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The relationship between delayed type hypersensitivity and protective immunity induced by mycobacterial vaccines in man

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Introduction

BCG is the most widely used vaccine in the world today (1). It is also the least reliable, at least insofar as controlled trials indicate that its protective efficary varies unpredictably from nil to 80 % against tuberculosis (2-10), and from 20 % to 80 % against leprosy (10-14) in different areas of the world (15). The determinants of BCG's protective efficacy are unknown, and constitute one of the more important practical problems in immunology and public health today.

It is expensive and time-consuming to assess the protective immunity imparted by mycobacterial vaccines in human populations. On the other hand, it is simple to evaluate the sensitization imparted by such vaccines, at least in terms of delayed type hypersensitivity (DTH) to mycobacterial skin test antigens. Because of this facility, measures of DTH have been used as surrogate measures of immunogenicity in vaccine development and in the monitoring of vaccine potency; and most mycobacterial vaccine trials have devoted considerable effort to pre- and post- vaccination skin testing. Their use in such contexts implies belief in a correlation – or at least an informative relationship – between delayed-type hypersensitivity and protective immunity. This paper reviews the epidemiological evidence bearing on this relationship and presents preliminary analyses of relevant data from the Lepra Evaluation Project in Malawi.

Two initial comments are in order. There is a large literature on this subject, based on animal studies, skin testing surveys and vaccine trials. This brief review cannot do justice to the literature as a whole, but concentrates mainly on the vaccine trials. This perspective is particularly relevant today, given that a new generation of anti-leprosy vaccine trials, at least two of them incorporating extensive skin testing, is now underway. Indeed, our own involvement in one of these trials has encouraged us to examine the subject afresh. In addition, we recognise that this presentation does not discuss certain major complexities of its subject, such as differences in BCG strains, in skin test reagents and in criteria for skin test «positivity». This is not to deny the importance of such issues. Their omission from this discussion is due in part to space constraints. In addition, it is hoped that we might thereby avoid distraction, and confront directly the crucial problem – is there an informative relationship between DTH and protective immunity as induced by mycobacterial vaccines?

Naturally acquired DTH and protective immunity

It is widely accepted that strong naturally acquired tuberculin sensitivity is an indicator of infection by – and of an immune response to – the tubercle bacillus, but that it is not a measure of protective immunity against clinical tuberculosis. Som of the best data relevant to this issue have come from BCG trials, which have involved the follow-up of large numbers of unvaccinated, control individuals of known prior tuberculin status. In all but one instance the incidence rates of tuberculosis were found to be higher among tuberculin «positive» than among tuberculin «negative» individuals (16). The exception obtained during the first 10 years (but not thereafter) of follow up in the British Medical Research Council (BMRC) trial begun in 1950. This finding has been interpreted as reflecting a bimodal incubation period of tuberculosis in Great Britain, the risk of disease among infected individuals being high during the first few months after primary infection and then falling to a very low level before rising again in old age (8). Whatever the explanation for this observation, the accumulated data from the various trials, and the simple fact that clinical tuberculosis is itself generally associated with strong tuberculin sensitivity, are evidence that natural specific DTH – at least as measured by the tuberculin reaction – is not a measure of protective immunity against tuberculosis.

Though strong natural tuberculin sensitivity does not appear to indicate protective immunity against tuberculosis, there is evidence that intermediate levels of tuberculin sensitivity are associated with reduced risk of tuberculosis in some (8) though not all (6) populations. This observation has been a major platform to the argument that infection with certain atypical or environmental mycobacteria – which induce low or moderate levels of tuberculin sensitivity – can induce some protection against tuberculosis (17). According to this hypothesis it is a heterologous sensitization which is associated with protective immunity against tuberculosis. Whether or not the moderate level of sensitivity to tuberculin is directly relevant to the protective mechanism is not known.

