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Genetic Regulation of Acquired Immune Responses to Antigens of *Mycobacterium tuberculosis:* a Study of Twins in West Africa

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The role of genetic factors in clinical tuberculosis is increasingly recognized; how such factors regulate the immune response to *Mycobacterium tuberculosis* in healthy individuals is unclear. In this study of 255 adult twin pairs residing in The Gambia, West Africa, it is apparent that memory T-cell responses to secreted mycobacterial antigens (85-kDa antigen complex, "short-term culture filtrate," and peptides from the ESAT-6 protein), as well as to the 65-kDa heat shock protein, are subject to effective genetic regulation. The delayed hypersensitivity response to intradermal tuberculin also demonstrates significant genetic variance, while quantitative T-cell and antibody responses to the 38-kDa cell membrane protein appear to be determined largely by environmental factors. Such findings have implications for vaccine development.

It is estimated that almost 8 million new cases of tuberculosis occur globally each year and that 2 to 3 million people die annually from the disease. The risks are compounded by factors such as overcrowding, concurrent human immunodeficiency virus disease, and the rise in prevalence of multidrug-resistant strains. The only vaccine currently available for human use (*Mycobacterium bovis* bacillus Calmette-Guérin [BCG]) remains highly controversial, with a protective efficacy against pulmonary tuberculosis varying from nil to 80%, possibly due to regional differences in exposure to environmental mycobacteria (12) or to genetic differences in host populations.

There is increasing evidence that host genetic factors are important determinants of the response to infection: twin studies have consistently demonstrated greater concordance for disease among members of monozygous (MZ) than among members of dizygous (DZ) pairs (17, 33). Racial (10) and HLA (23) differences have also been implicated. Recent studies have demonstrated that genetically distinct disorders that affect the production of, or response to, gamma interferon (IFN-γ) or interleukin-12 have been associated with increased risk of mycobacterial infection (26; D. A. Lammas, D. S. Kumararatna, R. de Joug, T. H. M. Ottenhoff, S. E. Dorman, S. M. Holland, F. Altare, and J. L. Casanova, Abstr. 4th. Int. Conf. Pathog. Mycobacterial Infect., p. 68, 1999), while genetic variation in *NRAMP1* and vitamin D receptor genes affects susceptibility to tuberculosis in West Africans (5, 6).

Tuberculosis may be regarded as a complex trait: a disease that results from multiple gene interactions with a major environmental component. Comparison of trait similarity between MZ and DZ twin pairs allows an estimate to be made of the relative genetic and environmental contributions to the phenotype in question.

To investigate the role of heritable factors in the regulation of cellular and humoral responses to specific mycobacterial antigens, we have studied a population of healthy twins residing in The Gambia, West Africa. The twin model is valuable, for it enables the genetic and environmental components of the phenotype under study to be dissected and the genetic component (if significant) to be partitioned into additive and nonadditive components. Healthy twins were chosen for this study (rather than individuals with tuberculosis) because progression to clinical disease may be associated with altered immune responses that could confound the relationship between genes and immunity.

Identification of the mycobacterial antigens that are targets of a protective immune response is clearly a priority if suitable candidates are to be incorporated into a subunit vaccine. Furthermore, an understanding of the genetic mechanisms that regulate the immune response in healthy (but exposed) individuals is clearly important if novel vaccine and other therapeutic targets are to be identified.

Our results demonstrate that heritable factors do influence cellular responses to specific *Mycobacterium tuberculosis* antigens and also appear to influence the delayed-type hypersensitivity (DTH) response that is provoked in primed subjects upon the intradermal injection of *M. tuberculosis* purified protein derivative (PPD). A combined genome screen and candidate gene study is in progress to identify genes linked to and/or associated with these responses.

