MAJOR ARTICLE

# *Mycobacterium africanum* Elicits an Attenuated T Cell Response to Early Secreted Antigenic Target, 6 kDa, in Patients with Tuberculosis and Their Household Contacts

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***Background.*** *Mycobacterium africanum,* a member of the *M. tuberculosis* complex that is infrequently found outside of western Africa, is the cause of up to half of the tuberculosis cases there.

***Methods.*** We genotyped mycobacterial isolates obtained from a study of patients with tuberculosis and their household contacts and compared T cell responses and tuberculin skin test results by infecting genotype.

***Results.*** The T cell response to early secreted antigenic target, 6 kDa (ESAT-6), was attenuated in patients with tuberculosis (odds ratio [OR], 0.41 [95% confidence interval {CI}, 0.19–0.89]; $P = .024$) and household contacts (OR, 0.56 [95% CI, 0.38–0.83]; $P = .004$) infected with *M. africanum,* compared with the response in those infected with *M. tuberculosis.* In these same groups, responses to culture filtrate protein, 10 kDa (CFP-10), were nonsignificantly attenuated ($P = .22$ and $P = .16$, respectively), as were tuberculin skin test results ($P = .30$ and $P = .46$, respectively). Sequencing of region of difference 1 of *M. africanum* revealed that Rv3879c is a pseudogene in *M. africanum;* however, this finding does not provide an obvious mechanism for the attenuated ESAT-6 response.

***Conclusions.*** This is the first evidence, to our knowledge, that strain differences affect interferon-$\gamma$–based T cell responses. Our findings highlight the need to test new diagnostic candidates against different strains of mycobacteria. Integrating additional immunologic and genomic comparisons of *M. tuberculosis* and *M. africanum* into further studies may provide fundamental insights into the interactions between humans and mycobacteria.

T cell–based assays using region of difference (RD) 1 antigens—such as early secreted antigenic target, 6 kDa (ESAT-6), and culture filtrate protein, 10 kDa (CFP-10)—have an evolving niche in the detection of *Mycobacterium tuberculosis* infection. The RD1 sequence is missing from *M. bovis* bacille Calmette-Guérin (BCG; figure 1), and this omission makes these antigens more specific for *M. tuberculosis* infection than is purified protein derivative (PPD) [3]. Preliminary results suggest that T cell–based assays, such as enzyme-linked immunospot (ELISPOT) assays, are not merely a modern version of the tuberculin skin test and that the quantity of cells generating interferon (IFN)–$\gamma$ against ESAT-6 and/or CFP-10 may also reflect the bacterial burden [4]. ELISPOT assays likely identify recently activated lymphocytes that have immediate effector memory and persist for a limited time in circulation once antigen is cleared [5, 6]. Because ELISPOT assays also have increased sensitivity for mycobacteria in patients with immune suppression [7–9], they are potentially very useful in areas where HIV is highly prevalent. However, their cost and technical requirements may limit an increase in their use. In The Gambia, the ESAT-6/CFP-10 ELISPOT response was attenuated [10]. The assay's lower sensitivity

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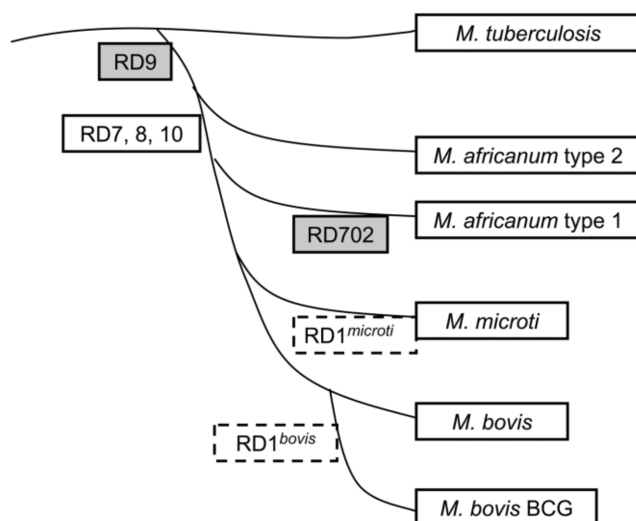


**Figure 1.** *Mycobacterium tuberculosis* complex, simplified in accordance with the data of Marmiesse et al. [1] and of Mostowy et al. [2]. Gray boxes indicate the region of difference (RD) for which polymerase chain reaction was performed, and the RDs that were sequenced in *M. africanum (boxes with dashed lines)* are indicated.

could be explained, in part, by the relatively high cutoff value that we used, but there may be other reasons for it.

