Kinetics of Delayed-Type Hypersensitivity to Tuberculin Induced by Bacille Calmette-Guérin Vaccination in Northern Malawi

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During 1986–1989, a bacille Calmette-Guérin (BCG) vaccine trial was carried out in northern Malawi. The effects of age, sex, and prevaccination delayed-type hypersensitivity (DTH) on the time course of the DTH response over 1–36 months after vaccination were studied in 2418 persons. DTH response increased rapidly, to peak at 31–90 days after vaccination, when most persons had a measurable response. This was followed by a marked decline by 181–365 days, particularly in those <15 years old at vaccination, followed by a more gradual decline. Prevaccination DTH was the single best predictor of postvaccination DTH. BCG-induced DTH responsiveness appears to decline more rapidly in tropical than in temperate environments. This may reflect high prevalence of exposure to other infections, which induce a Th2 bias or compete for "space" within the T lymphocyte compartment. The inability of some persons to mount a persistent DTH response probably reflects genetic background and/or environmental exposure history.

The discussion of the relationship between bacille Calmette-Guérin (BCG) vaccination and delayed-type hypersensitivity (DTH) to tuberculin has concentrated mainly on 3 issues. First, is the induction of tuberculin sensitivity an indication of induced protection against tuberculosis (TB)? It is now widely agreed that this is not so. Even though quality-control screening of BCG vaccines requires the induction of DTH in guinea pigs, there is no evidence that vaccine-induced DTH is a correlate of induced protection in humans [1]. The second issue concerns the implications of BCG-associated tuberculin hypersensitivity for control policies. The use and interpretation of tuberculin tests must take into account a person’s history of BCG vaccination [2]. The third issue concerns the implications of BCG-induced DTH when tuberculin surveys are used to estimate the annual risk of Mycobacterium tuberculosis infection. Such studies typically exclude persons with a history of BCG vaccination [3, 4].

Most discussion of these issues has neglected the fact that the degree of BCG-induced DTH depends on time and geography, as first noted long ago. In 1952, it was noted that the distribution of tuberculin sensitivity 1 year after BCG vaccination differed among populations in Denmark, Greece (Athens), and Egypt (Cairo). The Danish vaccinees had the strongest residual DTH, and the Egyptians had the least [5]. This observation is consistent with several others in the literature. For example, BCG-vaccinated Danish children who were tested for tuberculin either 10 weeks or 5 years after vaccination appeared to maintain their tuberculin hypersensitivity [6–8], whereas persons in a large south India BCG vaccine trial who were tested at 2.5 months or 2.5 years after vaccination showed considerable loss of hypersensitivity over time [9]. In a study in Malawi, only a small proportion (~10%) of BCG-vaccinated persons maintained BCG-attributable hypersensitivity for many years after vaccination [3].

These observations raise 3 important questions. What are the kinetics of the induction and persistence of DTH responsiveness after BCG vaccination? What are the implications of the kinetics of BCG-induced DTH for immunity and T cell memory? Finally, why do these kinetics appear to differ between populations? Published studies are limited in their ability to answer these questions. Few have measured the DTH response at several time points after BCG vaccination, few have included both adults and children, and only one has included persons who received a repeat BCG vaccination [10].

Here, we report tuberculin-sensitivity data from northern Malawi for >2000 children and adults. Their DTH responses were measured from 31 days to 36 months after either a first or a second BCG vaccination. We explore the implications of age, sex, initial BCG scar status, and prevaccination DTH for the magnitude and time trend of the postvaccination DTH...
response. We also assess detailed age trends in tuberculin sensitivity among young children as a function of BCG scar status, which reflects vaccination in infancy.

Methods

During 1986–1989, the Karonga Prevention Study implemented a randomized, double-blind vaccine trial of BCG-containing vaccine versus repeat inoculation with the BCG vaccine versus BCG vaccine plus killed M. leprae in the Karonga District of northern Malawi. The background, design, recruitment methods, and trial results are described in detail elsewhere [11, 12]. Trained vaccinators gave all vaccinations.

