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The Lepromatous Macrophage Defect as Related to Vaccine Development in Leprosy

It has become almost routine that with any allegation of *M. leprae* production in quantity, e.g., armadillo infection or *in vitro* culture, the word goes forth that now a leprosy vaccine is on the threshold. In part this represents newspaper attempts to interpret the need for and significance of the "breakthrough" which has finally made this possible. There is virtually no escape for the investigator trapped in this situation because any attempt at clarification of the real problem either vitiates the story or leaves the question, "So why do it?"

A vaccine in this sense is by definition a suspension of a modified fraction of or an attenuated living pathogen of any disease, incapable of producing a severe infection but affording protection, when inoculated, against the action of the unmodified pathogen.

Early in the modern era of leprosy investigation it was recognized that the then only known host of *M. leprae,* the human, responds in two major ways to the pathogen, either as "neural" or as "nodular" leprosy, and it was recognized in the 19th century that the latter was the malignant or bacilli-rich form in which the host was having difficulty in handling the pathogen. Slowly, and with much disputation, understanding developed to the point where Wade[1] in the early 1930's, morphologically delineated tuberculoid leprosy; and by 1938, largely on clinical studies, Rabello[2] advocated the concept of polarity, with tuberculoid and lepromatous leprosy representing opposite poles of host resistance to *M. leprae*. Congresses came to recognize this in the classification system and, by the 1950's, Lowe[3] elaborated the "polarity" concept into a detailing of the dichotomy in immunologic response which then began to correlate with developing concepts of cell-mediated immunity (CMI). From the late 1950's on it became increasingly evident that the major immune defense in leprosy is CMI and that its virtual absence is primarily responsible for the manifestations of lepromatous leprosy.

The question of a leprosy "vaccine" is, therefore, double-pronged. One aspect concerns the possibility of developing an agent which will stimulate CMI to protective levels in a nondeficient host and the second relates to the possibility of rectifying a host immune response deficiency. There is not assurance, or even reasonable indication, that both problems are amenable to the same approach.

In recent years, multiple technics have been developed and extensively used for the study and characterization of CMI and, to a considerable extent, these have been employed in the study of leprosy. As might be expected from the generally current concepts of CMI, much attention has been paid to the possibility that deficiency in the T cell/B cell lymphocyte system might be responsible for the lepromatous immunologic defect. A considerable number of significant publications have resulted; too numerous to be adequately presented in the present essay.[4] There have been demonstrated a number of generally altered or suppressed indicators of immunologic reactivity such as deficient skin sensitization to DNCB, depressed mitogenic effect of PHA on lepromatous lymphocytes, and prolonged survival time of transplants. These, and other findings, have led to consideration of the possibility that a classical tolerance on the part of both T- and B-cells is induced to a small number of *M. leprae* antigens important to CMI. Other data suggest an alternate possibility of selective T cell tolerance or a mechanism of immune deviation by which antibody deviates away from engaging T cells.

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[1]Wade, H. E. Tuberculoid changes in leprosy. I. The pathology of tuberculoid leprosy in South Africa. II. Lepra reaction in tuberculoid leprosy. III. The pathology of a nerve abscess. Int. J. Lepr. **2** (1934) 2-38, 279-292, 293-300.

[2]Rabello, F. E. A. Questões em dicussão sôbre a classificação das formas da lepra. Arq. Higiene **8** (1938) 59-76.

[3]Lowe, J. The leprosy bacillus and the host reaction to it. *In:* Experimental Tuberculosis with an Addendum on Leprosy. Ciba Foundation Symposium. London: J. A. Churchill, Ltd., 1955, pp 344-354.

[4]E.g., Myrvang, B. Immune responses to *Mycobacterium leprae*. J. Oslo City Hosp. **25** (1975) 3-24; Godal, T., Myklestad, B., Samuel, D. R. and Myrvang, B. Characterization of the cellular immune defect in lepromatous leprosy: a specific lack of circulating *Mycobacterium leprae*-reactive lymphocytes. Clin. Exp. Immunol. **9** (1971) 821-831.

Four problems immediately present themselves in these considerations:

1) The defect in lepromatous leprosy is highly specific.

2) The defect is evident in very early disease and does not develop later in response to massive accumulation of bacilli (antigen).