There is mounting evidence that strain differences affect host-pathogen interactions [11]. *M. africanum,* which is a member of the *M. tuberculosis* complex (figure 1), is known to have lost ~68 kb of its genome, compared with *M. tuberculosis* strain H37Rv [12]. First isolated in Senegal in 1968 [12], *M. africanum* accounts for up to half of all cases of pulmonary tuberculosis (TB) in western Africa [13]; however, it is rarely identified outside of this region [14]. Phenotypic comparisons between *M. tuberculosis* and *M. africanum* have been limited to early animal studies, which suggested that *M. africanum* may have decreased virulence [15].

Spoligotyping is a user-friendly polymerase chain reaction (PCR)–based approach to the genotyping of *M. tuberculosis* complex strains that makes it feasible to examine strain-specific differences in tuberculin skin test results and/or ELISPOT responses. We used spoligotyping to genotype isolates obtained from a study of patients with TB and their household contacts, to examine the tuberculin skin test results and ELISPOT responses to *M. africanum* and *M. tuberculosis.* We then used genomic analyses to search for underlying mechanisms for our findings.

## PARTICIPANTS AND METHODS

### Participants

TB index patients, ⩾15 years old, with smear-positive pulmonary TB were recruited from the TB clinics at the Medical Research Council (MRC) Laboratories and Serrekunda, an area with a population of ~450,000 and an incidence rate of smear-positive TB of ~80/100,000 population [16]. We included index patients in the study if they had 2 sputum samples that were positive for acid-fast bacilli by Ziehl-Neelsen (Z-N) stain. The patients were examined, were interviewed, had a chest radiograph, and were invited, after counseling, to have an HIV test.

Household contacts, at least 6 months old, were included in the study if they primarily lived in the same compound as the head of the household where the index patient lived and had not been treated for TB during the past year. Household contacts were excluded if they had been diagnosed with TB within 1 month of recruitment. After informed consent was obtained, household contacts were interviewed and examined, and a blood sample was obtained for HIV testing and use in the T cell assay. Fresh blood samples from all participants were processed on site at the MRC Laboratories. Household contacts were included in the ELISPOT assay on the basis of random selection of up to a maximum of 12 participants, which was the capacity for the processing of fresh blood by the laboratory for use in the ELISPOT assay. Household contacts were categorized according to their sleeping proximity to the index patient, as a surrogate of level of exposure [10], in a gradient from "sleeping in the same bedroom" to "sleeping in a different bedroom in the same house" to "sleeping in a different house in the same compound."

Index patients and household contacts underwent a tuberculin PPD skin test (2 tuberculin units of PPD RT23; Staten Serum Institut). Induration in millimeters was recorded at 48–72 h after testing. Household contacts with a positive skin test result (mean induration diameter, ⩾10 mm) were offered a chest radiograph, and those with symptoms underwent a clinical assessment. Household contacts who were found to have TB were referred to the National Leprosy and TB Control Programme of The Gambia for free treatment. There is no current standard protocol for treatment of latent TB in The Gambia. The Gambia Government/MRC Joint Ethics Committee and the Stanford University Administrative Panel on Human Subjects approved the present study.

### Laboratory Procedures

The different tests were performed in separate laboratories without access to each other's data.

***Sputum samples and culture.*** Sputum samples were prepared and stained with auramine-phenol, and results were confirmed using Z-N stain. Sputum samples that were positive for mycobacteria by Z-N stain were decontaminated and inoculated into 1 slope each of Lowenstein-Jensen medium containing glycerol or sodium pyruvate and 1 vial of BACTEC 9000 MB medium (Becton Dickinson). All mycobacterial strains were identified and their identities were confirmed using standard procedures, and cultures were stored at −70°C in glycerol [17].



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***Spoligotyping and PCR for RD9 and RD702.*** DNA was extracted using cetyl trimethyl ammonium bromide and chloroform, in accordance with previously published methods [18], and its concentration and purity were assessed by spectrophotometry (Eppendorf BioPhotometer). Spoligotyping was performed using membranes (Isogen Biosciences), in accordance with standardized methods [19]. Spoligotyping results were scanned and analyzed with software (D.J.J., unpublished data) designed in Matlab (Mathworks), after which manual editing and confirmation were performed.