Subjects were eligible for the trial if they were >3 months old, were born after 1914, had no evidence of past or present TB or leprosy, and had no evidence of current malnutrition or severe illness. Those with scar evidence of previous BCG vaccination at recruitment (“scar positive”) were given either a repeat BCG vaccination, a repeat BCG vaccination plus 6 × 10^7 killed M. leprae, or placebo (dextran). Persons with no evidence of a scar at recruitment (“scar negative”) were given either BCG vaccination, BCG vaccination plus 5 × 10^7 killed M. leprae, or BCG vaccination plus 6 × 10^7 killed M. leprae. BCG vaccine was provided by Glaxo (strain 1077) and was given in a standard dose (0.1 mL) in the deltoid region of the right arm.

Of >120,000 subjects in the trial, 2418 had tuberculin skin tests between 31 days and 3 years after receipt of a BCG-containing vaccine (6 subjects were tested twice), and 452 were tested after receiving placebo. Of these 2870 persons, 1725 were also tested 1 week before and 1 week after vaccination (table 1). These analyses gave virtually identical results to those that assumed independence of observations made on the same person (because most individuals were measured twice, once before and once after vaccination). These analyses gave virtually identical results to those that assumed independence. For simplicity, only those that assumed independence are presented here.

We also analyzed data for 14,666 BCG scar–positive children and 9220 BCG scar–negative children, who had tuberculin skin tests at age ≤10 years, during a population survey of Karonga district in 1980–1985 [13]. The percentage of children with a skin-test response above the thresholds of 5 and 10 mm was calculated by BCG scar status and age.

Results

Data are summarized in table 1 as the percentage of subjects with an induration diameter of >5 mm before vaccination and at 5 time points after vaccination. The trends were similar when we used 0- or 10-mm thresholds (data not shown). As expected, the prevalence of DTH responsiveness prior to vaccination increased with age (table 1 and figure 1). The time course of the DTH response was similar for persons who received BCG with or without killed M. leprae (table 1) and, thus, data from these groups are combined in subsequent analyses.

Figure 1 shows the frequency distributions of DTH response before and at 5 time points after vaccination for subjects <15 and ≥15 years old at vaccination among persons who lacked a BCG scar at the time of vaccination. There was a sharp increase in response 2–3 months after vaccination in both age groups, at which time almost all persons had a DTH response (≥0 mm). This was followed by a marked decline in DTH response that was larger among younger than among older subjects. The evidence that the time course of the postvaccination DTH response differed by age was strong (P = .012 and P < .001 for the 5- and 10-mm thresholds, respectively) and was also evident in the time trend of the median response (data not shown).

The sex difference in DTH response among those <15 years old was small and not statistically significant, but, at older ages, the responses were higher among men (figure 2A) both before and 4–36 months after vaccination (test for age-sex interaction 4–36 months after vaccination, P = .006 and P < .001 for use of 5- and 10-mm thresholds, respectively). The exception was at the peak period 2–3 months after vaccination when men and women had similar DTH responses. Of importance, the male excess after vaccination could be explained entirely by the DTH difference at the time of vaccination, because there was no evidence of an age-sex interaction in DTH response after vaccination after adjustment for the prevaccination response (analysis based on 1467 skin tests) or when analysis was restricted
Table 1. Subjects with a delayed-type hypersensitivity response to tuberculin of >5 mm, by time since vaccination, initial bacille Calmette-Guérin (BCG) scar status, age at vaccination, sex, and vaccine type.