3) Almost all these CMI studies have been done on well-established cases of leprosy and there are few studies of early disease. In studies from one laboratory involving early instances of leprosy[5,6] no nonspecific deficiency of CMI or abnormality of auto-antibody production was found. Thus, many of the abnormalities of reaction may be secondary rather than primary.

4) Due to the chronicity of this disease, there are as yet no consecutive follow-ups of the course of these determinations either with progress of the disease or in regression of the infection under treatment. When several of the tabulations from various studies are tabulated against each other (Table), in an attempt to overcome this deficit, it seems evident that the characteristic indicator deviations turn toward tuberculoid, or even normal values when the lepromatous infection regresses under treatment. This would suggest that they may be secondary rather than primary.

Though it is generally true that the macrophage is a nonspecific immune mechanism,[7,8] it is capable of being stimulated to enhanced activity. It is generally held that specificity in this enhancement lies with stimulated T cells which release signal substances (lymphokines) to which the macrophages respond. It would appear that the macrophage antimicrobial effect in response to this stimulus is largely a lysosomal enzyme response and must be somewhat specific insofar as variant enzymes are necessary for the disposal of the variant constituents of different pathogens. Despite the presumed relative lack of specificity in the CMI response as compared to antibody specificity it does have specificity at least insofar as delayed-type hypersensitivity responses to lepromin, tuberculin, histoplasmin, coccidioidin, etc., are CMI related responses. It is also evident that the CMI deficiency in lepromatous leprosy does not predispose to kala-azar.[9]

In these respects, the portion of the leprosy immunologic spectrum represented by the varying and graduated CMI responses of the tuberculoid through the dimorphous manifestations are largely comparable to many of the features of the CMI response to classic infectious granulomatous disease such as tuberculosis.[10] In the area of CMI activity represented in these and related models, there are variations in effectiveness of response but no specific deficiencies. Thus, disseminated miliary tuberculosis falls within the range of the former category but does not represent a specific immune deficiency. This assumes, for the present, that the graded response in dimorphous leprosy results primarily from biological variation in the efficacy of CMI enhancement rather than the converse as represented by a possible deficiency gradation in some macrophage enzyme system. In the light of the overall pathologic picture, it is probable that the eventual answer will encompass a complex of interrelated factors.

Attempts to develop a vaccine against tuberculosis have been vigorously pursued ever since Robert Koch produced tuberculin in such an effort. Much has been learned about the tubercle bacillus and its composition in the almost innumerable fractionation efforts pursued in the attempt to develop a practical vaccine from some portion of this bacillus. The fact that BCG, a viable, attenuated bacillus is the only "vaccine" currently considerably employed speaks to the general experience that the whole viable bacillus is generally a better immunogen than any of its components when used in a host/pathogen relationship where there is no deficiency in

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[5] Rea, T. H., Quismoro, F. P., Nies, K. M., Harding, B., Di Saia, P. J., Levan, N. E. and Friou, G. J. Intradermal antigen, epicutaneous haptenes, T cell counts, B cell counts and auto-antibodies in one group of patients with lepromatous leprosy. Int. J. Lepr. **42** (1974) 369-371.

[6] Rea, T. H., Quismoro, F. P., Harding, B., Neis, K. M., Di Saia, P. J., Levan, N. E. and Friou, G. J. Immunologic response in patients with lepromatous leprosy. Arch. Dermatol. **112** (1976) 791-800.

[7] Mackaness, G. B. Resistance to intracellular infection. J. Infect. Dis. **123** (1971) 439-445.

[8] McGregor, D. D. and Koster, F. T. The mediator of cellular immunity. IV. Cooperation between lymphocytes and mononuclear phagocytes. Cell. Immunol. **2** (1971) 312-325.

[9] Convit, J., Pinardi, M. E. and Arias Rojas, F. Some considerations regarding the immunology of leprosy. Int. J. Lepr. **39** (1971) 556-564.

[10] Skinsnes, O. K. Comparative pathogenesis of mycobacterioses. Ann. N.Y. Acad. Sci. **154** (1968) 19-31.

