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Strong Antibody Responses to *Mycobacterium tuberculosis* PE-PGRS62 Protein Are Associated with Latent and Active Tuberculosis▽

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***Mycobacterium tuberculosis* has a unique family of PE-PGRS proteins with conserved N-terminal domains (PE) containing site-specific proline-glutamine residues and polymorphic GC-rich repetitive sequences (PGRS). Tuberculosis (TB) patients produce antibodies against some such proteins, but it is not clear whether these responses correlate with disease. Clinical groups with different mycobacterium exposure were studied for their seroreactivity to PE-PGRS17 and PE-PGRS62 proteins and their respective PE domains. There were minimal antibody responses against both PE domains and full-length PE-PGRS17, even in patients with active TB. However, patients with active and latent TB showed significantly higher PE-PGRS62-specific immunoglobulin G antibody responses than treated TB patients and mycobacterium-reactive TB contacts without latent infection. Latently infected persons had high anti-PE-PGRS62 responses but low responses to the 38-kDa antigen commonly used for TB serology, while treated TB cases showed the opposite response. Thus, patterns of seroreactivity to PE-PGRS62 correlate with clinical status and are associated with latent TB infection.**

The genome sequence of the *Mycobacterium tuberculosis* H37Rv strain reveals a family of highly homologous PE-PGRS genes that encode a highly conserved N-terminal domain with Pro-Glu (PE) residues at specific sites and a polymorphic GC-rich repetitive-sequence (PGRS) domain at the C terminus (7). The abundance of these unique genes suggests that this gene family may have important roles, but many members have unknown functions. The PGRS domain of PE-PGRS proteins is generally rich in glycine-alanine (Gly-Ala) repetitive sequences (7). Comparison of some of the PE-PGRS gene sequences between an *M. tuberculosis* laboratory strain (H37Rv), an *M. tuberculosis* clinical isolate (CDC1551), and *Mycobacterium bovis* (2, 14) reveals that the repetitive regions of PGRS domains are prone to insertion-deletion mutations. Moreover, *M. tuberculosis* clinical isolates exhibit variations in restriction fragment length polymorphism patterns in their PE-PGRS genes (11). Hence, it has been speculated that the PGRS domain has a functional role in generating antigenic diversity to avoid host recognition.

Antibodies against the PGRS domains of some such proteins, e.g., wag22 ($Rv1759c^{PE-PGRS}$) and PE-PGRS51, have been detected in sera of tuberculosis (TB) patients (10, 21). Murine immunization with a DNA vaccine encoding PE-PGRS33 also elicits substantial antibody responses (9). Thus, at least some of the *M. tuberculosis* PE-PGRS proteins are expressed in vivo and are recognized by host immunity. However, it is not known whether latent versus active infection, the stage and extent of disease, or duration of treatment may influence immune reactivity to PE-PGRS proteins in human TB. The relevance of such reactivity to TB diagnosis and prognosis is also unknown.

In this study, the association of seroreactivity against PE-PGRS proteins with disease status was evaluated to uncover any differences in immune recognition of these proteins between healthy persons and persons with TB. We aimed to determine whether latent infection or prior mycobacterium exposure results in responses to these proteins similar to those in persons with active infection and whether such antibodies persist after treatment. As prior studies suggest that there are differential immune responses to the two domains (PE versus PGRS) and that the Gly-Ala repeats may inhibit immune recognition of the whole protein (3), we also wanted to know if these repetitive sequences influence the induction of antibodies against specific PE-PGRS proteins. Therefore, we studied two contrasting PE-PGRS proteins with only 20% identity—one rich in these repeats (PE-PGRS17) and one with a relative paucity of these repeats (PE-PGRS62). We also chose these two proteins because of evidence based on expression or knockout studies (19, 23) suggesting that they have relevance to host-pathogen interactions in vivo, which increases the likelihood that they may interact with host immunity in infected persons. Our study reveals markedly different antibody responses to PE-PGRS62 and to PE-PGRS17. We also demonstrate that strong responses to the former are particularly associated with latent and active TB infection but not treated TB, suggesting that immune recognition of this protein may be a feature of the acute response to TB infection.

MATERIALS AND METHODS

Identification of study subjects. According to methods detailed previously (1), gamma interferon (IFN-γ) production by human peripheral blood mononuclear cells, in response to 5-day stimulation with purified protein derivative (PPD), a