In what may be seen as a parallel to this link between heterologous sensitization and protection against tuberculosis, there has been interest in whether tuberculin sensitivity is associated with protective immunity against leprosy. This interest was encouraged by the recognition that BCG vaccines induce tuberculin sensitivity and they also induce variable degrees of protective immunity against leprosy. Three of the four randomized controlled trials of BCG against leprosy have explored the relationship between natural tuberculin sensitivity and leprosy incidence in control (unvaccinated) populations. No association was seen between leprosy incidence and prior tuberculin sensitivity in the Burma trial (13), and the appropriate tabulations have yet to be published from the Chingleput trial. On the other hand, the Uganda trial reported that individuals who were initially strongly tuberculin positive (Heaf grades 3 and 4) had a 58 % lower age standardised incidence rate of leprosy than did individuals who were initially tuberculin negative (12). Given the potential importance of this finding, it is of interest to read the authors' actual words: «This comparison, unlike that between the BCG-vaccinated and control groups, is not a randomized one, and so differences between the groups apart from their age and tuberculin sensitivity may have affected the incidence of leprosy. However, it seems most likely that the reduction in incidence was largely attributable to the strong tuberculin positivity of the one group of children. Strong natural positivity may therefor give a protection only slightly less than that of BCG vaccination› .

The initial part of this quotation deserves emphasis. It is recognized by the authors that their comparison is not a randomized one, and that age is the only potentially confounding variable which has been adjusted for in the analysis. The fact that the individuals differed in their tuberculin sensitivity is itself evidence that they came from epidemiologically different populations – e.g. perhaps urban versus rural? – which could in turn have been the «true» ex-

planation for the different incidence rates of leprosy. This points to a crucial paradox in all attempts to assess the implications of naturally acquired DTH, in that it is impossible to find comparable groups which differ «only» in their skin test sensitivity. Given their recognition of this problem, it is interesting that the authors were not more cautious in their conclusion.

Almost nothing is known of the implications of natural DTH to *M. leprae* antigens for leprosy incidence. Studies on leprosy incidence as a function of lepromin status (e.g. 18) may not be relevant, for at least three reasons. First is the problem of non comparability of lepromin negative and positive individuals, analogous to the problem discussed above. Second, the delayed nature of the Mitsuda response is unlike conventional measures of DTH. Finally, integral lepromin is itself a potent sensitizer, and thus a positive Mitsuda reaction may be interpreted as a successful vaccination rather than as an assessment of prior DTH (19). Soluble antigen skin test reagents (MLSA's) have recently been prepared from *M. leprae* but there is as yet no published information on the relationship between MLSA sensitivity and leprosy incidence.

BCG-induced DTH and protective immunity

The ability to induce DTH has been widely used as a criterion of potency in the development and quality control of mycobacterial vaccines. Thus Shepard used the ability to induce DTH as one measure of «immunity» in evaluating potential leprosy vaccine preparations in the mouse (20). Several IMMLEP-supported projects have used the stimulation of sensitivity to *M. leprae* soluble antigens in order to assay potential leprosy vaccines in humans (21). And the stimulation of tuberculin sensitivity has been widely used as a quality control measure for batch testing of BCG (e.g. 14, 22).

Despite this manifest interest in the DTH-stimulating ability of mycobacterial vaccines, and equivocal evidence in some animal studies (23), there is little epidemiological evidence that it is of relevance to protection in humans. The association has been examined at least twice, in human populations, by different approaches.

Comstock investigated the relationship between the BCG-attributable tuberculin «conversion» rates (proportion of prior tuberculin «negatives» who became tuberculin «positive» shortly after having received BCG) and the observed vaccine efficacies against tuberculosis, in all the trials for which appropriate data were available. No correlation was evident between protection and DTH by this method (24). In his words «the lack of correlation is obvious and underscores the futility of predicting potency from conversion rates».

Hart *et al* were able to study this relationship on an individual basis using the BMRC trial population (25). They grouped all vaccinees according to the degree of tuberculin sensitivity subsequent to vaccination, and found no difference in vaccine efficacy. They thus concluded that «with *highly effective* tuberculosis vaccines, the degree of protection conferred *on the individual* is independent of the degree of tuberculin skin sensitivity induced in that individual by the vaccination» (italics in original).

In this context it might also be noted that the tuberculin sensitivity induced by BCG wanes with time, at a rate which appears to differ between different populations (e.g. 6, 9). While there was some evidence of a fall in protection against tuberculosis over time in the BMRC trial (8), such a trend has not been observed everywhere. A parallel waning of tuberculin sensitivity and protective immunity has not been observed. The BMRC trial showed a slight increase (from 80 % to 87 %) in protection against tuberculosis over the first five years of the trial, followed by a gradual fall (8). An analogous initial rise in protection was observed over the first three years of the Burma trial against leprosy, followed by a plateau lasting 11 years (14). The Chingleput trial revealed a dramatic fall over three years in post vaccine tuberculin sensitivity but a rise after five years in protection against tuberculosis (9, 10).

