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EDITORIALS

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Lepromin and the Arthus Reaction

The lepromin reactions (early and late) have long been lamented as not being diagnostic of leprosy but mooted as of value in immunologic classification and therefore as having prognostic value.

Over the past two or three decades it has slowly come to be recognized that the lack of lepromin response in lepromatous patients, broadly often attributed to "anergy," reflects, in fact, the inability of patients predisposed to this disease manifestation to develop delayed-type hypersensitivity and concomitant cell-mediated immunity. These matters have been discussed voluminously over the past years.

Remarkably, there has been relatively little published regarding another peculiarity of the negative lepromin test in lepromatous leprosy. Abundant evidence has accumulated to the effect that the lepromatous, in contrast to the tuberculoid, patient produces and circulates large amounts of humoral antibodies in response to infection with *M. leprae*. Some of these antibodies are in response to class mycobacterial antigens and some are to *M. leprae* specific antigens. The significance of these humoral antibodies in the pathogenesis of *erythema nodosum leprosum* (ENL) has been increasingly recognized, to the point where ENL is considered by many investigators to be a manifestation of an antigen-antibody-complex reaction. Given these considerations, it would seem reasonable to expect that when a suspension of whole and disrupted *M. leprae* (lepromin) is introduced into the dermis, or other tissues, of lepromatous patients there would be an Arthus type reaction. There is not, and this is rather astonishing. It is almost equally astonishing that this peculiarity has received so little attention and that at least two Congresses (8th and 9th) have failed to record consideration of the possible significance of this remarkable fact while flatly listing a positive lepromin test as a requisite for the identification of possible *in vitro* cultures of *M. leprae*.

Four related considerations come to mind as requiring thought in this respect.

1. Mycobacterial class antigens and *M. leprae* specific antigens (probably lipo-polysaccharides) might be inactivated by the standard autoclaving of lepromin. Shepard recently reported[1] that antigens of *M. leprae*

[1]Shepard, C. C. Heat stability of *M. leprae's* immunogenicity. Int. J. Lepr. **44** (1976) 554.

are heat stable, but the experiments were not such as to define the nature of the heat stable antigens. In some of our own, as yet unreported experiences, autoclaving of alleged cultures of *M. leprae* does not abolish their stainability with FITC coupled, *M. leprae* specific, purified human lepromatous humoral antibody.[2] The same is essentially true for reactions with bacilli obtained from human and armadillo tissues, though in both cases fewer bacilli respond with immunofluorescence and with less intensity than do cultivated bacilli, even without autoclaving.

2. In general, *M. leprae* in human, mouse and armadillo tissues are slow growing and, for the most part, probably relatively "old" organisms. Lepromin prepared from them might, therefore, be analogous in this respect to old *in vitro* cultures. It is well known that a variety of microorganisms show marked variation in antigenic characteristics under varying conditions of cultivation[3] and that there may be a change of antigenic structure with change from vegetative to spore forms. BCG and *M. tuberculosis hominis* grown in Sauton's medium show antigenic variation associated with the age of their cultures.[4-8]

Again reverting to as yet unpublished findings of studies still in progress, utilizing the FITC-Ab determination previously reported as apparently specific for *M. leprae,*[2] a more striking response has been found with alleged cultures of *M. leprae* aged about seven days as compared with the same flask cultures aged eight weeks.

3. A third consideration may be of considerable importance in interpreting the problem under consideration. The humoral antibodies of lepromatous patients are, of course, not confined within the blood vascular system but get into the tissues, particularly in areas of inflammation, and are present in vessels within tissues, biopsied or otherwise removed for lepromin preparation. Prior to the removal of tissues, bacilli may well be exposed to such antibodies as well as complement, and almost certainly are so exposed during tissue maceration and grinding for lepromin preparation. It is not unlikely that the bacilli are coated with antibody, i.e., that their antigenic sites are considerably blocked by antibody coupling. If so, they would not have available antigenic sites to react with the lepromatous subject's antibodies and thus might not generate a reaction.