MATERIALS AND METHODS

Study population. The study was carried out in the Gambia between January 1996 and April 1997. There were 282 twin pairs identified aged 12 years and above who agreed to participate in the study: all were living in rural villages within a 4-h drive of one of the three Medical Research Council (MRC) field stations (based in Basse [a rural area 400 km inland], Fajara [on the coast and including the semiurban conurbations around Banjul] and Farafenni [on the north bank of the River Gambia]). A 10-ml heparinized blood sample was drawn from each donor, after which a Mantoux test was performed by injecting 2 tuberculin units of PPD (Statens Seruminstitut, Copenhagen, Denmark) intradermally into the dorsal aspect of the forearm: the area of induration was mea-

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TABLE 1. Demographic details of MZ and DZ twin pairs included in the final analysis

Characteristic	No. of pairs	
	MZ	DZ
Sex[a]		
Male	32	46
Female	30	78
Mixed		64
Area of origin		
Basse	20	83
Banjul	24	74
Farafenni	18	31
Ethnicity[b]		
Fula	16	45
Mandinka	27	74
Other	18	69
Total	62	193

[a] There were five pairs of DZ twins for whom the sex of the second twin was not recorded.

[b] there were six pairs of twins for whom the ethnic group had not been recorded.

sured at 48 h. The study protocol was approved by the Gambia Government/MRC Ethics Committee. Some of the twins were unavailable at the time of venipuncture, 257 full pairs were bled. A zygosity status (confirmed by minisatellite [37] or microsatellite [11] typing) was available for 255 pairs (193 DZ; 62 MZ). These pairs, aged 12 to 83 years (mean, 25 years), were included in the final analysis.

Lymphoproliferation assays. Peripheral blood mononuclear cells (PMBCs) were separated by density gradient centrifugation and cultured at a density of 10^5/well for 7 days according to methods described previously (30). [^{3}H]thymidine incorporation was measured by using a flat-bed liquid scintillation counter (1205 Betaplate; Wallac Oy, Turku, Finland). Each antigen or mitogen was tested in triplicate at a predetermined optimum concentration that was found to induce maximal secondary responses in the donor population (data not shown). Up to nine unstimulated control wells were included for each donor. A specific vector control antigen (*Escherichia coli* maltose-binding protein) was included in some plates to confirm the specificity of responses to the recombinant 10-kDa antigen. The following antigen stimuli were used: PPD-RT48 (Statens Seruminstitut), 1 μg/ml; Evans' PPD (Evans Medical, Horsham, United Kingdom), 50 U/ml (Fajara and Farafenni only); recombinant *M. tuberculosis* 70-, 38-, and 10-kDa proteins (World Health Organization Recombinant Protein Bank) all at 5 μg/ml; recombinant *M. bovis* 65-kDa protein (World Health Organization), 5 μg/ml; 30- to 32-kDa antigen 85 (Ag85) protein complex (kindly provided by Kris Huygen, Instituut Pasteur van Brabant, Brussels, Belgium), 5 μg/ml; purified short-term culture fluid (ST-CF) (kindly provided by Peter Andersen, Statens Seruminstitut), 1 μg/ml; three peptides from the ESAT-6 protein that were predicted to contain Th epitopes (E6G01, E6-2, and E6-3, kindly provided by Anne de Groot, Brown University, Providence, R.I.), 10 μg/ml; two 20-mer peptides from the *M. tuberculosis* 10-kDa heat shock protein (HSP) (1T and 8T; kindly provided by J. Ivanyi, Hammersmith Hospital, London, United Kingdom), 20 μg/ml; and a 20-mer peptide (38.G) containing residues 350 to 369 from the 38-kDa cell membrane protein (also kindly provided by J. Ivanyi), 25 μg/ml. Phytohemagglutinin (Wellcome Diagnostics, Dartford, England) at 5 μg/ml was included as a system control antigen.

38-kDa ELISA. Plasma immunoglobulin G antibody directed against the 38-kDa phosphate binding protein of *M. tuberculosis* was detected by enzyme-linked immunosorbent assay (ELISA) using plates that had been precoated with recombinant 38-kDa antigen (Omega Diagnostics, Alloa, Scotland) according to a method described previously (36). Defined control samples containing high and low concentrations of anti-38-kDa IgG were included on every plate and used to calculate the value of the cutoff absorbance (optical density [OD]) level at 450 nm (OD_{450}). The antibody index was calculated for each test sample using the formula test OD_{450}/cutoff OD_{450}.