To confirm the classification of strains as either *M. africanum* or *M. tuberculosis* by use of spoligotyping, DNA was amplified from 1 random isolate with each spoligotype pattern for RD9 and RD702, which delineate the branch of *M. africanum* type 1 (figure 1). For both RD702 and RD9, 10 ng of genomic DNA was amplified in a volume of 25 $\mu$L, which contained premixed *Taq,* Q solution (both from Qiagen), and each primer at a final concentration of 0.2 $\mu$mol/L [2]. Conditions used for the amplification of the RD702 sequence were as follows: 94°C for 3 min, followed by 35 cycles each of 94°C for 1 min, 64°C for 1 min, and 72°C for 2 min. The RD9 sequence was amplified using similar conditions, except that an annealing temperature of 55°C was used. PCR products of ∼2 kb each were visualized on a 0.8% agarose gel. Positive and negative controls were included in each experiment. Isolates were classified according to spoligotype and PCR results as being either *M. tuberculosis* or *M. africanum* type 1.

***ESAT-6/CFP-10 ELISPOT assay.*** The ex-vivo ELISPOT assays for IFN-$\gamma$ were performed with the antigens ESAT-6, CFP-10, and PPD, as described elsewhere [10]. Results were recorded as the number of spot-forming units (sfu) producing IFN-$\gamma$ in response to *M. tuberculosis* antigens. Positive wells were predefined as containing at least 10 more than and at least twice as many spot-forming units as negative control wells. For a positive combined ESAT-6/CFP-10 result, it was necessary for at least 1 pool of overlapping peptides to be positive. The negative control well was required to have <30 sfu.

***HIV testing.*** Testing for HIV-1 and HIV-2 infection was performed using competitive ELISA (Wellcome Laboratories) and Western blot (New LAV Blot I and New LAV Blot II; Diagnostics Pasteur).

***RD1 sequencing.*** A 19,744-bp area corresponding to RD1, which is deleted in *M. bovis* BCG and *M. microti* (figure 1), was sequenced at Stanford University (available at: http://cmgm.stanford.edu/pan/index.html) after PCR amplification (see table 1 for primers). Comparative genomic analysis between *M. tuberculosis* strain H37Rv, *M. bovis,* and the RD1 sequence of *M. africanum* was performed using SeqMan/Lasergene (DNAstar).

### Analysis

We used the $\chi^2$ test and the Wilcoxon rank-sum test to analyze differences between index patients infected with *M. africanum* and those infected with *M. tuberculosis.* We estimated the odds ratios (ORs) and 95% confidence intervals (CIs) to assess the association between genotype (*M. africanum* vs. *M. tuberculosis*) and the response to ESAT-6, CFP-10, PPD, and the tuberculin skin test. Covariates with $P \leqslant .10$ and biological plausibility in the univariate analysis were tested in a multivariate logistic regression model. A random-effects regression model, allowing for household clustering, was used to compare the characteristics of household contacts exposed to *M. africanum* versus *M. tuberculosis.* We also tested for significant interaction terms and used the Mantel-Haenszel test to look for confounding or effect modification by HIV status. Nonnormally distributed responses were appropriately transformed. All analyses were conducted using Stata (version 8SE; StataCorp).

## RESULTS

The 317 index patients had a total of 2381 participating household contacts, with a median of 6 contacts/household. The median ages were 28 years for index patients and 15 years for household contacts. Twenty-nine percent of the index patients and 53% of the household contacts were female. The majority of participants were of Mandinka (32%), Jola (26%), or Wolof (13%) ethnicity.

A cultured isolate was available from 300 of the 317 index patients. Blank spoligotype patterns were obtained repeatedly for 12 of these 300 isolates, suggesting that they were not members of the *M. tuberculosis* complex. Of the 288 isolates with an interpretable spoligotype result, 108 (38%) were classified as being *M. africanum,* on the basis of the absence of spacers 8, 9, and 39. The remainder of the isolates were *M. tuberculosis* sensu stricto, and no *M. bovis, M. canettii,* or *M. microti* isolates were found. No patterns suggestive of infection with a mixture of strains were identified. Two isolates with spoligotype patterns that were consistent with *M. tuberculosis* repeatedly had blank PCR results and were classified as being indeterminate. All of the isolates classified as being *M. africanum* by spoligotyping belonged to *M. africanum* type 1 [1] (figure 1), as evidenced by PCR-confirmed deletion of RD9 and RD702. None of the remaining isolates had a deletion of RD9, and all of the RD9-deleted isolates had deletions of RD702 as well, suggesting that *M. africanum* type 1 was the only RD9-deleted member of the *M. tuberculosis* complex that was present in our study.

