CORRESPONDENCE

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A Critique on the Interpretation of the Lepromin Reaction Using Heat-killed *M. leprae* Vaccine

TO THE EDITOR:

Skin reactivity elicited by various mycobacterial antigens serves as a surrogate measure of protective immune responses in leprosy almost impossible to measure with any conventional immunological test available today. It is principally on the basis of such inputs that large-scale clinical trials of several leprosy vaccine candidates involving substantial expenditure and infrastructure are initiated. The paper therefore by Gill, *et al.* in the March 1988 issue of the JOURNAL entitled "Vaccination of Human Volunteers with Heat-killed *M. leprae*: Local Responses in Relation to the Interpretation of the Lepromin Reaction" is a belated though welcome one in its search for an interpretation of the lepromin reaction.

The purpose of this communication is not to argue with the basic premises of vaccination and skin testing in mycobacterioses, valid though such arguments are, but to indicate flaws in the very conception and design of the above-mentioned study and to provide alternative explanations for the conclusions that have been drawn.

In our opinion, a significant error in the study appears to be the consideration of the armadillo-derived *Mycobacterium leprae* (AML) vaccine as a representative of standard Mitsuda lepromin. To quote an example, humoral antibody responses generated by Mitsuda lepromin are significantly more rapid than those elicited by AML vaccines ([2, 3]). The dose ranges of the two preparations are obviously different, the lowest dose of the AML vaccine being 10-fold higher than the standard dose of the Mitsuda lepromin. Even so, in engendering a late reaction at that dose, its efficacy is apparent in only 50% of normals at a cut-off point of 5 mm. Satisfactory skin-test conversion responses to *M. leprae* soluble antigens (MLSA) in normal individuals are also achieved only at the two highest doses of 1.5×10^8 and 5×10^8, which are 100- to 500-fold higher than the Mitsuda preparations. Conversely, the Mitsuda reaction is negative in most lepromatous patients, while the AML vaccine exhibits at the site of injection an optimal reaction/ulceration in the majority of such cases. A plausible explanation for the above discussion is dose-induced sensitization which, in fact, implies that the only factor a late lepromin reaction may measure is an individual's threshold of sensitization.

Nevertheless, an interesting fall-out of the present study which the authors fail to emphasize is why normals from nonendemic areas, a majority of whom reportedly should be responders to Mitsuda lepromin, fail to respond satisfactorily to AML vaccine except at substantially higher doses. This also points a finger to a major lapse in the study design, i.e., the comparison of a pre- and postvaccination Mitsuda lepromin response with the late reaction elicited by the AML vaccine. Considering the impossibility of interpreting responses in individuals subjected to four doses of AML vaccine, this step would have provided information on

the ability of a single vaccine dose to transform Mitsuda nonresponders to responders.

It is probable that this omission is due to the sensitizing capacity attributed to integral lepromin. Two alternatives may be explored to overcome this: a) Dharmendra antigen that is not a potent sensitizer and yet may be capable of eliciting both an early and a late response, and b) liposome-bound soluble antigen of Sengupta, *et al.* ([7]) which may mimic integral bacilli to a large extent.

As is also reported here, all vaccine candidates appear to work more efficiently in the higher dose ranges. On this basis, the authors suggest the use of a higher dose of Mitsuda challenge for testing vaccine potency. It is indeed a paradox if this were to be so since, in immunological systems, successful sensitization results in a response evoked to low doses of the challenge antigen. If the aim of induced sensitization is protection via memory, then a susceptible individual must be endowed with the ability to respond to lower doses of antigen, a situation that is analogous to *in situ* occurrences where the exposure to bacilli at the onset may be low and insidious rather than overwhelming. Therefore, a postvaccination, negative lepromin skin test should be accepted as a failure of vaccination rather than attempt coercion of a positive response through dose manipulation that would have little relevance to the infectious processes in the leprosy patient.

Despite theoretical reservations, a good test for any vaccine given to normal individuals would be to measure both the early and late reaction to a "Mitsuda like agent" at periodic time intervals after vaccination. In the event that a vaccine candidate is efficacious, we would then expect the early lepromin reaction to wane with time, while leaving Mitsuda reactivity fairly intact. This hypothesis is upheld by the present data which show that prevaccination MLSA responses do not correlate with the early response of the AML vaccine except at the highest vaccine dose. Moreover, this observation has been rightly interpreted by the authors to signify that integral preparations, such as AML vaccines, may contain variable or minimal amounts of soluble antigen. In an unrelated context, they also quote the example where in BCG-vaccinated populations, MLSA activity was positive immediately after vaccination but was found to be negative 90 days later ([6]). In fact, this quotation upholds their own conclusion of the capricious amount of soluble antigen and is not, as they imply, suggestive of the "non-effect" of an antigenic crossreactivity between *M. leprae* and BCG. It also vindicates the original interpretation of the Fernandez reaction as a measure of a pre-existent cell-mediated immune response to *M. leprae* resulting from active or crossreactive infections ([5]).

To most observers, the choice of using MLSA except as a measure of convenience in the field has long been intriguing. The standardization of batches has been suspected before to be less than optimal ([8]), whereas no published data exists on the matter of MLSA sensitivity and leprosy incidence ([1]) except perhaps in the pages of the World Health Organization-Tropical Diseases Research reports. With the computing of data from the present study, its credibility decreases further.

One is also reminded of earlier studies which demonstrated that the sharing of antigens between integral BCG and batches of PPD is extremely limited ([4]). We are not so far aware of any studies that seek to elucidate antigenic relationships between *M. leprae,* its heat-killed/irradiated integral counterpart, and the soluble antigens prepared therefrom.

It is imperative to implement studies such as these in order to have a firm scientific basis before undertaking expensive large-scale vaccine trials.

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