<table>
<thead>
<tr>
<th>BCG scar status at vaccination, variable</th>
<th>Before vaccination</th>
<th>Test time after vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>31–90 days</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4</td>
<td>9 (248)</td>
<td>82 (45)</td>
</tr>
<tr>
<td>5–9</td>
<td>15 (126)</td>
<td>95 (21)</td>
</tr>
<tr>
<td>10–14</td>
<td>33 (144)</td>
<td>86 (28)</td>
</tr>
<tr>
<td>15–24</td>
<td>47 (96)</td>
<td>88 (17)</td>
</tr>
<tr>
<td>25–44</td>
<td>51 (202)</td>
<td>90 (40)</td>
</tr>
<tr>
<td>≥45</td>
<td>64 (171)</td>
<td>100 (34)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>37 (434)</td>
<td>90 (83)</td>
</tr>
<tr>
<td>Female</td>
<td>33 (553)</td>
<td>89 (102)</td>
</tr>
<tr>
<td>Vaccine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCG</td>
<td>36 (456)</td>
<td>87 (78)</td>
</tr>
<tr>
<td>BCG + 5 × 10⁶ killed M. leprae</td>
<td>33 (135)</td>
<td>93 (29)</td>
</tr>
<tr>
<td>BCG + 6 × 10⁶ killed M. leprae</td>
<td>34 (396)</td>
<td>91 (78)</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4</td>
<td>16 (128)</td>
<td>100 (20)</td>
</tr>
<tr>
<td>5–9</td>
<td>28 (243)</td>
<td>97 (32)</td>
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<td>10–14</td>
<td>34 (134)</td>
<td>94 (17)</td>
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<td>57 (161)</td>
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<td>25–44</td>
<td>54 (69)</td>
<td>100 (13)</td>
</tr>
<tr>
<td>≥45</td>
<td>100 (3)</td>
<td>100 (1)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38 (370)</td>
<td>9 (61)</td>
</tr>
<tr>
<td>Female</td>
<td>34 (368)</td>
<td>100 (52)</td>
</tr>
<tr>
<td>Vaccine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCG</td>
<td>31 (292)</td>
<td>98 (46)</td>
</tr>
<tr>
<td>BCG + 6 × 10⁶ killed M. leprae</td>
<td>40 (184)</td>
<td>97 (67)</td>
</tr>
<tr>
<td>Placebo</td>
<td>38 (262)</td>
<td>56 (50)</td>
</tr>
</tbody>
</table>

NOTE. Data are percentage (no.) of subjects. M. leprae, Mycobacterium leprae.
Figure 1. Delayed-type hypersensitivity response to tuberculin, by age at vaccination and time after vaccination, among subjects who were bacille Calmette-Guérin scar negative at the time of vaccination. A. Age <15 years at vaccination. B. Age ≥15 years at vaccination.

increased at 31–90 days was not much greater than the percentage whose response had decreased: 23 (48%) had an increased response at 31–90 days, 18 (37%) had a decreased response, and 7 (15%) had no change.

It was not possible to identify the timing of the peak response more precisely than 31–90 days after vaccination because of sample size constraints.

Figure 5 summarizes the tuberculin sensitivity of the 23,886 children given skin tests during a total population survey in 1980–1985 at age ≥10 years. Each single-year age group contains ≥750 children. The percentage of children with a response of >5 and >10 mm increased with age for BCG scar-negative children \((P<.001)\). For BCG scar-positive children, the percentage with a response of >5 mm decreased between ages <1 and 2 years and then progressively increased. The percentage of BCG scar-positive children with a response of >10 mm was fairly stable between ages <1 and 4 years and then slowly increased. The evidence that age trends differed between BCG scar-negative and BCG scar-positive children was highly significant for both thresholds \((P<.001)\).

Discussion

Our findings provide important insights into the peculiarities of BCG-induced DTH response to tuberculin, as noted by others [5–10, 14, 15]. In Malawi, BCG-induced DTH peaks 2–3 months after vaccination, at which time most persons have a DTH response. The DTH response then declines, first sharply and then more gradually. The data further show that the level of tuberculin reactivity achieved is greater for older persons, greater for male persons than female persons ≥15 years old, and greater for persons with prior BCG scars. By far, the most important determinant of DTH status after BCG vaccination in this population is the DTH response before vaccination (itself
higher in older persons, male persons, and those with a prior BCG scar).