IFN-γ ELISA. The 6-day supernatants from those PBMCs exposed to PPD-RT48, ST-CF, Ag85 complex, and the 70-kDa *M. tuberculosis* HSP were assayed for IFN-γ using a specific human IFN-γ immunoassay (Quantikine; R&D Systems, Inc.). These antigens were found (upon preliminary testing) to elicit the greatest IFN-γ responses; supernatants from 42 MZ and 63 DZ pairs (chosen at random) were assayed.

Statistical methods. For the proliferation data, responses to replicate wells for the test and control antigens were log transformed to normality, and the stimulation index (SI) was calculated as the ratio of their geometric means (7). Individual donors who were ≥3.5 standard deviations from the mean for any antigen response were excluded from the analysis of that antigen (generally, the number of rejected donors was small, and it never accounted for >5% of the total). All variables were tested for equality of means and variances across sex and zygosity to ensure the validity of the twin model. Essentially, there are four major causes of phenotypic variation: additive (A) and dominant (D) genetic influences, common environment (C) (these three result in family members appearing to be more alike than random individuals), and unique environmental variance (including measurement error) (E). A twin model-fitting approach was used to estimate the components of variance (A, D, C, and E) that provided the most parsimonious model to account for the observations. Model fitting was performed using Mx (25). The variance parameters were estimated by normal-theory maximum-likelihood estimates as the models were fitted to the raw data. Since Mx uses raw data instead of variance-covariance matrices, the program does not give a goodness-of-fit statistic but does provide a model for the likelihood. The variance components C and D are negatively confounded, so they cannot be estimated concurrently in a study of MZ and DZ twins reared together.

For each variable, the full ACE or ADE model was fitted to the data. The significances of the variance components A, C, and D were tested by dropping them in specific submodels, eventually leading to the most parsimonious fit to the data. The fit of each submodel was assessed by the difference in log likelihood between the submodel and the full model (20). Twice the difference in log likelihood is distributed as $\Delta\chi^2$, with the degrees of freedom equal to the difference in degree of freedom between the full model and the submodel. The Akaike's Information Criterion, computed as $\chi^2 - 2$ df, can also be used as another indicator of the fit of submodels (1); the submodel with the most negative Akaike's Information Criterion is taken to be the best-fitting model. For some variables, there was no statistically significant effect of dropping variance components from the full model, and this is due to the lack of power associated with the small number of twin pairs (21). Therefore, only the saturated (ACE or ADE) model is listed, with 95% confidence intervals for each variance estimate for these variables.

The 38-kDa and IFN-γ ELISA data were analyzed using the same program after natural log transformation. The tuberculin responses (diameter of induration [R]) were categorized ($R = 0$, $0 < R < 10$ mm, and $R \geq 10$ mm) for analysis.

RESULTS

The demographic details of the study population are shown in Table 1. The mean age of the MZ pairs was 24.3 ± 1.11 years, and that of the DZ pairs was 25.2 ± 0.72 years.

Lymphoproliferative responses. The median SI value for each antigen for the two twin types is shown in Table 2; there was no significant difference between the two groups for any antigen (data not shown). The mean differences in the log(SI)

TABLE 2. Median SI values by twin type for each antigen

Antigen	Median SI (interquartile range)	
	MZ	DZ
PPD-RT48	80.62 (27.75–169.78)	63.45 (25.07–138.14)
Evans' PPD	40.95 (15.63–118.70)	39.48 (12.07–91.41)
70-kDa HSP	5.38 (1.56–12.52)	5.28 (2.02–13.14)
65-kDa HSP	3.51 (1.39–7.36)	3.94 (1.50–9.17)
10-kDa HSP	1.65 (1.13–2.93)	2.00 (1.24–3.80)
T1 peptide	1.19 (0.76–2.09)	1.15 (0.86–1.68)
T8 peptide	0.93 (0.70–1.46)	1.04 (0.81–1.50)
38-kDa protein	1.96 (1.13–3.97)	2.30 (1.26–4.35)
38G peptide	1.16 (0.71–1.71)	1.08 (0.81–1.64)
85-kDa protein	3.65 (1.86–11.94)	4.16 (1.78–13.28)
ST-CF	67.37 (21.52–132.15)	49.32 (18.14–106.79)
E6G01 peptide	1.42 (0.98–3.31)	1.29 (0.79–2.65)
E6-2 peptide	1.09 (0.66–2.36)	1.02 (0.74–1.72)
E6-3 peptide	1.17 (0.74–1.73)	1.19 (0.83–1.95)
Phytohemagglutinin	73.13 (31.84–138.01)	63.26 (23.67–153.23)