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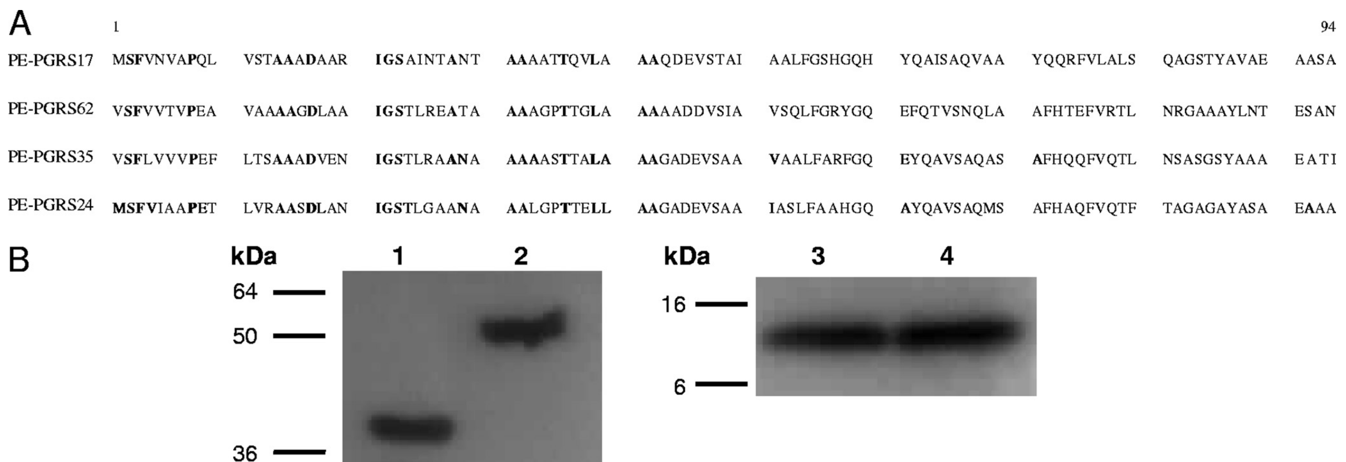

FIG. 1. (A) The N-terminal domains (amino acids 1 to 94) of both PE-PGRS17 and PE-PGRS62 proteins have significant homology with the PE domain consensus sequence found in products of other members of the PE multigene family. (B) Western blot analysis of the recombinant proteins using mouse anti-His monoclonal antibodies. Lanes: 1, PE-PGRS17; 2, PE-PGRS62; 3, PE-PGRS17^{PE}; 4, PE-PGRS62^{PE}.

mixture of overlapping peptides of *M. tuberculosis* early secretory antigen target (ESAT-6) protein and culture filtrate protein 10 (CFP-10) peptides, phytohemagglutinin (positive control), or phosphate-buffered saline (negative control), was tested in culture supernatants. Samples with IFN-γ levels four standard deviations above the mean for negative control wells were deemed to be positive for responses to the specific antigens. Healthy persons who were ESAT-6/CFP-10^{+} were defined in this study as having latent TB infection (LTBI^{+}), in accordance with many published studies (15).

Based on the above tests, out of a larger group of clinically healthy subjects, we selected 59 subjects to constitute three groups with distinct profiles for further antibody testing. There were 20 subjects who were nonreactive to PPD and ESAT-6/CFP-10 (i.e., LTBI^{-} PPD^{-}) and who had no TB exposure history (i.e., "nonexposed" group); this was the negative control group. The remaining 39 healthy subjects chosen had a recent TB exposure history ("TB contact" group); of these, there were 19 with the profile LTBI^{+} PPD^{+} and 20 who were LTBI^{-} PPD^{+}. Approximately 60% of the healthy subjects had a history of *M. bovis* BCG vaccination; in most cases the last vaccination occurred more than a decade ago.

We also recruited 40 study subjects with radiologically and sputum culture-proven TB; 20 had active pulmonary TB ("active-TB" group), and 20 had just completed 6 months of chemotherapy with clinical and radiological evidence of recovery ("treated-TB" group). All these TB patients had positive ESAT-6/CFP-10 and PPD responses, and all were human immunodeficiency virus negative. For the 20 active-TB cases, all were either untreated or had started treatment less than 2 weeks before the blood sample was taken. Of these, 13 were sputum smear positive and 5 had at least one cavity visible on chest X-ray. All subjects gave informed consent, and guidelines of the National University of Singapore review board for human studies were followed in the conduct of this research. The serum samples of all the above subjects were studied for reactivity to PE-PGRS proteins.

Recombinant proteins. Recombinant PE-PGRS17 and PE-PGRS62 proteins and their corresponding PE domains (PE-PGRS17^{PE} and PE-PGRS62^{PE}) were generated by expression in *Escherichia coli*. The coding regions of *M. tuberculosis* H37Rv genes Rv0978c and Rv3812 (7) and their corresponding PE regions were separately cloned into the pET-11a expression vector (Novagen) containing histidine tags to permit protein purification by affinity chromatography. The control antigen, *M. tuberculosis* 38-kDa antigen, was purchased from Biodesign International.

Western blotting. To verify the identity of the recombinant proteins, the purified samples were fractionated on sodium dodecyl sulfate-polyacrylamide gel and immobilized on a Hybond-P membrane (GE Biosciences), which was then incubated overnight with either mouse monoclonal antipolyhistidine (1 μg/ml; R&D Systems) or antihemagglutinin (50 ng/ml; Roche). Horseradish peroxidase-conjugated anti-mouse immunoglobulin G (IgG) (1:4,000 dilution; GE Biosciences) was used for detection of protein bands, which were visualized with the ECL chemiluminescence kit (GE Biosciences). The recombinant proteins were also analyzed by mass spectrometry to verify their identity.

