ controls from South Africa were enrolled from the same regions as cases at Edendale Hospital. Inclusion criteria for U.S. controls were (i) an age of ≥18 years, (ii) a normal chest X ray, and (iii) a recorded result for a prior TST. According to guidelines of the Centers for Disease Control and Prevention (CDC), a TST in HIV+ individuals is considered positive if the induration is ≥5 mm (27). As in other regions where TB is endemic, no routine TST (and no routine radiologic imaging) is performed in South Africa due to BCG vaccination at birth and high rates of M. tuberculosis exposure. Thus, inclusion criteria for S.A. controls were limited to (i) an age of ≥18 years and (ii) a known HIV test result. Exclusion criteria were (i) a history of treated TB within the year prior to enrollment and (ii) symptoms compatible with TB. All controls were enrolled from outpatient clinics and to our knowledge have remained asymptomatic within the several months following enrollment. Controls were categorized into four groups: U.S. HIV+ individuals with a negative TST (n = 22) or a positive TST (n = 21) and S.A. individuals with a negative HIV test (n = 18) and a positive HIV test (n = 24) (Table 2). Based on epidemiologic data, HIV immune suppression, and M. tuberculosis exposure risk, U.S. HIV+ TST+ controls (n = 22) were considered to have a negligible risk for LTBI or TB and their sera were used to calculate cutoff values for positive Ab detection, U.S. HIV+ TST+ patients (n = 21) were considered to have a moderate risk, S.A. HIV− patients (n = 18) were considered to have a low risk, and S.A. HIV+ controls (n = 24) were considered to have a high risk for TB development (28–35).

All subjects were bled at the time of enrollment, and sera were stored at −80°C until tested. Demographic and clinical information was obtained from interviews and medical records. Approval for human subject research for U.S. subjects was obtained from the Institutional Review Board of the Albert Einstein College of Medicine. Approval for S.A. subjects was obtained from the Biomedical Research Ethics Committees of the University of KwaZulu-Natal and by the KwaZulu-Natal Department of Health. Written informed consent was obtained from all subjects prior to enrollment.

Protein preparations. Recombinant bacterial expression plasmids pMRBL.38, encoding the protein MPT51 (Rv3803c), pMRBL.8, encoding...
the protein malate synthase (MS; also referred to as GlcB; Rv1837c), and pMRLB2A, encoding the 38-kDa protein (also referred to as PstS1; Rv0934c), were obtained from the Biodefense and Emerging Infectious Disease Research Resources Repository, Manassas, VA (BEI). A vector for the RD4 gene Rv0222, encoding the protein echA1, was generated using the high-throughput cloning strategy at the Macromolecular Therapeutic Development Facility at the Albert Einstein College of Medicine. Briefly, this method relies on highly efficient ligation-independent cloning, allowing for the parallel cloning of a single PCR product into all available expression platforms (36). The cloned construct demonstrating the most robust expression was selected for scaled-up production (37). The vectors were expressed in Escherichia coli, and the recombinant proteins were purified as described previously (18).

ELISAs. Enzyme-linked immunosorbent assays (ELISAs) were performed as previously reported (18). We focused on measuring reactivities for IgG, as this immunoglobulin has been shown to be the predominant isotype elicited in TB patients regardless of HIV status (reviewed in reference 19). Briefly, wells of 96-well microtiter plates (Immunolon 2HB; Dynax Technologies, Inc., Chantilly, VA) were coated with MS, Rv0222, and the 38-kDa protein at 4 µg/ml (50 µl/well) or MPT51 at 8 µg/ml (50 µl/well). Sera diluted 1:50 were added to the antigen-coated wells, and bound IgG was detected with a protein A-alkaline phosphatase (1:1,000; Sigma, St. Louis, MO), followed by detection with N5-nitrophenyl phosphate substrate (60 min at 37°C). The optical densities (OD) were measured at 405 nm. The mean OD of sera from 22 TST- control subjects plus 3 standard deviations (SDs) was used to determine the cutoff for a positive antibody response in each assay. As negative controls, two wells on each plate were processed as described above but without the serum. Sera from two patients with advanced TB and a known antibody response against the antigens were used as positive controls.

Laboratory measurements. Laboratory-based high-sensitivity CRP testing was performed using a Dimension RXL analyzer from Dade-Behring (Deerfield, IL, USA). The machine has a low limit of detection of 0.7 mg/liter, a normal range (from 0 to 5 mg/liter), and good precision compared to those of another laboratory-based high-sensitivity CRP test (38). CD4 counts were measured in cells/mm³ by standard flow cytometry.