These findings are consistent with other study results. Detailed studies of schoolchildren in Denmark, Egypt, India, Mexico, and Pakistan [6] and a study in Myanmar [16] also found that most persons have a DTH response 2–3 months after BCG vaccination. The dramatic decrease in DTH response observed in this population, which occurs after the peak at 3 months, is similar to decreases reported in several tropical or subtropical populations—for example, the (less detailed) results from the southern India BCG trial [9] and studies in the Solomon Islands [17], Sri Lanka [18], and the southern United States [19]. We are aware of only one apparent exception to this pattern: most children vaccinated in the BCG trial in Puerto Rico 1949–1951 maintained appreciable DTH responsiveness when tested 4–5 years later, with a distribution of response similar to that of TB cases and markedly different from the distribution among unvaccinated control subjects [20]. Thus, DTH response can be quite stable in some subtropical environments. A possible reason for this exception is that stability of BCG-induced DTH varies by vaccine strain (variation among BCG strains for post-vaccination DTH response is well documented [7]; the Birkhaug-Albany strain was used in the Puerto Rico trial). However, this is unlikely to be the full explanation. For example, the Copenhagen strain, which induced relatively strong DTH response to tuberculin 10 weeks after vaccination in Danish children [7], was used in southern India, where there was substantial waning of BCG-induced DTH.

That BCG-induced DTH wanes after vaccination in tropical environments is further supported by the Malawi data for children with skin tests during the population survey of 1980–1985 (figure 5). These children were vaccinated within an expanded immunization program either at birth or at initial health services contact (e.g., at the time of the first diphtheria–tetanus toxoid–pertussis vaccination at age 6 weeks). Only 19% of BCG scar–positive children <1 year old at testing had an induration response of >5 mm; this declined to just 14% among those 2 years old at testing, even though exposure to environmental mycobacteria and/or M. tuberculosis occurs between birth and age 2 years. This cumulative exposure is evident in the data for children who were BCG scar negative, for whom the percent-ages >5 and >10 mm increased progressively between ages <1 and 10 years.

In contrast, there is considerable evidence that BCG-induced DTH response is relatively stable among temperate-zone populations, as first noted in 1952 [5]. Examples include studies of Danish schoolchildren, for whom the response 5 years after vaccination was virtually identical to that 6–13 weeks after vaccination for children who had intermediate testing [6] and was only slightly reduced at 5 years for children with no intermediate tests [7, 8], and 2 studies of UK children [21, 22]. That prevaccination DTH status emerges as the most important determinant of the postvaccination DTH response is perhaps not surprising. A person’s DTH status represents cumulative exposure to mycobacterial (environmental, M. tuberculosis, or M. leprae) antigens over time, and a BCG vaccination can be interpreted as just one in a lifetime of mycobacterial exposures.

Figure 2. Proportion of subjects with delayed-type hypersensitivity (DTH) response to tuberculin of >5 mm, by age at vaccination (in years), sex, and time after vaccination among subjects who were bacille Calmette-Guérin scar negative at the time of vaccination. A, All individuals. Proportions were predicted by a logistic regression model. B, Individuals with a prevaccination DTH response of 0 mm. Raw data are shown.

Figure 3. Proportion of persons with delayed-type hypersensitivity response to tuberculin of >5 mm, by age at vaccination (in years), initial bacille Calmette-Guérin (BCG) scar status (+, positive; −, negative), and time after vaccination, as predicted by logistic regression model.
The relationship between pre- and postvaccination response is consistent with the evidence that the difference in DTH response between persons with and without a prior BCG vaccination persists after (a second) BCG vaccination, as reported among Danish children [10].

On the other hand, our data also suggest that at least some persons have a “preferred” DTH status to which they return after an exposure such as vaccination with BCG. This is clearly demonstrated by the tendency of persons who showed no tuberculin reactivity at the time of vaccination to mount a reasonable short-term response but then to return to the anergic state (figure 4). This indicates that these persons are somehow “programmed” to a particular DTH status. Such programming could, in theory, reflect either genetic determinants (there is some evidence for genetic influences on DTH reactivity in humans [23] and animals [24]) or prior immunologic experience. Our findings in this regard are consistent with 3 previous, although less detailed, studies [19, 25, 26].

The male excess in DTH response to tuberculin among adults is attributed by some authors to sex differences in exposure to \( M. \) \textit{tuberculosis} (or environmental mycobacteria) [27]; others have suggested that the male excess might reflect a sex difference in the ability to generate a DTH response [28]. The fact that postvaccination DTH responses were greater among male individuals than among female individuals at age \( \geq 15 \) years at vaccination in our study (figure 2A) shows that BCG vaccination does not eliminate the male excess in DTH response in this age group (which was evident before vaccination in these data). This finding is consistent with a biologic difference in DTH response between male and female persons, but it is also consistent with there being differences in cumulative exposure to mycobacteria between male and female persons, which is reflected in DTH responsiveness.