Index patients infected with *M. africanum* were less likely to mount an IFN-$\gamma$ response in the combined ESAT-6/CFP-10 ELISPOT assay (72% of *M. africanum*–infected index patients

**Table 1. Region of difference 1 primers used for sequencing.**

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vs. 84% of *M. tuberculosis*–infected index patients), but the difference was not significant (OR, 0.47 [95% CI, 0.20–1.1]; $P=.071$) (table 2). The attenuated response was primarily due to a significantly lower response to ESAT-6 (63% of *M. africanum*–infected cases had a positive ESAT-6 response, vs. 81% of *M. tuberculosis*–infected cases; OR, 0.41 [95% CI, 0.19–0.89]; $P=.024$), whereas the response to CFP-10 was nonsignificantly decreased (53% vs. 63%, respectively; $P=.22$). The PPD ELISPOT results were comparable (91% vs. 89%; $P=.75$). Moreover, the tuberculin skin test results were similar (median induration of 16 mm for both groups, with 79% and 84% positive results, respectively; $P=.30$).

Of the index patients, 273 (86%) consented to HIV testing, of whom 22 (8.1%) tested positive. Despite the association between *M. africanum* and HIV that we described earlier (13% HIV coinfection in *M. africanum*–infected cases vs. 4% in *M. tuberculosis*–infected cases; $P=.015$ [20]), HIV coinfection did not alter the association between *M. africanum* and ESAT-6 immunogenicity. When the analysis was limited to the 132 known HIV-negative index patients with ELISPOT results, the OR for having a positive ESAT-6 ELISPOT result when infected with *M. africanum* was 0.41 (95% CI, 0.18–0.92; $P=.031$).

Household contacts exposed to index patients infected with *M. africanum* were less likely to be ESAT-6/CFP-10 ELISPOT positive than were household contacts of index patients infected with *M. tuberculosis* (25% vs. 34%; OR, 0.64 [95% CI, 0.44–0.95]; $P=.025$) (table 3 and figure 2). ESAT-6 induced an IFN-$\gamma$ response in 23% of the household contacts of index patients infected with *M. africanum,* compared with 34% of the household contacts of index patients infected with *M. tuberculosis* (OR, 0.56 [95% CI, 0.38–0.83]; $P=.004$). The difference was less pronounced for CFP-10, which induced a response in 14% of the household contacts of index patients infected with *M. africanum,* compared with 18% of the household contacts of index patients infected with *M. tuberculosis* (OR, 0.74 [95% CI, 0.49–1.1]; $P=.16$). In contrast, responses to PPD, either in the tuberculin skin test (38% vs. 41%; $P=.46$) or in the ELISPOT assay (65% vs. 68%; $P=.47$), were similar between household contacts of index patients infected with *M. africanum* and of those infected with *M. tuberculosis.*

Of household contacts, 2037 (86%) consented to HIV testing, of whom 50 (2.5%) tested positive. The attenuated response to ESAT-6 in household contacts exposed to *M. africanum* was not explained by increased HIV infection rates (tables 2 and 3). The increased rates of HIV infection in household contacts of *M. africanum*–infected index patients resulted from household clustering of HIV infection, because the multivariate analysis indicated that the strongest predictor of HIV infection in household contacts was HIV infection in the index patient in the same household (OR, 6.4 [95% CI, 3.2–12]; $P<.001$), whereas older age and female sex were additional predictors of HIV infection.