TABLE 3. Mean interpair differences in log(SI) by twin type for each antigen

Antigen	No. of MZ pairs	Mean MZ within-pair difference (SD)	No. of DZ pairs	Mean DZ within-pair difference (SD)
PPD-RT48	54	0.92 (0.85)	162	1.05 (0.87)
Evans' PPD	32	0.88 (0.88)	87	1.16 (0.98)
70-kDa HSP	54	0.76 (0.68)	169	0.79 (0.61)
65-kDa HSP	50	0.57 (0.45)	166	0.74 (0.58)
10-kDa HSP	52	0.55 (0.53)	159	0.55 (0.52)
IT peptide	29	0.32 (0.37)	61	0.34 (0.34)
8T peptide	27	0.23 (0.25)	75	0.27 (0.25)
38-kDa protein	54	0.60 (0.58)	165	0.57 (0.49)
38G	33	0.37 (0.38)	91	0.34 (0.26)
85-kDa protein	35	0.78 (0.64)	92	0.97 (0.81)
ST-CF	46	0.91 (0.94)	150	1.19 (1.07)
E6G01 peptide	50	0.52 (0.53)	142	0.55 (0.55)
E6-2 peptide	28	0.39 (0.33)	75	0.38 (0.36)
E6-3 peptide	27	0.37 (0.39)	70	0.39 (0.40)
Phytohemagglutinin	50	0.89 (0.81)	160	1.00 (0.87)

values between pairs of MZ and DZ twins are shown in Table 3. The mean within-pair differences are generally smaller for the MZ pairs than for the DZ pairs (the exception being the responses to the 38-kDa protein and the derived 38G peptide). Some antigens were made available after the study had commenced, so the numbers of twins tested against these are smaller than the whole cohort; in other cases, small venous bleeds meant that it was not possible to test every donor against every antigen. The estimates of the variance components, confidence interval, and most parsimonious model for each antigen are shown in Table 4. None of the antigen responses demonstrated a significant dominance component, so the value of the additive genetic variance component (*a*) equals the heritability. If no value of *a* is shown, there is no evidence of a significant heritable component.

It is apparent that environmental rather than genetic factors are the major determinants of the quantitative cellular responses to the 70- and 10-kDa HSPs, possibly as a result of the widespread occurrence of closely related HSP molecular chaperones in common environmental prokaryotes. The fact that heavy environmental exposure may mask a genetic effect (through inflation of the common [shared] environmental component) provides an explanation for the apparent lack of genetic variance in the response to PPD-RT48 (which was tested in all areas); a significant additive variance in response to the Evans' PPD (which was utilized in the more urban areas of Fajara and Farafenni but not in Basse) was demonstrated, although it could be argued that the difference reflects nonspecific variation in the compositions of the two preparations.

Unlike the response to the 70-kDa HSP, proliferative responses to the *M. bovis* 65-kDa HSP demonstrate a significant heritable component: the additive genetic variance accounts for 71% of the total phenotypic variance.

T-cell responses to the whole recombinant 38-kDa phosphate binding antigen and to the permissively recognized immunodominant 20-mer peptide (38.G) at the carboxy terminus of the molecule (35) did not appear to be subject to genetic regulation, with environmental components accounting for most of the phenotypic variation. Humoral responses to this membrane protein also appear to be determined largely by nongenetic factors (95% confidence intervals are shown in

TABLE 4. Results of univariate analysis and path model for antigen-induced proliferation assays[a]

