

CORRESPONDENCE

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In Vitro Stimulation of Human Lymphocytes With Mycobacterial Antigens

TO THE EDITOR:

In mycobacterial infections, the cell-mediated immune mechanisms seem to be the most important for resistance to or recovery from infection⁽¹⁾. It would be desirable to know which antigens, either specific to *M. leprae* or cross-reactive with other species, might be crucial to the generation of such protective responses. Most of the studies directed at dissection of the important antigens of *M. leprae* have employed antibodies to detect and distinguish the antigens (for example, ^(3,6,7,9)). However, this is not necessarily the most direct approach, since these are not necessarily the same antigens which are important for the cell-mediated responses which are responsible for the control of mycobacterial growth and spread. These responses have been studied at a gross level by skin testing and by lymphocyte transformation tests (LTTs)^(4,7,10,12), but both of these tests have the disadvantages of depending on *in vivo* immunization of the individual and that they can be influenced greatly by physiological factors which may or may not be related to the antigens of interest ⁽⁵⁾. It could be important also to know the reasons for the poor cell-mediated immunity in potential or actual lepromatous leprosy patients. At present there is no good animal model to study these problems, so the most useful information may come from *in vitro* studies on human material.

Recently, Hensen and Elferink reported the successful "*in vitro* priming" of human peripheral lymphocytes, which apparently permits selection and expansion *in vitro* of clones reactive to any given antigen, even to synthetic antigens such as TGAL, to which the donor was not, presumably, previously sensitized ⁽⁸⁾. We think that this technique could be very valuable in the assessment of the reactions of human lymphocytes to mycobacterial antigens, and we have done some preliminary experiments to confirm that besides PPD (used by Hensen and Elferink ⁽⁸⁾), whole BCG and various fractions of it can be used to prime and to restimulate the cells *in vitro*.

The technique consists essentially of culturing Ficoll-Hypaque-separated blood lymphocytes with antigen at very low concentrations (compared to the standard LTT) for 10 days, then washing and reculturing these "primed" cells in the presence of the same or different antigens and measuring their responsiveness by thymidine incorporation 2 days later ⁽⁸⁾. At the same time that the priming culture is set up, a standard LTT test is also initiated to ascertain whether the donor had been sensitized *in vivo*. Typical data are shown in the table for an experiment involving BCG, fractions thereof, and tetanus toxoid as an unrelated antigen; the cross reacting antigens can be easily distinguished. It is important to note a) the low concentrations of antigens used

TABLE. ³H-Thymidine incorporation in lymphocytic cultures primed with BCG and recultured with various antigens.^a

Primed with:	Restimulated with:						
	—	BCG	PPD	Pellet	Cytosol	Cell wall	Tetanus
—	820	829	2321	525	2125	1719	847
10 ³ BCG	1545	5050	4434	216	3789	832	270
10 ⁴ BCG	1356	6908	7109	113	3559	1846	527
10 ⁶ BCG	1533	3186	9555	87	5747	3113	41
10 ⁷ BCG	915	4346	9375	127	2435	5425	1366

^a Incorporation of ³H-thymidine into cultures of lymphocytes primed and then recultured with BCG (10⁴/ml), PPD (100 µg/ml), tetanus toxoid (RIV, Bilthoven, Holland, diluted 1/5) or with fractions of BCG prepared by cell disruption and ultracentrifugation (Binkhuysen and Das, manuscript in preparation). The numbers given are means of cpm from duplicate or triplicate cultures after an incubation from 30 until 48 hours of culture; the variation between replicates was usually less than 5% and never more than 10%. The fractions were lyophilized and reconstituted in culture medium and used at 10 µg/ml except for pellet, which was in this case only 2 µg/ml.

in priming and b) that in the secondary response, few cells per well give a response in a short time.

This enrichment of specific antigen-reactive cells could be either a real "antigenic priming" or simply a selective expansion of memory cells induced by cross-reactive antigens. Since little is known about the events involved in priming it is probably not important to distinguish between the two in order to use the technique for the purposes we shall suggest. In a donor who had been previously exposed, there could be both priming and secondary clonal expansion; in either case there would be an enrichment of reactive cells of interest which would permit their further study as to immunological functions, cross-reactivity, ability to recognize different bacteria, susceptibility to humoral influences, etc. We feel that this assay offers an approach to the study of *M. leprae* antigens and the responses of different human lymphocytes to them which could be exploited in a number of ways, among which we suggest, for example, the following:

1) The reactive cells in a given priming culture could be expanded in number by culture in the presence of Interleukin II (T cell growth factor). This would permit their expansion into numbers which could then be diluted so as to have small pools or even individual clones of cells reactive to one or a few antigenic determinants. A panel of reactive cells could be accumulated which could be used like antisera to in-

vestigate the important antigens and their distribution among the different species and within the organism itself. This sort of approach has been used successfully with both mouse and human cells for other antigens (1,2,11).

2) Since the responses developed in this system are somewhat removed from the environment of the donor, it would be interesting to investigate the ability of cells from lepromatous patients to generate reactivity. In the standard LTT they are known to be generally anergic but this is no proof that they have no reactive cells nor that they are fundamentally unable to respond.

3) Since the donor's adherent cells could be treated separately with antigens of interest and later recombined with the unexposed lymphocytes, experiments could be done to determine the relative roles of these cell types in the responses that both healthy and lepromatous persons make to these antigens.

4) If clones of reactive cells could be developed, it would also be of great interest to investigate the immunological functions that they carry out. This might make it possible to identify the antigens responsible for the induction of different responses which could be very useful information for vaccination program development. Thus, we feel that this technique could be exploited to make important contributions to the understanding of the immunological problems in the response of the human host to infection with *M. leprae*. We hope that some of

this may indeed be carried out by some others in a position to do it.

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DDS 100 mg Daily, the Cornerstone in the Management of Reversal Reaction

TO THE EDITOR:

A retrospective study was performed to prove our hypothesis that the improvement in treatment response reported in the article "Reversal reaction, the prevention of permanent nerve damage. Comparison of short and long-term steroid treatment" (1) was not due solely to longer steroid treatment but mainly to the increase of the dapsone (DDS) dosage. Our hypothesis is based on

the fact that in the above mentioned article the patients with the better treatment response received not only longer steroids but also DDS 100 mg daily, whereas the patients with the poor treatment response received DDS 200 mg weekly or less besides the short-term steroids (2). Our retrospective study was designed to find borderline leprosy patients in reversal reaction with nerve damage who had been treated for