Enzyme-linked immunosorbent assay. To measure serum antibodies specific for the PE-PGRS and 38-kDa antigen proteins, 96-well microplates (Corning) were coated with diluted PE or PE-PGRS proteins (2.5 μg/well) or the 38-kDa antigen (0.4 μg/well) overnight at 4°C. Bovine serum albumin was used to coat negative control wells to determine the level of nonspecific reactivity. Plates were washed with phosphate-buffered saline containing 0.05% Tween 20 (PBS-T) prior to being blocked with PBS-T containing 1% bovine serum albumin for 1 h at room temperature. After a washing, 50 μl of diluted sera (1:10 for PE-PGRS17, 1:50 for PE-PGRS62 and the 38-kDa antigen) was added to the microplates in replicates and incubated at 37°C for 2 h. Bound IgG was detected with horseradish peroxidase-labeled anti-human IgG (1:20,000 dilution; Sigma) and 3,3′,5,5′-tetramethylbenzidine substrate (BD Biosciences). The colorimetric reaction was read at 450 nm, and each absorbance (optical density [OD]) reading was normalized against the respective serum dilutions to obtain relative OD units per milliliter of serum.

Statistical analysis. Statistical analysis software SPSS 13.0 was used. In Spearman rank correlation analysis, P values that were <0.05 were considered significant. The Kruskal-Wallis test was used in multigroup comparisons for testing for overall differences between groups. Where there was a significant overall difference, pairwise comparisons between groups were performed with the nonparametric Mann-Whitney U test. The Bonferroni correction was applied, and only pairwise comparisons for which P was <0.005 were considered statistically significant.

RESULTS

Expression of PE-PGRS recombinant proteins. Figure 1A illustrates the first 94 amino acids of a subset of *M. tuberculosis* PE-PGRS proteins; this contains the common conserved PE regions of these proteins, based on pfam v11.0 software (NCBI). These were the regions of PE-PGRS17 and PE-PGRS62 proteins expressed as truncated proteins (the PE domains alone) for our study. The identity and purity of full-length and truncated recombinant proteins were confirmed by Western blotting (Fig. 1B). We assayed the serum of our five subject groups for IgG antibodies specific for these proteins.

Humoral immune response of clinical subjects to PE-PGRS proteins. All groups showed generally low anti-PE-PGRS17 responses (Fig. 2A). The LTBI^{+} TB contacts had the lowest response. Those with active and treated TB showed greatest intragroup variability (Fig. 2A). Relative to responses to the full-length protein, responses to the PE-PGRS17^{PE} domain were weaker (Fig. 2B). There was no evidence that TB infection increased recognition of this protein, no difference between persons with active TB and nonexposed persons was

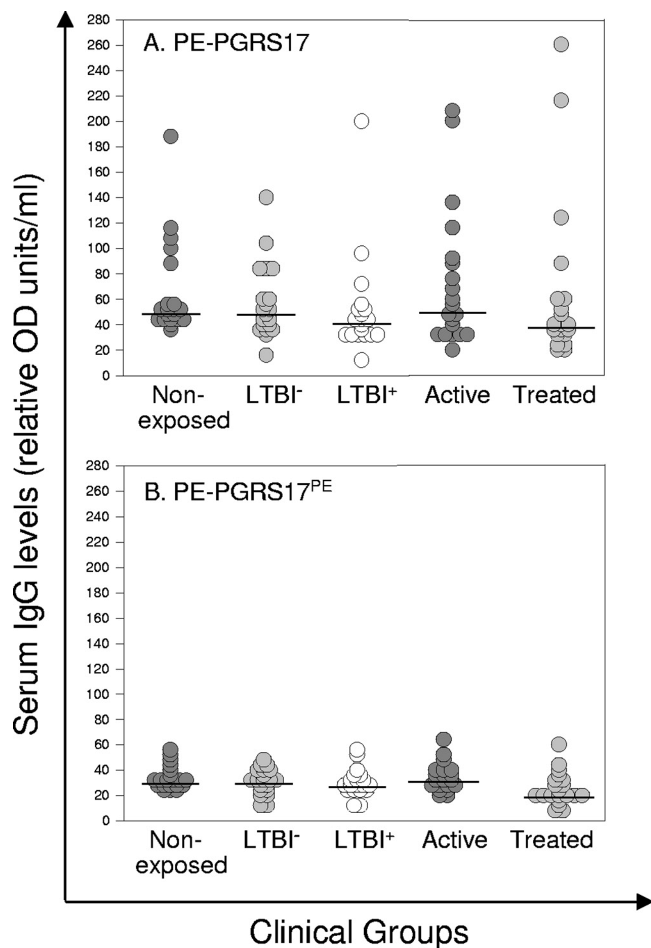

FIG. 2. Serum IgG responses to (A) PE-PGRS17 and (B) PE-PGRS17^{PE} proteins. Group medians are shown as horizontal bars. By the Kruskal-Wallis test there was no significant difference overall between groups.