We thus find little if any evidence in the literature that vaccine-induced tuberculin sensitivity and protective immunity against either tuberculosis or leprosy are correlated, let alone causally linked. Causality aside, the lack of correlation is surprising, in that one might expect both DTH and protective immunity to be a function of vaccine dose. Several studies have shown a clear positive correlation between BCG dose in terms of *viable* (but not killed) bacilli and post vaccinal tuberculin response (22, 26, 27). Evidence for a correlation between BCG dose and protection has been reported in guinea pigs (28), but there are only hints of such an association in human studies. The South India-Chingleput trial compared two doses (0.1 mg and 0.01 mg) of both Paris and Copenhagen BCG's, and found slightly but consistently greater protection with the higher dose against leprosy, but not against tuberculosis (10, 29). The most recent report from the Burma trial suggests that the two different batches of BCG used in that trial differed in viable count and that the higher dose material imparted greater protection against leprosy (14). (It must be admitted that the relatively small differences in dose, approximately 0.38×10^6 versus 0.45×10^6, and the fact that the two batches were given at different times and in slightly different places, makes this inference less than convincing on its own.) Furthermore, a breakdown of the British MRC trial results by vaccine batch revealed a trend (not statistically significant) between viable count and protection against tuberculosis imparted by the different batches (25). Taken together, these results suggest that BCG vaccines are generally given near the top of their dose response curve, and thus it is some other factor which determines variations in vaccine-induced DTH and/or protective immunity. Perhaps this is why we do not find a clear correlation between vaccine induced DTH and protection.

Natural DTH and BCG-induced protective immunity

Despite the equivocal evidence concerning the implications of naturally acquired tuberculin sensitivity for protective immunity against tuberculosis or leprosy, there is a widespread belief that BCG cannot provide protection to individuals who are already naturally tuberculin positive. Thus most of the controlled trials of BCG excluded tuberculin positives at the start, the only major exceptions being the Burma and South India/Chingleput trials (9, 10, 14). Only the Chicago trial did not include an initial tuberculin test, but as the participants were aged less than 3 months it can be presumed that virtually all were negative (3). Furthermore, in many countries such as the United Kingdom, primary and secondary BCG vaccination is given only to individuals who are tuberculin negative.

The South India Chingleput trial recognized that this assumption had never been confirmed, and thus did not exclude tuberculin positives, in order to test the hypothesis «that BCG might increase the specific immunity in people infected so many years ago that their naturally acquired immunity (from the original, virulent infection) might have › (9). Though detailed results have yet to appear, the report covering the first 7½ years follow-up of that trial provided no evidence of protection against tuberculosis by BCG in any group regardless of initial tuberculin status (9). Subsequent analyses have suggested that protection began to appear after 5 years in those who were initially tuberculin › and under 15 years of age (10). What happened in the other groups has not been reported.

It has been argued that measurable vaccine efficacy may be reduced, or «masked», in individuals who have already received partial protection from infection by › mycobacteria (23). There is evidence that incidence rates of tuberculosis are reduced in individuals with skin test reactivity to certain non-tuberculous mycobacteria (17), and thus this masking hypothesis does appear reasonable. Although the masking effect has been demonstrated in guinea pigs (23), we are aware of no data from human vaccine trials actually demonstrating the effect (e.g. demonstrating a lower efficacy among individuals with low or moderate in-

itial levels of tuberculin sensitivity compared to the efficacy in those with no initial sensitivity at all) (e.g. 6).

With reference to leprosy, neither the Chingleput nor the Burma trials excluded tuberculin positive individuals. Published reports from the Burma trial (14) and unpublished preliminary reports from Chingleput (29) indicate that in neither trial was there a relationship between prior tuberculin sensitivity and protection imparted by BCG. The Uganda trial excluded strong tuberculin positives, but found no difference in BCG's protection amongst those with no or «weak» tuberculin positivity (Heaf grades 0, 1, 2) (12).