In the final logistic regression model—which examined household contacts, had household as a random effect, included proximity and age, and was limited to known HIV-negative persons—the OR for ESAT-6 positivity when infected with *M. africanum* versus *M. tuberculosis* was 0.57 (95% CI, 0.40–0.80; $P=.001$). When this final model had HIV included as an explanatory variable, the OR changed minimally, to 0.55 (95% CI, 0.39–0.77; $P=.001$). The Mantel-Haenszel *P* value for homogeneity between ORs for HIV-positive and HIV-negative household contacts was .62, suggesting that there was no effect

**Table 2. Results of univariate and multivariate analyses to determine the clinical and immunological characteristics of index patients infected with *Mycobacterium africanum* or *M. tuberculosis.***

| | *M. africanum*–infected index patients | | *M. tuberculosis*–infected index patients | | Univariate analysis | | Multivariate analysis[a] | |
|---|---|---|---|---|---|---|---|---|
| Characteristic | No. (%) | Total no. | No. (%) | Total no. | OR (95% CI) | *P* | OR (95% CI) | *P* |
| Female sex | 30 (27) | 110 | 54 (30) | 178 | 1.2 (0.69–2.0) | .58 | | |
| BCG scar positive | 31 (30) | 110 | 44 (27) | 178 | 1.2 (0.68–2.0) | .58 | | |
| HIV positive | 12 (13) | 104 | 7 (4) | 165 | 3.3 (1.3–8.8) | .015 | 3.1 (1.2–8.3) | .024 |
| ESAT-6 positive | 33 (63) | 90 | 72 (81) | 159 | 0.41 (0.19–0.89) | .024 | 0.41 (0.19–0.89) | .024 |
| CFP-10 positive | 28 (53) | 52 | 57 (63) | 89 | 0.65 (0.33–1.3) | .22 | | |
| ESAT-6/CFP-10 positive[b] | 38 (72) | 53 | 76 (84) | 90 | 0.47 (0.20–1.1) | .071 | 0.47 (0.20–1.1) | .071 |
| PPD ELISPOT positive | 48 (91) | 53 | 80 (89) | 90 | 1.2 (0.39–3.7) | .75 | | |
| TST positive | 76 (79) | 96 | 134 (84) | 159 | 0.71 (0.37–1.4) | .30 | | |

**NOTE.** For the 110 individuals infected with *M. africanum,* the median age (range) was 30.5 (15–86) years, and for the 178 individuals infected with *M. tuberculosis,* the median age (range) was 27 (14–75) years ($P=.035$, univariate analysis; $P=.20$, multivariate analysis). BCG, bacille Calmette-Guérin; CFP-10, culture filtrate protein 10; CI, confidence interval; ELISPOT, enzyme-linked immunospot; ESAT-6, early secreted antigenic target 6; OR, odds ratio; PPD, purified protein derivative; TST, tuberculin skin test.

[a] Variables assessed were age, sex, presence of a BCG scar, and HIV positivity.

[b] Data are for individuals who had a positive result for either ESAT-6 or CFP-10. This model is separate from the ones analyzing ESAT-6 or CFP-10 alone.



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**Table 3. Results of univariate and multivariate analyses to determine the clinical and immunological characteristics of household contacts exposed to *Mycobacterium africanum* or *M. tuberculosis***

| | *M. africanum* | | *M. tuberculosis* | | Univariate analysis | | Multivariate analysis[a] | |
|---|---|---|---|---|---|---|---|---|
| Characteristic | Value | Household contacts, total no. | Value | Household contacts, total no. | OR (95% CI) | *P* | OR (95% CI) | *P* |
| Household contacts, median (range), no. | 6 (1–22) | 806 | 6 (1–30) | 1340 | | .95 | | |
| Age, median (range) | 15 y (2 mo–100 y) | 806 | 15 y (2 mo–89 y) | 1340 | | .67 | | |
| Female sex | 428 (53) | 806 | 713 (53) | 1340 | 1.0 (0.84–1.2) | .96 | | |
| BCG scar positive | 337 (48) | 698 | 558 (50) | 1123 | 0.95 (0.78–1.1) | .56 | | |
| Proximity to index patient | | 806 | | 1340 | | .13 | | |
| Same room | 264 (33) | | 390 (29) | | | | | |
| Same house | 356 (44) | | 642 (48) | | | | | |
| Different house | 186 (23) | | 302 (23) | | | | | |
| HIV positive | 25 (3.5) | 708 | 20 (1.8) | 1117 | 2.1 (0.90–4.9) | .085[b] | | |
| ESAT-6 positive | 143 (23) | 625 | 309 (34) | 905 | 0.53 (0.37–0.75) | <.0001 | 0.56 (0.38–0.83) | .004 |
| Spot-forming units, median (range) | 3 (0–443) | 625 | 4 (0–298) | 905 | | .17 | | |
| ESAT-6 response if ESAT-6/CFP-10 positive, median sfu | 25 | 159 | 27.5 | 315 | | .81 | | |
| CFP-10 positive | 91 (14) | 638 | 169 (18) | 944 | 0.74 (0.49–1.1) | .16 | | |
| Spot-forming units, median (range) | 1.5 (0–303) | 638 | 2 (0–301) | 944 | | .31 | | |
| CFP-10 response if ESAT-6/CFP-10 positive, median sfu | 10.75 | 160 | 10 | 323 | | .71 | | |
| E/C[c] positive | 160 (25) | 638 | 324 (34) | 945 | 0.60 (0.42–0.86) | .005 | 0.64 (0.44–0.95) | .025 |
| PPD ELISPOT positive | 417 (65) | 638 | 638 (68) | 945 | 0.87 (0.60–1.3) | .47 | | |
| Spot-forming units, median (range) | 15.5 (0–370) | 638 | 19 (0–384) | 945 | | .61 | | |
| PPD ELISPOT response if ESAT-6/CFP-10 positive, median sfu | 65.75 | 160 | 48.5 | 324 | | .15 | | |
| TST positive | 290 (38) | 765 | 521 (41) | 1277 | 0.87 (0.60–1.3) | .46 | | |
| Induration, median (range), mm | 0 (0–32) | 765 | 0 (0–30) | 1277 | | .21 | | |
| TST result if induration >0, median, mm | 15 | | 15 | | | .41 | | |