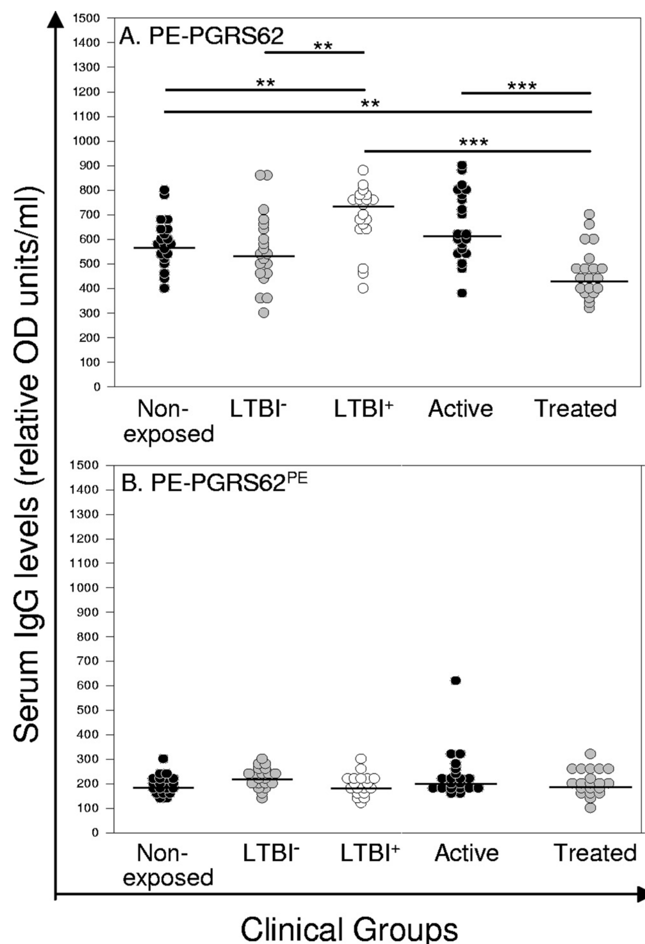

FIG. 3. Serum IgG responses to (A) PE-PGRS62 and (B) PE-PGRS62^{PE} proteins. Group medians are shown as horizontal bars. By the Kruskal-Wallis test, there was an overall significant difference between groups. Medians were compared pairwise using the Mann-Whitney U test with the Bonferroni correction. **, $P < 0.005$; ***, $P < 0.0005$.

noted, and LTBI$^+$ subjects showed even lower responses than the nonexposed (Fig. 2A). The treated-TB group had the lowest responses to both full-length and truncated proteins. Overall, antibodies to the PE-PGRS17 protein were not discriminatory between infected and noninfected groups.

In contrast, antibody responses to PE-PGRS62 were markedly higher in all groups, suggesting that this protein was more immunogenic. *M. tuberculosis*-infected subjects responded most strongly overall. LTBI$^+$ contacts had the highest median PE-PGRS62-specific responses, statistically similar to those of the active-TB group (Fig. 3A) and significantly higher than those of the nonexposed ($P < 0.005$) and LTBI$^-$ contacts ($P < 0.005$). The treated-TB patients had the lowest median responses, significantly lower than those of all the other four groups. Based on a cutoff antibody level of 620 relative OD units/ml of PE-PGRS62 IgG, a significantly higher proportion of LTBI$^+$ (infected but not diseased) subjects had anti-PE-PGRS62 antibodies than active-TB patients (84% versus 50%; chi-square test, $P < 0.05$). As with PE-PGRS17^{PE}, anti-PE-PGRS62^{PE}, responses were much weaker than responses to the full-length protein, minimal difference between groups was seen, and there was little intragroup variability (Fig. 3B). Overall, anti-PE-PGRS62 responses, but not anti-PE-PGRS62^{PE} responses, showed clear clinical correlates, with differential recognition in infected and noninfected persons.

The 38-kDa antigen is an immunogenic *M. tuberculosis* secretory protein (24). Some serodiagnostic studies using this antigen have shown good sensitivity and specificity for the diagnosis of TB although this opinion is not universally supported (5, 12, 22). We chose the 38-kDa antigen as an established, serodominant control antigen to determine whether reactivity in our panel of sera relates to the clinically predicted level of *M. tuberculosis* infection in the different clinical groups. The treated- and active-TB groups had the highest median responses to the 38-kDa antigen (Fig. 4), and the nonexposed group had the lowest. Thus, the groups showed levels of seroreactivity consistent with their relative levels of *M. tuberculosis* exposure. With a cutoff value of 300 relative OD units/ml for defining positive anti-38-kDa antigen responses, the response rates for TB and LTBI$^+$ groups were not significantly different. However, there was a marked difference in response rates between the

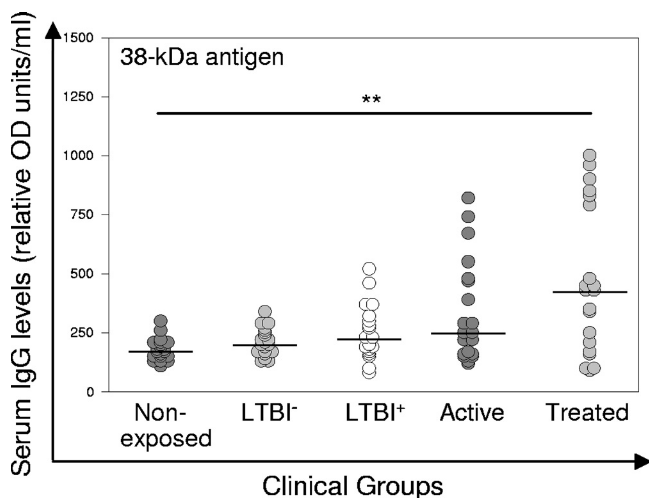

FIG. 4. Serum IgG responses to the *M. tuberculosis* 38-kDa protein. By the Kruskal-Wallis test, there was an overall significant difference between groups. Medians were compared pairwise using the Mann-Whitney U test with the Bonferroni correction. **, $P < 0.005$.

diseased group (combining active and treated TB) and the combination of the three healthy groups (50% versus 10%, respectively; chi-square test, $P < 0.0001$).