Despite this lack of evidence for a relationship between prior tuberculin sensitivity and vaccine efficacy, at least two trials have provided evidence that the efficacy of BCG was highest among the youngest recipients, and that it fell with increasing age at vaccination. Such a trend is implicit in Tripathy's report of BCG's protection against tuberculosis in Chingleput (10), and is explicit in the latest reprot of the Burma trial against leprosy (14). Such a trend is not surprising, insofar as the proportion of individuals naturally infected with *M. tuberculosis* or *M. leprae* should increase with age and one might expect a vaccine to protect better if given before, rather than after, natural infection. But what *is* surprising is the manifest absence of any such trend in the Uganda trial (17), and the absence of data demonstrating that this trend, when observed, correlates specifically with measurable skin test sensitivity (e.g. within narrow age groups).

Analysis of data from the Lepra Evaluation Project

We have recently reported evidence from the Lepra Evaluation Project that routine vaccination with (Glaxo, freeze dried) BCG is providing at least 50 % protection against leprosy in Karonga District, Northern Malawi (30). This conclusion was based upon case-control analysis of prevalent cases first ascertained in a total population survey and cohort analysis of incident cases arising after the initial survey. It indicated that the vaccine's protection was independent of age, sex, socio-economic status or location within the project area. We present here an extension of the published analyses, using skin test sensitivity as an additional variable.

The background and methods of analysis are published elsewhere (30) Tuberculin (RT23, 21U) and/or *M. leprae* soluble antigen (various batches, here called MLSA) skin tests were carried out on most individuals when they were first encountered in the total population survey carried out 1980–1984. BCG scar status was also recorded at the initial examination. Incident cases represent biopsy confirmed disease which had onset after the initial examination. Data on incidence cases identified (mainly passively) by the end of 1985 are reported here. Table 1 shows the distribution of prior BCG and skin test status in incidence cases and in the population at risk. Table 2 presents the relative risks of leprosy between different groups, combining both sexes and using the Mantel Haenszel procedure to standardize for age (31). Thus the first line of Table 2 suggests that individuals who had a BCG scar and who were tuberculin «negative» when first examined had a risk of leprosy which was 31 % that of individuals with no BCG scar and a negative tuberculin test, and that this reduction is statistically significant. (The conventional vaccine efficacy measure is 1 – relative risk expressed as a percent, and would thus be 69 % in this example.)

Table 1. Distribution of incident leprosy cases (numerators) and of population at risk (denominators) by age and by BCG scar and tuberculin or MLSA status prior to onset of disease. These data refer to individuals less than 35 years of age. BCG+ and BCG – indicate presence and absence of BCG scar, respectively. Criterion of positivity for both skin tests taken as a 48 to 72 hour induration greater than 5 mm. Data from Lepra Evaluation Project, Karonga District, Northern Malawi, 1980–1985.

Prior status	Age at initial examination							Total
	0–4	5–9	10–14	15–19	20–24	25–29	39–34	
BCG- TUB-	0/3720	6/3976	4/2579	2/804	4/796	2/1189	0/1342	18/14406
BCG+ TUB-	2/6277	1/5115	1/2587	2/2133	0/1275	1/309	0/266	7/17962
BCG- TUB+	0/263	0/592	2/720	1/377	0/474	3/1100	2/1341	8/4867
BCG+ TUB+	0/1268	1/1436	4/1862	0/2369	0/1574	1/494	1/407	7/9410
BCG- MLSA-	0/1348	6/1335	6/797	2/251	2/293	1/460	0/566	17/5050
BCG+ MLSA-	1/2135	0/1794	1/1008	2/778	0/529	1/127	0/143	5/6514
BCG- MLSA+	1/132	0/271	0/297	1/173	2/168	1/305	1/354	4/1700
BCG+ MLSA+	0/439	1/505	3/628	0/910	0/512	0/115	0/111	4/3220

Table 2. Relative risk of developing clinical leprosy as a function of BCG and tuberculin or MLSA skin test status, in Karonga District, Northern Malawi 1980–1985. Relative risk were calculated on males and females combined, using Mantel Haenszel method to standardize for age (31).