Statistical analysis. Statistical analysis was performed using Prism software, version 6.0a (GraphPad Software, Inc., San Diego, CA) and STATA software, version 9.2 (StataCorp, College Station, TX). The t test was used when variables were normally distributed, and when they were skewed (e.g., all OD values), the Mann-Whitney U test was used for two-group comparison or the Kruskal-Wallis test was used for multiple-group comparison. The Spearman rank test was used for correlations of Ab reactivities. Categorical variables were compared using the chi-square test or the Kruskal-Wallis test was used for multiple-group comparisons (36). The cloned construct demonstrating the most robust expression was selected for scaled-up production (37). The vectors were expressed in Escherichia coli, and the recombinant proteins were purified as described previously (18).

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RESULTS
Sera from 56 HIV+ smear-negative pulmonary TB patients were evaluated and compared to those from 3 control groups with different risks for TB development. Except in having higher CRP values and more hilar adenopathy, subjects with culture-conferred TB (n = 39) did not differ significantly in demographics or other clinical parameters from subjects with clinically confirmed culture-negative TB (n = 17) (Table 1).

Ab reactivities in smear-negative HIV+ TB patients. IgG reactivity against the 4 proteins showed a broad range of sensitivities for HIV-associated smear-negative TB. Reactivity against MPT51 occurred most frequently (73%), followed by reactivity against echA1 (59%), MS (36%), and the 38-kDa protein (13%) (Fig. 1A to D and Table 3). The low sensitivity of the reactivity against the 38-kDa protein was due to the overall high cutoff value caused by considerable reactivity in the U.S. HIV+ TST+ control group, which was also observed in the other control groups. Importantly, Ab reactivity against both MPT51 and/or echA1 was seen in the majority of HIV+ subjects with smear-negative TB (both culture positive and culture negative). Interestingly, Ab reactivity against MPT51 and echA1 did not significantly correlate with each other in TB patients (Fig. 2A). Combining Ab reactivity against MPT51 and echA1 resulted in a detection of 88% of culture-positive and 100% of culture-negative TB patients, with also increased Ab detection in the HIV+ control groups (Table 3). A weak but significant correlation was observed between Abs against MPT51 and MS, and no significant correlation was observed between echA1 and MS in culture-positive TB patients (Fig. 2B and C). Ab reactivity against the 38-kDa protein did not correlate significantly with any of the other proteins (P > 0.1 for all correlations). CD4 counts did not correlate significantly with Ab reactivity against any of the 4 proteins (P > 0.05 for all correlations).

Ab reactivities in control groups. Ab reactivities against MPT51 and echA1 were significantly higher in the S.A. HIV+ controls (high TB risk) than in the U.S. HIV+ TST+ (moderate TB risk) and S.A. HIV- controls (low TB risk) (Fig. 1A and B). MPT51, in contrast to echA1 and MS, best discriminated between TB cases and controls (Fig. 1A to C and Table 2). While no Ab reactivity against MPT51 was observed in HIV- S.A. controls, it was detected in a few U.S. HIV+ TST+ controls (14%) and several S.A. HIV- controls (25%). Similar observations were made with echA1 but with a much higher proportion of positivity seen in the S.A. HIV+ controls (45%). No significant correlation was observed between Ab reactivity against MPT51 and echA1 or between MPT51 and MS in the US and S.A. HIV+ control groups (P > 0.1), while strong and significant correlations were seen for both comparisons in the S.A. HIV- control group (P < 0.01).

DISCUSSION
Serology data for smear-negative HIV-associated TB are limited, and many immunogenic mycobacterial antigens that elicit Ab responses in HIV- TB patients do not elicit the same magnitude of response in HIV+ TB patients (reviewed in reference 19). Our study shows that Ab reactivity against two proteins, particularly MPT51 and to a lesser extent echA1, can be detected in the majority of South African smear-negative HIV+ TB patients, including those who are culture negative and diagnosed based on clinical response to antituberculous drugs. Although Abs against MPT51 and echA1 were also detected in a quarter and in almost half of the South African HIV+ controls, our data suggest that serology with these proteins may help identify smear-negative patients with HIV-associated TB. These data are critical, as they not only validate serologic data with certain proteins in patients with HIV-associated TB from other settings of TB endemicity and nonendemicity (18, 22, 23) but also demonstrate a potential adjunctive value of serology to other common TB diagnostics, such as sputum microscopy and mycobacterial culture. Furthermore, given that echA1 is an RD-encoded protein that is absent in many other mycobacteria, such as BCG, and given the increased reactivity in the high- compared to the low-TB-risk control groups, it is conceivable that Ab reactivity against echA1, and possibly MPT51, reflects increasing M. tuberculosis infection activity in some of the controls. In our study, MS and the 38-kDa protein have little and no value, respectively, in the serodiagnosis of TB in S.A. HIV+ patients. This is contradictory to our and others’ prior results for MS in HIV+ TB patients from other regions of endemicity and nonendemicity (18, 22) and in accordance with the results of other studies for the 38-kDa protein (23).
Adjunctive diagnostic methods are urgently needed for sputum smear-negative HIV-associated TB, which is challenging to diagnose with currently available rapid diagnostics. An even bigger challenge is the identification of culture-negative patients with TB, which in the absence of biopsy and microbiologic or characteristic histological findings can be diagnosed only on the basis of responses to empirical antituberculosis treatment. Therefore, a positive Ab detection in 77% of smear-negative culture-positive (Cx+) and culture-negative (Cx−) TB patients and asymptomatic control groups categorized by country, tuberculin skin test (TST) results, and HIV status. Cutoff values for each protein were derived from the mean optical densities (OD) of the U.S. HIV+ TST+ controls plus 3 SDs. Bars show median ODs with interquartile ranges. The Mann Whitney U test was used for two-group comparison of median antibody reactivities.