A general pattern linking clinical status and antibody responses was noted among the clinically healthy persons. LTBI$^-$ contacts and nonexposed groups had low responses to PE-PGRS62 and the 38-kDa antigen (PE-PGRS62^{lo} 38-kDa^{lo}), LTBI$^+$ contacts had high responses to PE-PGRS62 and low responses to the 38-kDa antigen (PE-PGRS62^{hi} 38-kDa^{lo}), and posttreatment TB patients were PE-PGRS62^{lo} 38-kDa^{hi}. Those with active disease fell between the LTBI$^+$ contact and treated-TB groups in response to these two antigens.

Correlation of responses to full-length and truncated PE-PGRS62 proteins. Reactivity to the PE domain was generally proportional to reactivity to the full-length protein; thus, a positive correlation between responses to PE-PGRS62 and PE-PGRS62^{PE} in all groups was noted (Fig. 5). The exception was the active-TB group, in which there was no apparent relationship between the two parameters. Interestingly, there were only two active-TB patients in this cohort ($n = 20$) with extensive disease (i.e., with radiographic lesions exceeding one-third of one lung volume), and both fell within the group with strong responses to the full-length PE-PGRS62 but weak responses to the PE domain. Thus, within the limits of our small sample, patients with greater extent of disease were those with disproportionately poor antibody responses to PE-PGRS62^{PE} compared to responses to PE-PGRS62.

DISCUSSION

Our main purpose for investigating antibody responses of different clinical groups to the PE-PGRS proteins was to examine whether these proteins could be expressed in vivo during human infection (latent, active, and treated) and whether differences in immune recognition of these proteins may be influenced by prior mycobacterium immune exposure in healthy persons or by differences in Gly-Ala repeats in the PGRS domain. This provides insight into the extent and nature of immune interactions between PE-PGRS proteins and the host.

PE-PGRS17 and PE-PGRS62 proteins, like many *M. tuberculosis* PE-PGRS proteins, are generally GC rich, with conserved N-terminal PE regions (7). However, PE-PGRS17 and PE-PGRS62 proteins differ particularly with respect to the glycine and alanine repeats in their PGRS domains (3), with the former having eight GGA and eight GGX repeats, whereas the latter has only one of each. If the marked difference in the seroreactivities of TB patients to these two PE-PGRS proteins is due to this, then Gly-Ala repeats in the PGRS domain could be impeding recognition of PE-PGRS proteins, as suggested by others (4); however, this remains speculative at present.

We were initially interested in the potential of PE-PGRS17 to be involved in host-pathogen interactions because it is expressed in vivo during murine *M. tuberculosis* infection (23) and upregulated within IFN-γ-activated macrophages (20). However, the generally low antibody response in all groups (Fig. 2A) does not discriminate between those with and without disease. Perhaps this protein is not expressed in *M. tuberculosis* in infected humans, or clinical strains may have undergone genetic alteration in the associated gene (from the published sequence), thereby resulting in altered immunogenicity. The last hypothesis would support a view that the Gly-Ala repeats of the PGRS domain have a role in antigenic variation (6, 7). It would also account for the relatively wide variation of responses to this protein in the TB group. There is one contrasting report suggesting significant differences between IgG responses in first-time adult TB patients and healthy controls (18), but no differences between the same controls and extrapulmonary-TB patients or relapsed patients who had previously completed one course of treatment. It is possible that the strength of this response may decline with increasing disease severity and/or time since first acquisition of disease, which may account for differences in our study outcomes. The PE-PGRS62 protein is a cell wall-associated protein (4), but the location of the PE-PGRS17 protein is not known. We considered it possible that low anti-PE-PGRS17 responses could be because this protein is not exposed on the mycobacterium cell surface, but in silico analysis of this protein based on TMpred (Prediction of Transmembrane Regions and Orientations; available at http://www.ch.embnet.org/software/TMPRED_form.html) predicts the presence of four transmembrane helices, which supports a cell membrane location for this protein.