**NOTE.** Data are no. (%) of household contacts, unless indicated otherwise. BCG, bacille Calmette-Guérin; CFP-10, culture filtrate protein 10; CI, confidence interval; ESAT-6, early secreted antigenic target 6; ELISPOT, enzyme-linked immunospot; mo, months; OR, odds ratio; PPD, purified protein derivative; sfu, spot-forming units; TST, tuberculin skin test; y, years.

[a] Variables assessed were age, sex, presence of a BCG scar, HIV positivity, and proximity to the index patient.

[b] The borderline significant association between *M. africanum* and HIV in household contacts is the result of an association between *M. africanum* and HIV infection in index patients, with HIV infection clustering by household (see text).

[c] Data are for individuals who had a positive result for either ESAT-6 or CFP-10. This model is separate from the ones analyzing ESAT-6 or CFP-10 alone.

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modification by HIV infection status of the association between *M. africanum* and ESAT-6 positivity.

The 2 primary diagnostic tools for the determination of TB—that is, the tuberculin skin test and the combined ESAT-6/CFP-10 ELISPOT assay—are shown for *M. africanum*– and *M. tuberculosis*–exposed household contacts across the gradient of proximity to the index patient (figure 2). Differences between tuberculin skin test results were not significant, whereas differences between ELISPOT results were significant.

Lowering the cutoff for positivity of ESAT-6 to 8 sfu or 5 sfu in the ELISPOT assay made the difference in responses to *M. africanum* versus those to *M. tuberculosis* less pronounced, but they were still significant: at 10 sfu, the OR was 0.56 (95% CI, 0.38–0.83; $P = .004$); at 8 sfu, the OR was 0.62 (95% CI, 0.44–0.86; $P = .004$); and, at 5 sfu, the OR was 0.70 (95% CI, 0.50–0.99; $P = .042$). Quantitative responses to ESAT-6 and CFP-10 were not significantly lower in household contacts exposed to *M. africanum,* even when the analysis was limited to those who had a positive ELISPOT response (table 3).

Sequencing of the extended RD1 of *M. africanum* (including both the RD1 sequence that is deleted in *M. bovis* BCG and the overlapping RD1 sequence that is deleted in *M. microti*) (figure 1) revealed fully conserved *esat6* and *cfp10* genes (GenBank accession number 725900) (table 4). Overall, the close relationship between *M. africanum* type 1 and *M. bovis* was confirmed by 3 small insertions/deletions and 3 single-nucleotide polymorphisms (SNPs), which distinguish *M. tuberculosis* strain H37Rv from *M. bovis* and *M. africanum.* Two SNPs distinguish *M. bovis* from *M. tuberculosis* strain H37Rv and *M. africanum,* and 4 SNPs (1 synonymous and 3 nonsynonymous) are characteristic of *M. africanum.* Of note is a 1-bp deletion in Rv3879c that leads to a frame shift and a truncated protein: Rv3879c is a pseudogene in *M. africanum,* and this information is also supported by the finding that 3 of the 4 *M. africanum*–specific SNPs that were identified in the whole 20-kb fragment occur in this gene. The deletion in Rv3879c was confirmed in 5 additional Gambian isolates of *M. africanum* type 1 with different spoligotype patterns.