Antigen	No. of MZ pairs	No. of DZ pairs	*a*	*c*	*e*	Model
Mixed antigens						
PPD-RT48	56	186		0.40 (0.28, 0.51)	0.60 (0.49, 0.72)	CE
Evans' PPD	35	102	0.59 (0.39, 0.74)		0.41 (0.26, 0.61)	AE[b]
HSPs						
70 kDa	56	186		0.44 (0.32, 0.54)	0.56 (0.46, 0.68)	CE
65 kDa	53	179	0.71 (0.59, 0.79)		0.29 (0.21, 0.42)	AE
10 kDa	55	182	0.00 (0.00, 0.46)	0.21 (0.00, 0.33)	0.79 (0.53, 0.92)	CE
10-kDa 20-mer peptides						
T1	32	71		0.40 (0.21, 0.55)	0.61 (0.45, 0.79)	CE
T8	29	85	0.24 (0.00, 0.55)	0.07 (0.00, 0.40)	0.69 (0.45, 0.95)	ACE
Cell membrane proteins						
38 kDa	55	182		0.18 (0.05, 0.30)	0.82 (0.70, 0.95)	CE
38 kDa 20-mer peptide (38G)	35	101			1.00	E
Secreted proteins						
85 kDa	36	101	0.52 (0.30, 0.69)		0.48 (0.31, 0.70)	AE
ST-CF	55	179	0.50 (0.32, 0.65)		0.50 (0.35, 0.68)	AE
ESAT-6 peptides						
E6G01 (13-mer)			0.45 (0.23, 0.62)		0.55 (0.38, 0.77)	AE
E6-2 (20-mer)	34	93	0.26 (0.00, 0.60)	0.14 (0.00, 0.48)	0.60 (0.40, 0.83)	ACE
E6-3 (16-mer)	35	84	0.00 (0.00, 0.43)	0.24 (0.00, 0.41)	0.76 (0.56, 0.93)	CE
System control mitogen						
phytohemagglutinin	54	184		0.54 (0.44, 0.63)	0.46 (0.37, 0.56)	CE

[a] 95% confidence intervals are in parentheses. *a*, *c*, and *e* represent the values of additive genetic, shared environmental, and unique environmental components of the total phenotypic variance, respectively. No dominance components were detected.

[b] Fajara and Farafenni areas only (see the text).

TABLE 5. Results of univariate analysis and path model for antigen-induced secretion of interferon-γ[a]

Antigen	No. of MZ pairs	No. of DZ pairs	*a*	*c*	*e*	Model
PPD-RT48	41	61	0.39 (0.00, 0.68)	0.11 (0.00, 0.51)	0.50 (0.32, 0.76)	ACE
70 kDa	41	61	0.00 (0.00, 0.56)	0.32 (0.00, 0.53)	0.69 (0.42, 0.93)	ACE
85 kDa	24	37	0.40 (0.00, 0.86)	0.24 (0.00, 0.73)	0.35 (0.14, 0.71)	ACE
ST-CF	40	63		0.46 (0.28, 0.60)	0.55 (0.40, 0.73)	CE

[a] 95% confidence intervals are in parentheses. *a*, *c*, and *e* represent the values of the additive genetic, shared environmental, and unique environmental components of the total phenotypic variance, respectively. No dominance components were detected.

brackets) ($c = 0.19$ [0.01, 0.36]; $e = 0.81$ [0.64, 0.99],) indicative of a CE model, based upon 32 MZ and 93 DZ pairs), possibly as a result of the homology between this molecule and the PhoS protein of *E. coli* (14).

The cellular responses that appear to be highly heritable are those directed against the secreted proteins of the Ag85 complex and the mixture of secreted proteins found within the ST-CF. One of the predominant components of the ST-CF is the low-molecular weight protein ESAT-6, which has been found primarily in *M. tuberculosis* but not in most environmental mycobacteria or in BCG (15); it elicits strong T-cell-proliferative and IFN-γ responses in tuberculosis patients and their contacts (9). T-cell mapping of the molecule has defined different epitopes in different human populations, perhaps reflecting variation in HLA restriction (29). The responses to E6G-01 (which incorporates a sequence of 13 amino acids from the first 20 of the *M. tuberculosis* ESAT-6 sequence) and E6-2 (a 20-mer which partially overlaps with E6G-01) demonstrate significant additive genetic variance.