Cell Mediated Immunologic Determinants in Leprosy

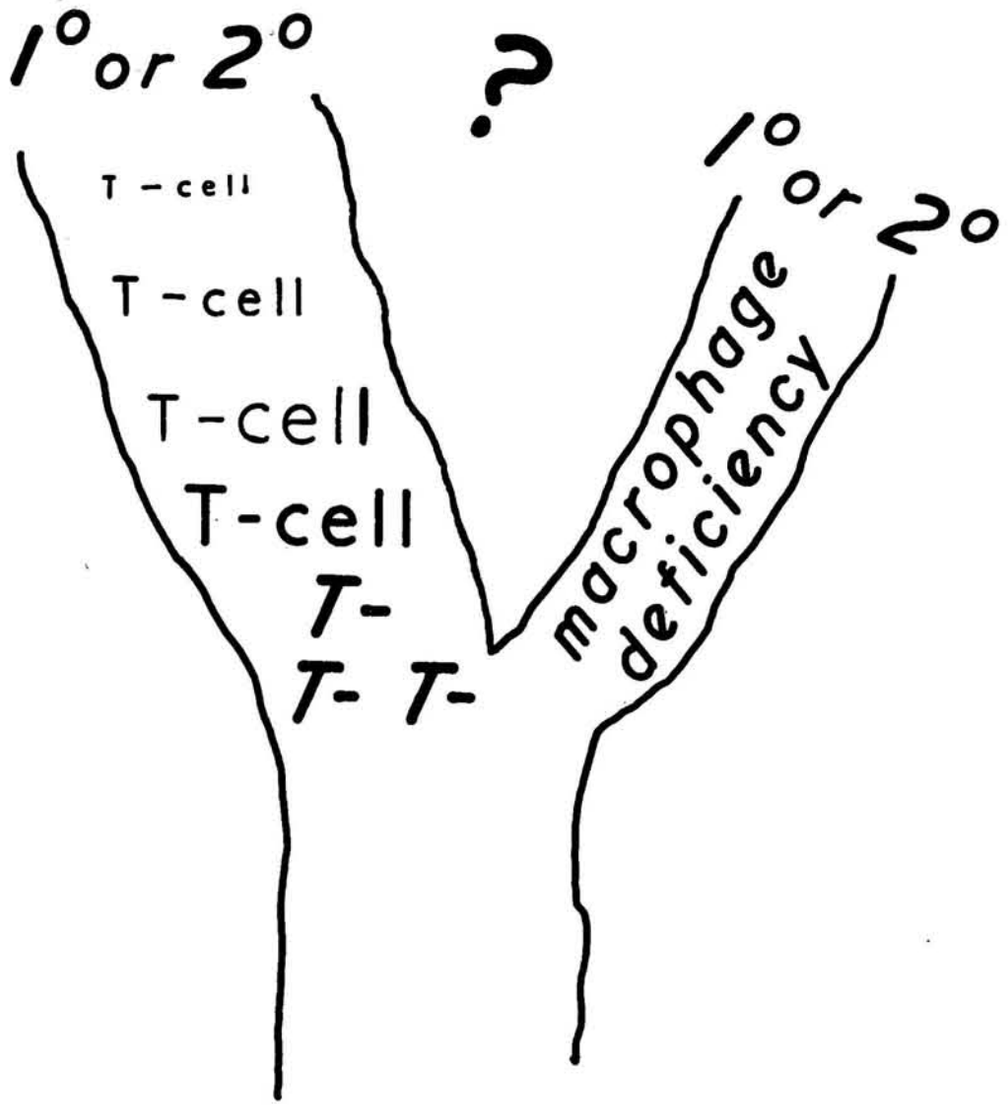
Determinant	Lepromatous		Tuberculoid		Normal
	active or short treatment	inactive or long treatment	active	inactive (treated)	
Skin hypersensitivity					
a) DCNB	24% +	80% +	80% + (dimorphous)		95% +
b) picryl chloride	32% +	53% +	52% +	58% +	93% +
c) oidiomycin	50% +	71% +	46% +	55% +	73% +
d) trichophytin	21% +	33% +	47% +	50% +	61% +
e) PPD–I	20% +	37% +	23% +	45% +	58% +
MIF (leprolin stim.)	slight inhibition		marked inhibition		
Lymphocyte transformation					
a) leprolin stim.	O	++	++		O
b) PPD stim.	O		++		O
c) PHA–M stim.	±	++	++		++++
Lymphotoxin production (PHA stimulation)	severely reduced		slightly reduced		+
Skin allograft survival	15.2 days		13.4 days		11.2 days
B–cells	35%				27%
T–cells	34%				58%