## DISCUSSION

The present study provides the first evidence, to our knowledge, that strains affect the response to T cell assays for TB. The attenuated immunogenicity of ESAT-6 that we describe for household contacts exposed to *M. africanum* leads to a marked further decrease in sensitivity of the ESAT-6/CFP-10 ELISPOT assay, yet it does not fully explain the decreased response described in The Gambia, compared with that in other countries [10]. ESAT-6 is the dominant immunogen in The Gambia and elicits a response in 81% of *M. tuberculosis*–infected patients (table 2), compared with >90% of these patients in most other studies [3]. An attenuated response was also reported in Ethi-

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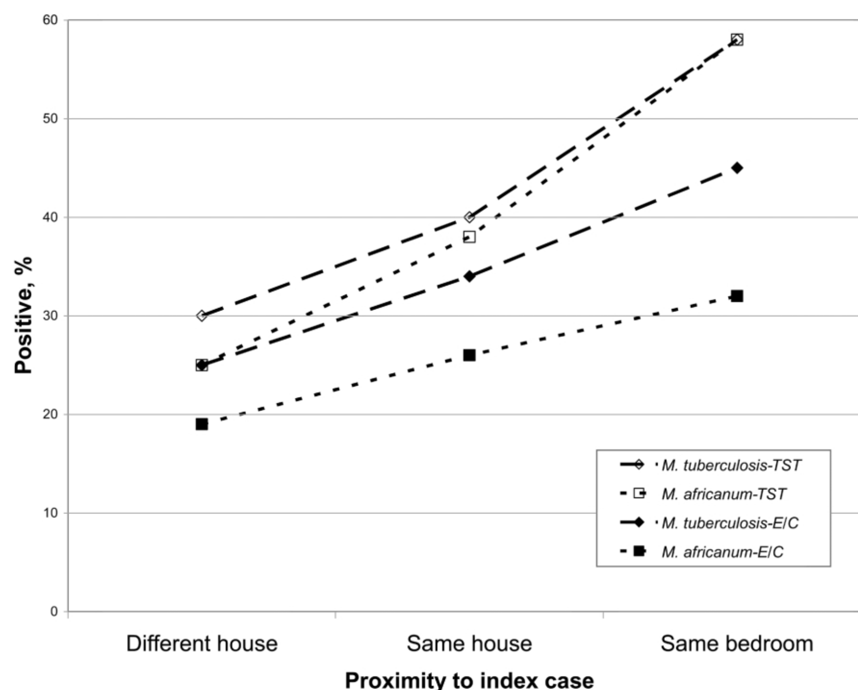


**Figure 2.** Tuberculin skin test (TST) and early secreted antigenic target 6 (E) and culture filtrate protein 10 (C) ELISPOT results in household contacts of tuberculosis (TB) index patient infected with *Mycobacterium africanum* or *M. tuberculosis,* by gradient of proximity to the TB index patient. For both diagnostic tests for TB, the TST results were not significantly different between *M. africanum*– and *M. tuberculosis*–exposed household contacts, whereas the differences in the E/C results were significant.

**Table 4. Three-way comparison of extended region of difference 1 sequences (19,744 bp) in *Mycobacterium* strains.**

The table is available in its entirety in the online edition of the *Journal of Infectious Diseases.*

opian patients with TB, compared with that in Danish patients with TB, when both ESAT-6 and CFP-10 antigens were tested in an IFN-$\gamma$–based ELISA [22]. The additional decrease in response that we describe in *M. africanum* infection could affect the diagnostic value of T cell–based assays that are restricted to these 2 antigens in areas where *M. africanum* is endemic. The lower likelihood of an ESAT-6 response occurs independently of HIV infection—the rates of which are low in The Gambia—because the analysis that was limited to known HIV-negative individuals showed very similar results. Other studies with larger numbers of HIV-infected individuals are needed to address the sensitivity of TB-specific ELISPOT assays in HIV-infected individuals and their household contacts.

Various mechanisms could explain the strain-specific lower likelihood of a response to ESAT-6. That both index patients and household contacts exhibited this phenomenon, in the absence of any difference in the tuberculin skin test responses, argues against a difference in transmissibility between *M. africanum* and *M. tuberculosis.* People from various ethnic backgrounds are less likely to respond to *M. africanum,* although we cannot rule out unmeasured differences in other host factors, such as HLA types, as determinants of ESAT-6/CFP-10 immunogenicity.