Preclinical TB serum samples, but not serum of healthy persons, have antibodies against PE-PGRS51 (21). The elevated anti-PE-PGRS62 responses in our *M. tuberculosis*-infected groups (latent- and active-TB groups), as distinct from the responses in clinically healthy groups (nonexposed, LTBI$^-$ contacts, and treated TB) suggest that immune recognition of this protein is specific to the acute response to infection. If PE-PGRS62 is, like *Mycobacterium marinum mag24* (19), expressed during *M. tuberculosis* dormancy and active replication within macrophages, then higher expression during acute infection would account for higher seroreactivity in persons with latent and active TB and waning responses in persons with resolving disease (treated-TB group). The higher seroreactivity in LTBI$^+$ (infected but not diseased) subjects than in ac-

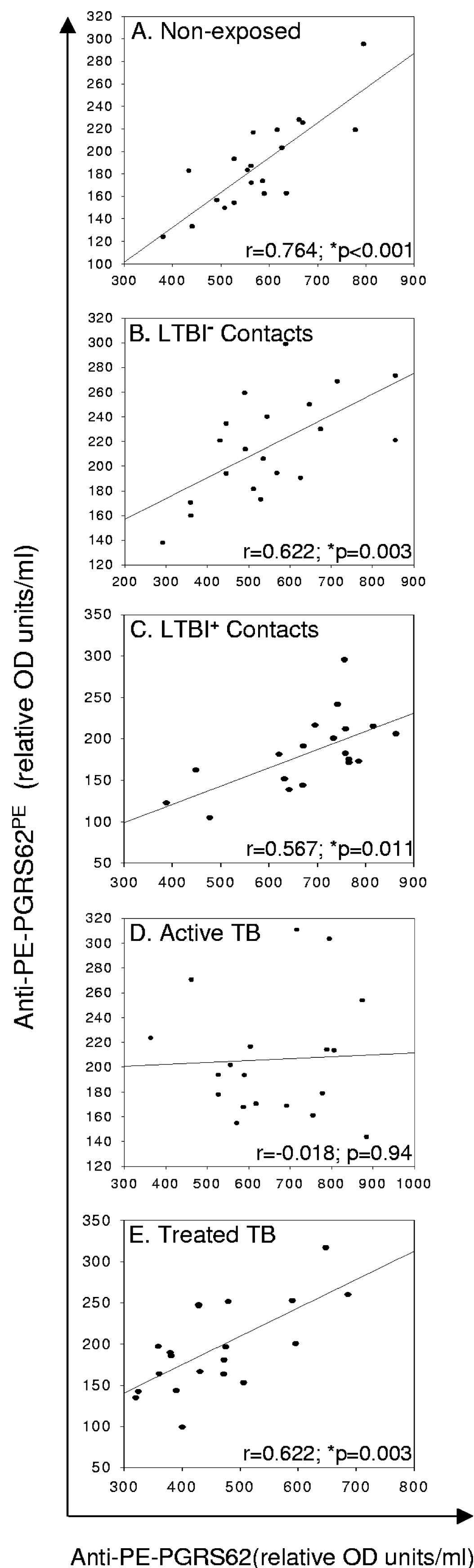

FIG. 5. Correlations between responses to PE-PGRS62 and PE-PGRS62^{PE} proteins. Regression lines and correlation coefficients are shown; statistics are based on the Spearman rank correlation.

tive-TB patients could imply that anti-PE-PGRS62 antibody responses in latent infection are relevant to *M. tuberculosis* containment. An alternative explanation relates to PE-PGRS62 being a cell surface protein; thus, PE-PGRS62-specific antibodies could form immune complexes with *M. tuberculosis*. Since our assay measures free antibodies, active TB patients could have increased immune complex formation related to higher bacterial burdens, accounting for lower antibody levels in the active-TB group relative to the LTBI group.

There has been interest in the domain specificity of responses to PE-PGRS proteins. TB patients have antibodies against PGRS domains of wag22 (Rv1759c$^{PE\text{-}PGRS}$) and PE-PGRS51 (10, 21). One report suggests that full-length PE-PGRS33 elicits antibodies on immunization in mice but that its PE domain elicits mainly cellular immune responses when not associated with PGRS (9). We also find that both the full-length PE-PGRS proteins studied yielded stronger antibody responses than their respective PE domains, particularly for PE-PGRS62, suggesting that humoral responses to this protein were mainly directed toward epitopes in the PGRS domain. The minimal differences between infected and noninfected persons in antibody response to PE domains of both proteins also suggest that immune recognition of this domain was generally weak and unrelated to bacterial load. However, since PE-PGRS62^{PE} and PE-PGRS62 protein responses were generally correlated in magnitude (Fig. 5), anti-PE antibodies probably contribute partially to the PE-PGRS62 response. The notable exception to this correlation was the active-TB group (Fig. 5D). Since the two active-TB patients with antibodies recognizing epitopes in PE-PGRS62, but not in PE-PGRS62^{PE}, were the two with the most extensive disease out of all cases studied, a relative lack of response to the PE domain may be linked to greater disease susceptibility, although a causative relationship cannot be directly inferred. Consistent with this hypothesis, the LTBI^{+} contacts (exposed but not diseased) did not show a dissociation of responses to the PE domain and full-length protein.

Antibodies against the *M. tuberculosis* 38-kDa antigen have been consistently associated with active TB, hence its inclusion in this study as a control antigen. However, its overall sensitivity and specificity levels in meta-analysis are insufficient for its routine use in diagnosis (22). Responses in TB patients reportedly increase 2 months after treatment (16), consistent with our observations. Whether this test discriminates latent from active TB is unclear. Some studies show that the levels are higher in active-TB subjects than healthy PPD^{+} subjects (8), others find no significant difference in levels in the progression from latent to active TB on serial serum testing (13), and yet others show higher response rates in PPD^{+} subjects (17). There is a paucity of studies comparing TB patients and ESAT-defined LTBI groups. Our study shows that this test did not discriminate

specifically between latent and active TB, but the clear differences in response rates between the diseased and healthy groups were consistent with other reports (22).