Groups compared			Relative risk	95 % Confidence limits
BCG+/TUB-	vs	BCG-/TUB-	0.31*	0.11, 091
BCG+/TUB+	vs	BCG-/TUB-	0.57	0.17, 1.91
BCG+/TUB+	vs	BCG-/TUB+	0.77	0.26, 2.34
BCG+/TUB+	vs	BCG+/TUB-	1.47	0.36, 6.08
BCG- /TUB+	vs	BCG-/TUB-	1.01	0.32, 3.21
BCG+/MLSA-	vs	BCG-/MLSA-	0.21*	0.06, 0.77
BCG+/MLSA+	vs	BCG-/MLSA-	0.33*	0.11, 0.93
BCG+/MLSA+	vs	BCG-/MLSA+	0.50	0.06, 3.91
BCG+/MLSA+	vs	BCG+/MLSA-	1.25	0.21, 7.32
BCG- /MLSA+	vs	BCG-/MLSA-	0.54	0.15, 1.98

* Relative risk significantly less than unity (P 0.05).

Given the small numbers of cases, multiple comparisons and broad confidence intervals, we do not wish to overstate these results. But two findings seem of particular relevance to the theme of this presentation.

1. Statistically significant reduction of risk is observed for vaccinated but skin test negative individuals when compared to unvaccinated skin test negative individuals. It appears that

a BCG scar in tuberculin *negative* individuals was associated with 69 % protection against leprosy (BCG+/TUB- vs BCG-/TUB-), whereas a BCG scar in MLSA *negative* individuals was associated with 79 % protection (BCG+/MLSA- vs BCG-/MLSA-). Lower levels of protection were associated with a BCG scar in skin test positive individuals when compared with either skin test negative or skin test positive individuals lacking a BCG scar. There is thus no evidence that the protection imparted by BCG in this population is related to tuberculin or MLSA sensitivity.

2. The relative risk of leprosy in scar negative but tuberculin positive individuals, compared to scar negative but tuberculin negative individuals was almost exactly (actually slightly above) unity, suggesting that natural tuberculin sensitivity did not affect – let alone reduce – the risk of leprosy. This finding is in direct contrast to the Uganda result discussed above.

Conclusions

The prominent position of skin testing in the literature on BCG against tuberculosis and leprosy and in the design of mycobacterial vaccine trials implies a belief that skin test results are informative with regard to the protective action of such vaccines. This review of the literature and analysis of data from Malawi have failed to find evidence of the usefulness of skin tests in this context. In particular:

1. There is little evidence that either pre-vaccination or postvaccination skin tests, e.g. with tuberculin, are predictors of vaccine efficacy against either leprosy or tuberculosis.
2. The observation that natural strong tuberculin sensitivity was associated with «protection» against leprosy in Uganda has not been confirmed elsewhere, and may have been an artifact attributable to other characteristics of the tuberculin positive group in the Uganda trial (12).
3. There is no evidence that the waning of post BCG vaccination tuberculin sensitivity is associated with waning protective immunity. Thus there is no justification for repeating a BCG vaccination solely on the basis of waning tuberculin sensitivity.

Two additional points arise from this discussion which are of particular relevance to the planning and analysis of mycobacterial vaccine trials. First, the observation that dose may be more strongly related to protection than is post-vaccination skin test sensitivity should be considered when formulating vaccines for trials in man. Secondly, it should be recognised that there are several different ways of expressing vaccine efficacy, depending on the criteria defining the groups to be compared – e.g. in terms of skin test status. It is conventional to describe BCG's efficacy with respect to individuals who were skin test negative at the time when vaccines were distributed. If initial skin test status does not affect vaccine efficacy, then this restriction is unnecessary. If it does, then other measures of protection may be of interest insofar as BCG has been and continues to be given to skin test positive individuals in many countries. The data collected in the South India – Chingleput trial are of tremendous potential importance in this regard, given that tuberculin and Battey bacillus skin tests were widely applied but not used as exclusion criteria, and incidence data were obtained on both leprosy and tuberculosis. The detailed analysis of those data is eagerly awaited.

This paper is intended to raise questions about the emphasis and interpretation of current mycobacterial skin tests in vaccine studies. But the critical tone is not to imply that skin tests are not important and useful. Indeed, by providing a simple, and reasonably sensitive and specific indicator of infection, tuberculin testing has taught us an immense amount about the epidemiology of tuberculosis, and is the key to control by case finding and chemoprophylax-

is in several countries. Even if vaccine-attributable tuberculin conversion does not correlate with protection, its monitoring may be justified as a means of ensuring that viable BCG was administered at all. On the other hand, we find that, despite its incorporation in most vaccine trial protocols, skin testing has thus far proved of very little help in solving the persistent and extremely important puzzle of predicting BCG's efficacy.

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