Skin test DTH responses. Skin test DTH responses to tuberculin were tested and measured in 396 subjects; of these, 246 individuals (62.1%) produced an area of induration with a diameter of 5 mm or greater (Mantoux positive). Of the nonresponders (PPD diameter, <5 mm), almost 40% were under 18 years old while only 3% were 60 years old or above. Definite evidence of a BCG scar was apparent in 303 subjects (76.5% of the study population), of whom 190 (62.7%) were Mantoux positive. Following categorization of the Mantoux responses as described above (data were available for 62 MZ and 155 DZ pairs), model fitting provided evidence that this response is under genetic control in this population, for the most parsimonious model was that in which 71% of the residual variation was explained by additive genetic variance (AE: $a = 0.71$ [0.48, 0.85]; $e = 0.30$ [0.15, 0.53]).

There was a significant positive correlation between the quantitative Mantoux response and the proliferation response to PPD-RT48 ($r = 0.16$; $P = 0.02$) and also between the Mantoux and the ST-CF responses ($r = 0.23$; $p < 0.01$), between the Mantoux and ESAT-6 peptides ($r = 0.27$ and $p < 0.01$ for E6G01; $r = 0.22$ and $P = 0.02$ for E6-2; and $r = 0.21$ and $P = 0.03$ for E6-3), and between the Mantoux and the 10-kDa HSP peptide T8 ($r = 0.34$; $P = 0.01$). There were no other significant correlations.

IFN-γ responses. High levels of IFN-γ were detectable in the supernatants of PBMCs stimulated by PPD-RT48 and the ST-CF (median concentrations, 540 and 359 pg/ml, respectively, with >96% positive responders); rather lower levels were detected in response to the 70- and 85-kDa antigens (median concentrations, 34 and 13 pg/ml, with 81 and 61%, respectively, of donors tested having detectable levels). The estimates of the variance components of these responses are shown in Table 5. With the exception of the ST-CF response (for which no genetic variance was apparent), stimulated release of this cytokine may be subject to a low level of genetic regulation, although environmental factors generally account for a greater proportion of the phenotypic variance, largely through the influence of shared (common) environmental experience. The IFN-γ responses induced by each of the antigens are highly correlated (data not shown); furthermore, the responses induced by the 70-kDa HSP correlate significantly with the proliferation responses to the 70-, 65-, and 10-kDa proteins ($r = 0.49$, 0.39, and 0.21, respectively).

DISCUSSION

Heritable factors are known to influence susceptibility to tuberculosis; this study extends current understanding by defining some of the individual anti-mycobacterial immune responses (in healthy donors) that demonstrate a significant genetic component. These results may account, in part, for the described clinical observations, and they highlight the relevance of twin studies for dissecting the genetic regulation of immunological mechanisms that are likely to underlie observed variations in clinical disease susceptibility.

The hsp60 protein is an immunodominant target of both B and T cells in human and animal studies (19, 40). Recent studies have demonstrated that HSPs may exert independent stimulatory effects upon immunocompetent cells and that more than one signaling pathway may be involved (4); such findings may explain why the immune responses to different HSPs demonstrated different levels of genetic regulation.

The 38-kDa antigen is a phosphate binding protein that has been found to be a potent stimulus of both T and B cells in humans (39). T cells from humans with tuberculosis have been shown to have a selective anergy to both the whole protein (36) and the immunodominant 38.G peptide (35), suggesting that recognition of this antigen may be an important component of the protective immune response. Our study implies that cellular and humoral responses to this antigen are not subject to marked genetic restriction in humans.