PE-PGRS62 IgG levels could have relevance to the diagnosis of LTBI or active TB. As PPD^+ $LTBI^-$ contacts had significantly lower responses than the $LTBI^+$ subjects (Fig. 3A), the heightened reactivity in the latter group is a specific response to TB infection and not due to cross-reactivity with antibodies against other common mycobacterium antigens. It would appear from the strong responses in the healthy $LTBI^+$ group that high bacterial loads are not required for good seroreactivity. With a positive ESAT-6/CFP-10 response in healthy TB contacts as the definition of LTBI in our study, a cutoff antibody level of 620 relative OD units/ml of PE-PGRS62 IgG gives a sensitivity of 84% and specificity of 70% for diagnosis of LTBI if applied to the clinically healthy subjects in our study. However, with regard to the utility of this assay in diagnostics, our observation must be considered preliminary as we have not performed receiver-operator characteristic curve studies because it was not a primary objective of this study to develop this test for LTBI diagnostics.

Considering the three groups with *M. tuberculosis* exposure (latent, active, and treated TB), $LTBI^+$ contacts had the highest reactivity to PE-PGRS62 and lowest to the 38-kDa antigen, whereas the treated-TB group reacted most strongly to the 38-kDa antigen but least to PE-PGRS62 (Fig. 3 and 4). A plausible explanation for this pattern is that reactivity to the 38-kDa antigen increases with chronicity of *M. tuberculosis* exposure, whereas reactivity to PE-PGRS62 occurs in the acute stages of infection. This could happen if PE-PGRS62 is mainly expressed during acute infection, while the 38-kDa antigen is expressed continuously in vivo. Future investigations should be directed at examining seroreactivity of patients who pose a diagnostic challenge related to having relevant symptoms without obvious radiological evidence of disease but who are later proven by sputum culture to have active TB. We predict that such patients would show a pattern of reactivity similar to the $LTBI^+$ contact group. If so, this test could be useful for rapid TB diagnosis as well.

This study has shown that not all members of the *M. tuberculosis* PE-PGRS family elicit similar seroreactivities in infected persons and that such proteins have differential immunogenicities, despite the shared antigen domains. Antibodies specific for the PE-PGRS62 protein, in particular, have clear correlations with clinical status. Anti-PE-PGRS62 is associated with latent and active TB, but not treated TB or PPD reactors without LTBI; thus, this protein must be expressed in the human host during infection. The response appears to be more associated with epitopes in the PGRS domain; however, dissociation of responses to $PE\text{-}PGRS62^{PE}$ and PE-PGRS62 proteins is linked to those active-TB cases with relatively more extensive disease. The combination of low responses to the 38-kDa antigen with high anti-PE-PGRS62 responses deserves consideration as a potential tool for diagnosing LTBI. That full recovery in treated-TB patients is associated with reversal of these two responses supports its specificity as a marker of acute infection.

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We declare no conflicts of interest.