Pathogen-related factors seem most likely to be responsible for the lower response. First, we compared the RD1 sequences in *M. africanum* and *M. tuberculosis* to see if polymorphisms in the *esat6* gene or in the genes involved in expression and secretion of ESAT-6 could possibly explain the observed differences in immunogenicity. However, examination of the RD1 sequence did not reveal a clear-cut mechanism: we found that Rv3879c is a pseudogene in *M. africanum,* but this gene is not known to be involved in expression or secretion of ESAT-6 or CFP-10. Rv3879c encodes a proline- and alanine-rich protein that is polymorphic across members of the *M. tuberculosis* complex (table 4) and was found to be immunogenic in 45% of patients with TB and in *M. bovis*–infected cattle [23]. An *M. bovis*–Rv3879c knockout strain did not have a distinguishable phenotype in mice, although the immunogenicity of RD1 antigens was not studied [21]. Moreover, a natural Rv3879c knockout strain has also been described, but there was no mention of clear phenotypic differences between it and strains with the intact gene [24].

Second, the lower ESAT-6 immunogenicity could be due to differences in the extensive posttranslational modifications that the cotranscribed *esat6* and *cfp10* genes undergo [25]. The proteins of these genes are excreted as a complex, and posttranslational modification could differ between *M. africanum* and *M. tuberculosis,* resulting in a lower amount of secretion or lower immunogenicity of the ESAT-6/CFP-10 complex, despite the lack of an obvious explanation based on the RD1 sequence.

Third, *M. africanum* lacks RD8, an area of *M. tuberculosis* that contains 1 of the 11 copies of the *esat6* family of genes. The *esat6* and *cfp10* genes are part of the *esat6* family of genes, with 23 *esat6* family members located in 11 genomic loci on the *M. tuberculosis* genome [25]. These family members differ quite widely in their degree of sequence similarity, and BCG-vaccinated individuals do not recognize the RD1-derived ESAT-6 protein, although they respond to other antigens in the ESAT-6 family, such as Tb10.4 [26]. This situation suggests that antigens encoded by these non-RD1 genes of the *esat6* family do not induce cross-reactive antibodies with the ESAT-6 or CFP-10 antigens encoded by RD1. We compared the amino acid sequence of the RD8 *esat6* family member with the "original" *esat6* gene on RD1. Although the closest match consisted of a sequence of a mere 8 identical amino acids, this finding does not completely exclude the possibility of cross-reactivity between the *esat6* homologue on RD8 and the "original" RD1-based *esat6* gene in *M. tuberculosis.*

Further studies, including those of in vitro *esat6* and *cfp10* expression in *M. africanum,* and animal models in which the ESAT-6 and CFP-10 responses to *M. africanum* are compared with those to *M. tuberculosis,* may elucidate the specific mycobacterial differences underlying our observations. Furthermore, the lower immunogenicity of *M. africanum* might not be limited to RD1-derived proteins, and other antigens of *M. africanum* could also be less immunogenic than are those of *M. tuberculosis,* despite *M. africanum* eliciting similar responses to PPD in vivo and in vitro.

The lower specificity of the tuberculin skin test argues against its use as the reference standard to determine ESAT-6 and CFP-10 sensitivity. Despite this, the tuberculin skin test shows the steepest slope across the proximity gradient (figure 1) in household contacts.

*M. africanum* has not spread beyond western Africa, and its association with HIV infection suggests that it behaves like an opportunist. Some members of the ESAT-6 family of proteins are thought to be involved in the interplay between host and pathogen by either antigenic variation or antigenic drift [25], and lower ESAT-6 immunogenicity might reduce the selective pressure against *M. africanum.* Recent observations suggest that the evolution of *M. canettii* and the *M. tuberculosis* complex started in Africa ~3 million years ago [27], at around the same time that humankind evolved there. Coevolution between humans and mycobacteria might explain why *M. africanum* found a niche in western Africa without establishing itself elsewhere.

Our findings highlight the need to compare different mycobacterial genomic sequences that encode similar antigens and

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to evaluate diagnostics in different settings. It is important to assess other strains of mycobacteria that predominate in particular locations for their effect on T cell assays.

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