4. A fourth consideration must be borne in mind if "lepromin" from cultured bacilli is to be a comparative test agent. That is standardization of antigen concentration equivalence. When lepromin is derived from human or animal tissue, standardization is commonly done by counting bacilli and the technic has worked reasonably well for this type of material. With cultivated mycobacteria there may be tremendous variations in length of bacilli and this, in turn, may vary with the age and other conditions of the culture. Scanning electron microscopy reveals these variations more dramatically than either light or transmission electron microscopy. Theoretically, a bacillus five times as long as those commonly found in leprous tissues might contain at least five times as much protein, five times as much lipid, five times as much "etc." This rough armchair calculation does not, of course, allow for the probability of differing proportions of the various bacillary constituents at varying ages. The proportional lipid content of *homo sapiens* may vary with age and Osselinean[9] found tuberculosis bacilli to behave similarly. It

[2]Matsuo E. and Skinsnes, O. K. Immunologic identification of *M. leprae.* Immunofluorescence and complement fixation. Int. J. Lepr. **44** (1976) 301-314.

[3]Wilson, Sir G. S. and Miles, A. A. *Topley and Wilson's Principles of Bacteriology and Immunity,* 5th ed., Baltimore: Williams and Wilkins, 1964, pp 335-339.

[4]Turcotte, R. The variations in the antigenic composition of *Mycobacterium tuberculosis* during the growth cycle as measured by the passive hemagglutination and precipitation reactions. Can. J. Microbiol. **15** (1969) 681-688.

[5]Turcotte, R. and Quevillon, M. Asparaginase activity of BCG and its phenotypes in relation to culture phases. Ann. Microbiol. **126B** (1975) 181-185.

[6]Castelnvovo, G., Duncan, M. E. and Bellezza, G. Mycobacterial antigens: a study of antigens in bacterial extracts and in culture filtrates of different ages. Tubercle **45** (1964) 246-254.

[7]Magnusson, M., Kim, H. K. and Bentzon, M. W. Tuberculin production. 5. Relationship between age of the culture of *Mycobacterium tuberculosis* and tuberculin activity of culture filtrate. Acta Pathol. Microbiol. Scand. **60** (1964) 557 ff.

[8]Turcotte, R. and Des Ormeaux, Y. Influence of the age of mycobacterial cultures on the protein and carbohydrate composition of tuberculins. Can. J. Microbiol. **18** (1973) 637-645.

[9]Osselinean, J. Sur la variation de la teneur en lipidas du Bacilli Tuberculeux, en fonction de l'age de le culture. Ann. Inst. Pasteur **81** (1951) 306 ff.

would seem that there is a strong probability that not only protein but "etc." variations in concentration must be taken into consideration. As a beginning, in comparing "culture lepromins" with standard lepromins, both for evaluation of delayed-type hypersensitivity response as well as for possible Arthus type reaction, it would seem advisable to standardize by protein content determinations utilizing the protein concentration of standard lepromin as a guide. In so doing, the determinations should be made before preservative phenol is added to the lepromin preparations since phenol will give a strong false positive reaction in the Folin-Ciocalteu method and the biuret method of protein determination is not sensitive enough to be accurate at these levels of concentration.

At this point in the considerations, however, point 4 comes in conflict with point 3. If antibody protein is indeed adsorbed to the bacilli then determinations of the protein content of tissue derived bacilli will include the adsorbed antibody protein and not just the bacillary protein. This may also be true for the protein content of tissue-derived Dharmendra type lepromin. Some rough preliminary determinations and calculations suggest that the adsorbed antibody protein may amount to as much as twice the protein content of the bacilli. If this be anywheres near the mark, and even if it isn't, it is evident that lepromin standardization by protein content, theoretically desirable, cannot be accepted without careful evaluation of the possible factors involved. Thus, potential development of lepromin from *in vitro* culture, as weighed against the long experience with *in vivo* derived lepromin, is not just a matter of throwing some bacilli into a phenol-saline vehicle and labeling it "lepromin."

Among the possibilities here discussed, the second and third seem to be particularly viable in explanation of the absence of Arthus type reaction to lepromin in lepromatous patients. We are not aware of experimental work directed at this problem. Ridley[10] reported that the lepromin reaction in tuberculoid patients was eliminated if lepromin was treated with lepromatous serum. This was promptly refuted by Davey[11] and by Dharmendra and Mukerjee.[12] None of these studies significantly utilized lepromatous subjects, but if they had there probably would not have been any significant findings if the lepromin bacilli, as hypothesized, are already coated with antibody. Davey did also use Dharmendra type antigen, but this is "purified" for the delayed-hypersensitivity response protein antigen and most of the other antibody eliciting antigens are probably removed in preparation.