There was no evidence of a male-female difference among persons \( \geq 15 \) years old who lacked a BCG scar and who made no DTH response (0 mm) at the time of vaccination (figure 2B), although numbers were relatively small and the power of the analysis was low. If the absence of a prevaccination DTH response simply reflects lack of prior exposure to \( M. \) \textit{tuberculosis} or to environmental mycobacteria, this finding would be evidence against the hypothesis of a male-female difference in DTH responsiveness. However, it is possible that the lack of

**Figure 4.** Delayed-type hypersensitivity (DTH) response to tuberculin, by prevaccination DTH response (A, 0 mm; B, 1–5 mm; C, 6–10 mm; D, >10 mm) and time after vaccination, among subjects who were bacille Calmette-Guérin scar negative and \( \geq 15 \) years old at the time of vaccination.
In some temperate environments [3, 5, 7, 9]. Returning to the siveness decreases more rapidly in tropical environments than findings in several other studies indicating that DTH respon-
to tuberculin increases rapidly to a peak at 2–3 months after
at 48 or 72 h [31].

The prevalence of human immunodeficiency virus (HIV) in Karonga District was 3% among adults 15–49 years old at the time of the surveys [30]. Thus, our findings refer effectively among persons with an initial BCG scar than among those without such a scar. A plausible explanation is an excess of false-negative responses among those who were BCG scar positive at vaccination, compared with those who were BCG scar negative.

This is a large data set, and the trends are clear and convincing. However, we could not study trends in individuals over time. Ideally, one would like to test subjects repeatedly to map individual trajectories. This would enable assessment of whether, for example, individual DTH responses fluctuate appreciably. If so, data such as these would reflect only the proportion of persons who happened to be in a certain state at successive times after vaccination. However, such an approach is not possible because of boosting attributable to repeated tuberculosis tests [29].

The prevalence of human immunodeficiency virus (HIV) in Karonga District was <3% among adults 15–49 years old at the time of the surveys [30]. Thus, our findings refer effectively to HIV-negative subjects. There were no differences in results when our data were stratified by whether the tests were read at 48 or 72 h [31].

Our central finding was that, in Malawi, BCG-induced DTH to tuberculin increases rapidly to a peak at 2–3 months after vaccination and then decreases markedly. This is consistent with findings in several other studies indicating that DTH responsivens decrease more rapidly in tropical environments than in some temperate environments [3, 5, 7, 9]. Returning to the second question posed in the introduction about the implications of the kinetics of BCG-induced DTH for immunity and T cell memory, it is tempting to relate the difference in kinetics between temperate and tropical environments to the fact that BCG is known to provide better protection against TB in temperate than in tropical environments [32]. In this context, we note that the Puerto Rican data, which, in contrast to several other studies in tropical or subtropical environments, indicated relative stability of postvaccination DTH, also showed that BCG imparted a moderate level of protection (∼30%) against TB in that population [20]. The relationship is not a simple one, however, because there is no evidence that tuberculosis sensitivity (i.e., as measured 1 year after vaccination) is a correlate of BCG-induced protection [1, 33].

In regard to the third question posed in the introduction, which asked why do the kinetics of BCG-induced DTH appear to differ between populations, the reasons for the geographic differences are not clear. A possible explanation is that they reflect the high prevalence of exposure to other infections in tropical (but not in nontropical) environments, including parasites, which bias the immune system away from Th1 and DTH responsiveness [34] or which may compete for attention or space within the T lymphocyte compartment [35, 36]. A better understanding of the kinetics of the immune responses underlying these observations will provide an important clue to the natural history of mycobacterial infections.

References


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9. Trial of BCG vaccines in South India for tuberculosis prevention. Tubercu-


12. Randomized controlled trial of single BCG, repeated BCG, or combined

Figure 5. Proportions of children ≤10 years old tested in a popu-
lation survey who had a DTH response to tuberculin of ≥5 mm or ≥10 mm, by bacille Calmette-Guérin (BCG) scar status (+, positive; −, negative) and age.