There is evidence that important protective epitopes are to be found among the secreted antigens of growing mycobacteria. The mouse model has demonstrated that major targets for Th1 cytokine secretion by memory $CD4^+$ cells include Ag85B and ESAT-6 (2, 9a). T cells from healthy household contacts of tuberculosis patients produce significantly higher levels of IFN-γ upon exposure to Ag85B than those from patients with active tuberculosis (39), while patients with active minimal disease produce stronger Th1-type responses to low-molecular-weight (<10-kDa) secreted antigens than patients with

active pulmonary disease (9). These low-molecular-weight proteins are important vaccine candidates, for their early recognition may limit bacterial replication: mice vaccinated with Ag85A DNA were able to resist challenge with live *M. tuberculosis* and *M. bovis* BCG (15a). Recent data suggest that Ag85 complex-specific IFN-γ-secreting cells localize to the lungs in healthy contacts of tuberculosis patients, providing further evidence for a role of these cells in the early response to *M. tuberculosis* infection (31). The fact that these cellular responses appear to be genetically restricted is significant and may explain some of the clinical variation in response to infection.

The DTH response to intradermal tuberculin essentially represents the summed effects of a Th1-type response to mycobacterial antigens (8). Exposure to these antigens may occur through contact with environmental mycobacteria or through BCG vaccination. The relationship between the magnitude of the Mantoux response and protective immunity to *M. tuberculosis* is unclear (12, 28), and evidence now exists from murine studies that the cellular targets of the DTH and those of immunity to *M. tuberculosis* are different: protective T cells recognize low-molecular-weight (<15-kDa) ST-CF antigens, while cells recruited during the DTH response recognize predominantly Ag85B and the 65- and 70-kDa HSPs (27). While the DTH response identifies individuals sensitized by mycobacteria, it may also be used to assess immunity conferred by BCG vaccination, although epidemiological studies indicate variable persistence of sensitivity (18, 22, 24). In The Gambia, BCG vaccination in childhood has been included in the expanded immunization program since 1980, and previous countrywide BCG vaccination programes have targeted individual populations, accounting for the high proportion of scar-positive individuals in our study. The boosting effect of exposure to environmental mycobacteria is therefore likely to be significant in this rural population, where the proportion of Mantoux-positive individuals increases with age. Demonstration that the magnitude of the DTH response appears to be heritable in this population is of interest, therefore, and supports our laboratory findings that cellular responses to the Ag85 complex proteins and to the 65-kDa HSP are genetically restricted. A previous study has reported that a genetic influence was apparent in the tuberculin responses of older children who had received two BCG immunizations (13) but not in a group of young twins vaccinated at birth, probably owing to the immaturity of the immune response (32).

Each of the phenotypic characteristics that were examined in this study represents the end point of a complex series of cellular and cytokine interactions which may include several genetically regulated components; it is possible that genetically regulated steps operate at any point from antigen processing and presentation through the cytokine production or DNA synthesis recorded in this study. Furthermore, different antigens may be processed through different pathways and be subject to different coregulatory signals; distinct pathways for cytokine gene promoters may also exist (38). It was not the aim of this report to identify individual candidate genes but rather to provide an indication that cellular immune responses to defined groups of mycobacterial antigens are likely to be genetically determined.

It may be argued that the sharing of HLA alleles by the MZ twins could explain our results; we have observed in a previous study that the contribution of HLA-encoded genes is smaller than that of the non-HLA-encoded genes (16). Although the new twins in the current study were not HLA typed, it was possible to identify 12 pairs of HLA-identical DZ twins in this study that had also been included in the previous study: although the numbers are small, comparison of the intraclass correlation coefficients for these twins with those for the whole DZ cohort for ST-CF did not indicate a major class II-mediated effect (data not shown). Arend et al. recently demonstrated that T-cell responses to a recombinant ESAT-6 preparation and to ST-CF did not differ among patients with different HLA-DR types (3), although recognition of different immunodominant ESAT-6 peptide epitopes has been reported in different ethnic groups (29). Data describing the influence of individual candidate genes on the cellular and Mantoux responses will be presented elsewhere (A. Fowler, unpublished data).

In summary, therefore, this study demonstrates that heritable factors may regulate the magnitude of the cellular proliferative responses to the 65-kDa HSP and to secreted antigens of *M. tuberculosis*; they also influence the size of the Mantoux response in healthy individuals. Monokine responses stimulated by cell wall components also constitute an important early defense against infection, and we have evidence that some of these, too, are genetically determined (Fowler, unpublished). The secreted antigens are likely targets of a protective immune response, and our results have implications for further understanding of the genetic mechanisms that underlie disease susceptibility.

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