In considering the lepromin test as a specific means of identifying *M. leprae* in culture, it would seem that both these factors, and perhaps others also, require consideration since cultured bacilli thus present the probability of differences from tissue derived bacilli.

This presentation is not directed specifically at establishing the validity of any cultivation attempts for *M. leprae* but is directed toward clarification of the nature of the response to lepromin. Nevertheless, it is recalled that one major factor in the nonacceptance, for example, of the bacilli cultivated by Sister Marie-Suzanne and those of Kedrowski as being *M. leprae* was the failure of these cultures to "elicit" anergy in lepromatous patients.[13-15] Though these bacilli, as is generally held, perhaps were not cultivated *M. leprae,* they may still in major part have been rejected on the basis of an incomplete and narrow premise.

A final consideration comes to mind. Bacilli derived from human tissues as well as from armadillo tissues are pathogens which have survived for unknown times in macro-

[10]Ridley, D. S. The significance of antibody in the pathogenesis of leprosy. Trans. R. Soc. Trop. Med. Hyg. **48** (1954) 400-405. (Abstract: IJL **23** [1955] 353-354.)

[11]Davey, T. F. The effect of treating lepromin with lepromatous serum. Lepr. Rev. **26** (1955) 65-71. (Abstract: IJL **23** [1955] 354.)

[12]Dharmendra and Mukerjee, N. Lepromin not inactivated by lepromatous serum. Lepr. Rev. **26** (1955) 111-116. (Abstract: IJL **24** [1956] 364.)

[13]Dubois, A., Gavrilov, W. and Van Breuseghem, R. Intradermal injection of Kedrowski bacillus in lepers and nonlepers. Ann. Soc. Belg. Med. Trop. **16** (1936) 483. (Abstract: IJL **6** [1938] 136-137.)

[14]Chatterjee, K. R. and Bose, R. Immunological skin test in leprosy with a new antigen prepared from Kedrowski's bacillus; a preliminary note. Bull. Calcutta Sch. Trop. Med. **4** (1956) 127-132. (Abstract: IJL **24** [1956] 364.)

[15]Blanc, M. and Prost, M. Clinical and therapeutic study of an antigen prepared with *Mycobacterium marianum* applied to 457 leprosy patients. Int. J. Lepr. **23** (1955) 23-31.

phages. Even though the lepromatous human and lepromatoid armadillo macrophages seem to be strikingly deficient in their ability to destroy *M. leprae,* they do have lysosomes. In fortuitous electron micrographs almost selective erosion can be seen on the surfaces of bacilli contained in phagolysosomes. It is probable that many of the humoral antibody stimulating antigens, and therefore Arthus reaction eliciting, of *M. leprae* are surface antigens and the question arises as to what modifications in these may be caused by the bacillary sojourn in macrophages.

In vitro cultivated bacilli, derived from a totally different nutritional and enzymatic environment as compared to tissue isolated bacilli, as well as being devoid of the tissue products accompanying the latter, may well be expected to provide skin test responses apparently differing from expectations derived primarily from use of the classical Hayashi/Mitsuda type lepromin. There is the probability of obtaining an Arthus type reaction, a Fernandez reaction (early) and the Mitsuda reaction. Unless the problem is studied analytically, differentially and selectively it may be too readily said that the cultures are not *M. leprae* because their saline suspension may produce the gamut of reactions since even tuberculoid patients are not devoid of anti-*M. leprae* antibodies. Rather than quick judgements either way, the problem is complex enough to call for careful study and judicious judgement.

—OLAF K. SKINSNES

Note: *Fortuitously, as this issue of the IJL was "in press" the interesting letter from Dr. Claude Reich, page 379, came to hand. It would seem to have a distinct bearing on this editorial and to present a technical approach not only for throwing light on the problem here discussed but also on the related problem of* erythema nodosum leprosum.—*Editor*