FIG 1 Antibody reactivities against MPT51 (A), echA1 (B), MS (C), and the 38-kDa protein (D) in South African (S.A.) smear-negative (Sm−) culture-positive (Cx+) and culture-negative (Cx−) TB patients and asymptomatic control groups categorized by country, tuberculin skin test (TST) results, and HIV status. Cutoff values for each protein were derived from the mean optical densities (OD) of the U.S. HIV+ TST+ controls plus 3 SDs. Bars show median ODs with interquartile ranges. The Mann Whitney U test was used for two-group comparison of median antibody reactivities.
in resource-limited settings, where culture and molecular detection methods are typically not available. The high rate of detection of Abs against MPT51 in S.A. HIV+ TB patients is in accordance with prior results from both ourselves and others, demonstrating 77% sensitivity, regardless of smear results, with culture-positive U.S. HIV+ TB patients (18) and 80 to 95% sensitivity with smear-positive Indian HIV+ TB patients (18, 22). Our data now show that this high Ab reactivity can be detected regardless of sputum smear and culture results and is not limited to certain regions where TB is endemic. We also validated the theory that Ab reactivity was not influenced by CD4 counts, a finding consistent with our prior studies (18). Furthermore, we have previously shown that MPT51 also has clinical value in distinguishing HIV-associated TB from other HIV-associated respiratory diseases (18), making this protein an attractive candidate for potential multitarget assays.

It is well established that HIV− TB patients have heterogeneous Ab responses against mycobacterial antigens (39). Our prior studies with subjects living in the United States demonstrated a more homogeneous Ab response against MPT51 and MS in HIV− TB than in HIV+ TB patients (17). In this study, Ab responses to the proteins MPT51 and echA1 did not correlate significantly in HIV+ TB patients, while in accordance with our prior studies, the ones against MPT51 and MS did, although to a lesser extent (17). These findings are consistent with the differences in gene and protein expression during various levels of M. tuberculosis infection activity, which may be reflected by the subjects’ Ab responses (40). The lack of significant correlation had the benefit of an increased sensitivity of 88% for smear-negative, culture-positive TB patients and 100% for culture-negative TB patients when responses against MPT51 and echA1 were combined. This is in accordance with several studies demonstrating that combinations of antigens compared to single antigens often increase the sensitivity of TB serologic assays (reviewed in reference 19). However, such an approach typically leads to a reduced specificity, which in our case decreased to below 50% in the S.A. HIV+, high-TB-risk control group. On the other hand, increasing a high sensitivity even further is an attractive option that could lead to high negative predictive values, which are clinically quite useful, even in the absence of high specificities. Furthermore, the known limited sensitivities/specificities of ~50 to 70% for chest X-rays and clinical evaluation often results in overtreatment of smear-negative HIV+ TB patients (41–43). Thus, a highly sensitive serodiagnostic test, even in the absence of a high specificity, could contribute valuable information to the diagnostic algorithm for HIV-associated TB in resource-limited settings of high TB endemicity.