CMI response. It is recalled that Sabin and associates noted tubercle formation in animals which had received a total injection of 192 mg of the fatty acid derived from tuberculophosphatide, and Rich[11] commented that it would take 19 gm of tubercle bacilli to yield that amount of fatty acid. On the other hand, he further noted that a single tubercle bacillus can cause giant cell formation and several bacilli will cause the formation of a tubercle. The intact bacillus thus possesses far greater power of evoking characteristic inflammatory response than that which has been shown to be possessed by any or all of the tuberculolipids. Thus, both morphology and the immunologic response carry the same witness to the greater reaction-evocative effect of bacilli as compared to their fractions.

In lepromatous leprosy the concern lies with an immunodeficiency not usually seen in tuberculosis but similar in many respects to that seen in disseminated, often fatal forms of kala-azar, histoplasmosis, blastomycosis, coccidioidomycosis and other disease entities. In well-established lepromatous leprosy the host is loaded with live bacilli—far more bacilli and their dead and degenerating products than are ever likely to be introduced in any vaccine preparation. Nevertheless, they do not develop effective immunity. It is highly unlikely that the lepromatous manifestation is the result of this antigen overload. The lepromatous patient has a generally slowly developing infection. There are long periods of development between the time of initial infection with an adequate challenge dose and the appearance of widely diffused and heavy concentrations of bacilli. This period of time should be adequate for the development of CMI and delayed-type hypersensitivity before antigen overload occurs, if the host were capable of such immune developments. Something is wrong from the beginning. It is specific and from it flow the subsequent unique lepromatous manifestations.

It has not been determined whether the primary immunologic defect in lepromatous leprosy lies with the lymphocyte or with the macrophage. Currently, possibly in part as a reflection of major interest in the T cell-B

[11] Rich, A. R. *The Pathogenesis of Tuberculosis,* 2nd edit., Oxford: Blackwell Scientific Publications, 1951, pp 17-18.



Die Sektion ist das Fundament der Pathologie
Ludwig Aschoff

IMMUNO-ENZYMOLOGY

cell mechanisms in immunology, majority opinion among leprosy workers favors the concept of a defect in the T cell population as being the most likely locale of the problem.[4] This possibility receives the most attention and most study in the spectrum of current investigations (Fig. 1) and is widely discussed. An editorial on this aspect of leprosy immunity has been promised to this JOURNAL. This essay will, therefore, not review that area of thought but will consider the macrophage contribution.

The possible role of the macrophages deserves more attention. Pathomorphology, though no longer the chief means of study and interpretation, nevertheless is evidence. Thus, Ludwig Aschoff, on a visit to Japan, wrote with a brush in calligraphic style, *"Die Section ist das Fundament der Pathologie"* (Fig. 2, courtesy of Department of Pathology, Kyorin University School of Medicine). When coupled with available and developing histochemical technics, both for light and electron microscopy, broad areas of investigational possibility are opened. These may give direction to various technics of biochemical and immunochemical analysis.

The most basic and truly striking histopathologic difference in host response between tuberculoid and lepromatous lesions is the presence of a structured epithelioid cell granuloma in tuberculoid leprosy and its absence in lepromatous leprosy while in the latter the lesion consists of nodular agglomerations of macrophages loaded with acid-fast bacilli in their earlier stages and later with persistent lipoidal debris.[12] Both non-infected individuals and tuberculoid patients yielding positive Mitsuda reactions show such epithelioid granuloma formation within a month of Mitsuda lepromin inoculation. Morphologic evidence strongly suggests that there is variant macrophage ability to handle living *M. leprae* from the very beginning of inoculation with a challenge dose. From the point of view of morphologic analysis, there is variant ability to handle bacilli and, therefore, probable significant variation in initial and ongoing processing of antigen by the macrophages in the two immunologically polar types of leprosy. This is reflected in the effective development of CMI in tuberculoid leprosy and a magnificent production of ineffective humoral antibodies in lepromatous leprosy. These are antibodies to polysaccharide antigens, common also to other mycobacteria, such as the β and δ antigens, as well as to more specific antigens which are probably glycolipoproteins; in any case, almost certainly not pure proteins. If the antigenic stimulus to CMI is a relatively pure protein, it is possible that a metabolic defect in the lepromatous macrophage related to the handling of complexed lipocarbohydrate, probably polysaccharide, components of the bacilli might result in a failure to produce the necessary CMI stimulant but permit antigenic response to the partially degraded