REFERENCES

1. **Arend, S. M., A. Geluk, K. E. van Meijgaarden, J. T. van Dissel, M. Theisen, P. Andersen, and T. H. Ottenhoff.** 2000. Antigenic equivalence of human T-cell responses to *Mycobacterium tuberculosis*-specific RD1-encoded protein antigens ESAT-6 and culture filtrate protein 10 and to mixtures of synthetic peptides. Infect. Immun. **68:**3314–3321.
2. **Betts, J. C., P. Dodson, S. Quan, A. P. Lewis, P. J. Thomas, K. Duncan, and R. A. McAdam.** 2000. Comparison of the proteome of *Mycobacterium tuberculosis* strain H37Rv with clinical isolate CDC 1551. Microbiology **146**(Pt. 12)**:**3205–3216.
3. **Brennan, M. J., and G. Delogu.** 2002. The PE multigene family: a 'molecular mantra' for mycobacteria. Trends Microbiol. **10:**246–249.
4. **Chaitra, M. G., R. Nayak, and M. S. Shaila.** 2007. Modulation of immune responses in mice to recombinant antigens from PE and PPE families of proteins of *Mycobacterium tuberculosis* by the Ribi adjuvant. Vaccine **25:** 7168–7176.
5. **Chiang, I. H., J. Suo, K. J. Bai, T. P. Lin, K. T. Luh, C. J. Yu, and P. C. Yang.** 1997. Serodiagnosis of tuberculosis. A study comparing three specific mycobacterial antigens. Am. J. Respir. Crit. Care Med. **156:**906–911.
6. **Cole, S. T.** 1999. Learning from the genome sequence of *Mycobacterium tuberculosis* H37Rv. FEBS Lett. **452:**7–10.
7. **Cole, S. T., R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C. E. Barry III, F. Tekaia, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, A. Krogh, J. McLean, S. Moule, L. Murphy, K. Oliver, J. Osborne, M. A. Quail, M. A. Rajandream, J. Rogers, S. Rutter, K. Seeger, J. Skelton, R. Squares, S. Squares, J. E. Sulston, K. Taylor, S. Whitehead, and B. G. Barrell.** 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature **393:**537–544.
8. **Davidow, A., G. V. Kanaujia, L. Shi, J. Kaviar, X. Guo, N. Sung, G. Kaplan, D. Menzies, and M. L. Gennaro.** 2005. Antibody profiles characteristic of *Mycobacterium tuberculosis* infection state. Infect. Immun. **73:**6846–6851.
9. **Delogu, G., and M. J. Brennan.** 2001. Comparative immune response to PE and PE_PGRS antigens of *Mycobacterium tuberculosis*. Infect. Immun. **69:** 5606–5611.
10. **Espitia, C., J. P. Laclette, M. Mondragon-Palomino, A. Amador, J. Campuzano, A. Martens, M. Singh, R. Cicero, Y. Zhang, and C. Moreno.** 1999. The PE-PGRS glycine-rich proteins of *Mycobacterium tuberculosis*: a new family of fibronectin-binding proteins? Microbiology **145**(Pt. 12)**:**3487–3495.
11. **Flores, J., and C. Espitia.** 2003. Differential expression of PE and PE_PGRS genes in *Mycobacterium tuberculosis* strains. Gene **318:**75–81.
12. **Foulds, J., and R. O'Brien.** 1998. New tools for the diagnosis of tuberculosis: the perspective of developing countries. Int. J. Tuberc. Lung Dis. **2:**778–783.
13. **Gennaro, M. L., M. Affouf, G. V. Kanaujia, P. N. Brusasca, B. Mangura, and L. Reichman.** 2007. Antibody markers of incident tuberculosis among HIV-infected adults in the USA: a historical prospective study. Int. J. Tuberc. Lung Dis. **11:**624–631.
14. **Gordon, S. V., K. Eiglmeier, T. Garnier, R. Brosch, J. Parkhill, B. Barrell, S. T. Cole, and R. G. Hewinson.** 2001. Genomics of *Mycobacterium bovis*. Tuberculosis (Edinburgh) **81:**157–163.
15. **Kunst, H.** 2006. Diagnosis of latent tuberculosis infection: the potential role of new technologies. Respir. Med. **100:**2098–2106.
16. **Lee, J. S., E. K. Jo, Y. K. Noh, A. R. Shin, D. M. Shin, J. W. Son, H. J. Kim, and C. H. Song.** 2008. Diagnosis of pulmonary tuberculosis using MTB12 and 38-kDa antigens. Respirology **13:**432–437.
17. **Lopez-Vidal, Y., S. P. de Leon-Rosales, M. Castanon-Arreola, M. S. Rangel-Frausto, E. Melendez-Herrada, and E. Sada-Diaz.** 2004. Response of IFN-gamma and IgG to ESAT-6 and 38 kDa recombinant proteins and their peptides from *Mycobacterium tuberculosis* in tuberculosis patients and asymptomatic household contacts may indicate possible early-stage infection in the latter. Arch. Med. Res. **35:**308–317.
18. **Narayana, Y., B. Joshi, V. M. Katoch, K. C. Mishra, and K. N. Balaji.** 2007. Differential B-cell responses are induced by *Mycobacterium tuberculosis* PE antigens Rv1169c, Rv0978c, and Rv1818c. Clin. Vaccine Immunol. **14:**1334–1341.
19. **Ramakrishnan, L., N. A. Federspiel, and S. Falkow.** 2000. Granuloma-specific expression of *Mycobacterium* virulence proteins from the glycine-rich PE-PGRS family. Science **288:**1436–1439.
20. **Schnappinger, D., S. Ehrt, M. I. Voskuil, Y. Liu, J. A. Mangan, I. M. Monahan, G. Dolganov, B. Efron, P. D. Butcher, C. Nathan, and G. K. Schoolnik.** 2003. Transcriptional adaptation of *Mycobacterium tuberculosis*

within macrophages: insights into the phagosomal environment. J. Exp. Med. **198:**693–704.
21. **Singh, K. K., X. Zhang, A. S. Patibandla, P. Chien, Jr., and S. Laal.** 2001. Antigens of *Mycobacterium tuberculosis* expressed during preclinical tuberculosis: serological immunodominance of proteins with repetitive amino acid sequences. Infect. Immun. **69:**4185–4191.
22. **Steingart, K. R., N. Dendukuri, M. Henry, I. Schiller, P. Nahid, P. C. Hopewell, A. Ramsay, M. Pai, and S. Laal.** 2009. Performance of purified antigens for serodiagnosis of pulmonary tuberculosis: a meta-analysis. Clin. Vaccine Immunol. **16:**260–276.
23. **Talaat, A. M., R. Lyons, S. T. Howard, and S. A. Johnston.** 2004. The temporal expression profile of *Mycobacterium tuberculosis* infection in mice. Proc. Natl. Acad. Sci. USA **101:**4602–4607.
24. **Verma, R. K., and A. Jain.** 2007. Antibodies to mycobacterial antigens for diagnosis of tuberculosis. FEMS Immunol. Med. Microbiol. **51:**453–461.

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