One of the historical caveats of TB serology has always been limited specificity. This lack of specificity is commonly attributed to cross-reactivity with antigens from BCG, nontuberculous, or environmental mycobacteria due to the considerable genetic homology to M. tuberculosis. However, estimating the true specificity is challenging, if not impossible, as there are no available tests that can quantify the level of M. tuberculosis infection activity prior to the occurrence of disease (1). Although LTBI and TB are commonly seen as binary states, it is now well accepted that reactivation is preceded by a continuum of increasing infection activity prior to the development of disease and the onset of symptoms (reviewed in references 9, 44, and 45). Worsening immunosuppression and a concomitantly reduced capacity to control M. tuberculosis infection activity translates into a high risk for progres-

### TABLE 3 Positive Ab reactivities against mycobacterial proteins in S.A. HIV+ smear-negative TB cases and controls

| Protein(s)       | All TB+ cases (n = 56) | TB+ Sm− CX− cases (n = 39) | TB+ Sm− CX+ cases (n = 17) | U.S. HIV+ TST+ controls (n = 21) | S.A. HIV− controls (n = 18) | S.A. HIV+ controls (n = 24) |
|------------------|------------------------|-----------------------------|-----------------------------|---------------------------------|----------------------------|-----------------------------|
| MPT51            | 41 (73)                | 30 (77)                     | 11 (65)                     | 3 (14)                          | 0 (0)                      | 6 (25)                      |
| echA1            | 33 (59)                | 21 (54)                     | 12 (71)                     | 2 (10)                          | 1 (6)                      | 11 (46)                     |
| MPT51 and/echA1  | 49 (88)                | 32 (82)                     | 17 (100)                    | 5 (24)                          | 1 (6)                      | 14 (58)                     |
| MS               | 20 (36)                | 15 (38)                     | 5 (29)                      | 2 (10)                          | 5 (28)                     | 5 (21)                      |
| 38-kDa protein   | 6 (11)                 | 5 (13)                      | 1 (6)                       | 0 (0)                           | 0 (0)                      | 0 (0)                       |

a Sm−, smear negative; CX−, culture positive; CX+, culture negative; TST+, tuberculin skin test positive; S.A., South African.

![FIG 2](http://cvi.asm.org/) Correlations between antibody reactivities against MPT51 and echA1 (A), MPT51 and MS (B), and echA1 and MS (C) in South African HIV+ smear-negative TB patients. Spearman rank correlation was used to test for statistical significance.
sion to TB, which is particularly evident in HIV+ individuals (reviewed in reference 45). Epidemiologic studies show that in M. tuberculosis-infected HIV+ individuals, the risk of development of TB can be over 10% per year (28–30), while in HIV– individuals, it is only around 10% over a lifetime (31, 32). Furthermore, the risk of disease increases as CD4 cell counts decrease (33–35). For example, TB incidence rates in S.A. HIV– antiretroviral-treatment-naive individuals with CD4 cell counts of >350, 200 to 350, and <200 were 3.6, 12.0, and 17.5 cases per 100 person years, respectively (33). Our S.A. HIV+ controls had median CD4 counts of around 600 and no history of prior TB, making it unlikely that their Ab reactivity was due to disease. Considering that the S.A. HIV+ compared to the S.A. HIV– controls were enrolled at the same site and likely had similarly high M. tuberculosis exposure rates, it is conceivable that the Ab reactivity against some mycobacterial proteins in the S.A. HIV+ controls reflects a true serologic response indicative of increasing M. tuberculosis infection activity due to HIV coinfection. This hypothesis is further supported by (i) echA1 being encoded by one of the RD which have been deleted from BCG but are present in M. tuberculosis (24, 25); (ii) animal studies indicating that Ab responses are associated with antigen burden rather than pathology and can be detected prior to culture positivity (46, 47); (iii) several, mostly small, studies demonstrating an increased Ab reactivity against a number of mycobacterial antigens, including MPT51 and ESAT-6, and to a lesser degree against the M. tuberculosis-specific proteins ESAT-6 and CFP 10, months to years prior to the development of TB in HIV+ individuals (21, 48–53); and (iv) identification of echA1 via mass spectrometry in M. tuberculosis-infected guinea pig lungs 30 days but not 90 days postinfection (54), suggesting that this protein might play a role in early TB pathogenesis. Studies on a larger scale with prospectively collected samples are warranted to further explore the potential biomarker value of Abs against echA1 as a measure of increasing M. tuberculosis infection activity and a potential correlate of risk for TB development in HIV+ individuals.

In summary, our results contribute important as well as novel data to the field of TB serology and offer promise that Ab detection against certain mycobacterial antigens, specifically MPT51 and echA1, may have adjunctive value in the identification of HIV+ TB. Although serology should not be anticipated to replace the sputum smear, it might, especially if further improved, contribute to the clinical algorithm of identifying smear-negative HIV-associated TB, which has limitations in its current form (55). Our data also indicate that Ab reactivity against echA1 and possibly MPT51 might be a marker for M. tuberculosis infection activity in HIV+ individuals prior to the development of disease. If validated by larger studies with prospectively collected samples, these findings could lead to the development of screening tests identifying those M. tuberculosis-infected HIV+ individuals that are at risk for developing disease, which in turn could lead to early therapeutic interventions.

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