[4] *Op. cit.*

[12] Skinsnes, O. K. Leprosy and the concept of *granuloma.* Int. J. Lepr. **38** (1970) 203-206.

products. The recent report[13, 14] of β- glucuronidase deficiency in LL macrophages as compared to TT epithelioid cells may relate to this possibility.

A further hint of this possibility is found in a demonstration by Dailey and Hunter.[15] They found that dinitrophenyl conjugated bovine serum albumin when doubly conjugated with medium chain fatty acids stimulated the production of delayed-type hypersensitivity for the haptene dinitrophenol with minimal antibody response to dinitrophenol and no detectable response to the carrier albumin (BSA). On the other hand, animals immunized with dinitrophenyl conjugated BSA produced antibody to the dinitrophenol and delayed-type hypersensitivity to the BSA. They suggested that this production of delayed-type hypersensitivity to haptenes and proteins is largely a function of the lipophilic nature of the entire immunogen and is not dependent upon properties of individual antigenic determinants. This indicates that the same antigen complexed with lipid calls forth a response in the CMI scale while without the complexed lipid the chief response is in the humoral antibody range. There may be some similar relationship in the polysaccharide lipoprotein conjugates derived from *M. leprae* in their breakdown by macrophages.

If there be merit in this concept, then there is the possibility of accomplishing, with an adequate source of bacilli, that which the lepromatous macrophage is incapable of accomplishing. A "vaccine" could then be developed but it would be a unique concept in vaccination for it would aim at replacing a deficiency in immunogenic stimulation and bringing the subject's resistance to a par with that available to those who develop or are capable of developing the tuberculoid variety of the disease.

A major problem with this concept, however, is still the fact of the deficient macrophage. Since CMI in leprosy appears to be primarily an enhancement of macrophage functional capacity, there is a large question as to whether the lepromatous macrophage has the capacity for effective response to adequately stimulated T cells. In view of its initial defect, probably not.

If the primary macrophage defect is indeed a genetically determined enzyme deficiency, such as the reported deficiency in β-glucuronidase[14] analogous to the deficiencies found in the lipidoses, then the necessary approach may be that of repairing the enzyme deficiency on the assumption that the immunologic responses will then revert to normal. In this regard the recent report by Dean *et al*[16] on enzyme restoration in patients afflicted with mucopolysaccharidosis is of considerable interest. They treated children having this defect with injections of histocompatible fibroblasts from tissue cultures. In one instance these cells continued to produce, at least for nearly a year, adequate quantities of the required enzymes to cause significant amelioration of the disease manifestations. T lymphocytes have been reported to contain β-glucuronidase[17] and it is possible that the reported[18] improvement in lepromatous patients might be related.

If there be no primary deficiency in the macrophage, but if the basic deficiency lies with the T cell in lepromatous leprosy, then the possibilities of a useful vaccine appear more remote and may be an untenable dream or a problem in genetic engineering or possibly a problem related to transfer factor, which is currently being studied. The possibilities relative to these concepts will be discussed in forthcoming presentations in these columns.

This leaves for consideration the question of classical type of vaccine—a vaccine such

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[13] Skinsnes, O. K. and Matsuo, E. Acid mucopolysaccharide metabolism in leprosy. 1. Storage of hyaluronic acid and its possible significance in the pathogenesis of leprosy. Int. J. Lepr. **42** (1974) 392-398.

[14] Matsuo, E. and Skinsnes, O. K. Acid mucopolysaccharide metabolism in leprosy. 2. Subcellular localization of hyaluronic acid and β-glucuronidase in leprous infiltrates suggestive of a host-*Mycobacterium leprae* metabolic relationship. Int. J. Lepr. **42** (1974) 399-411.

[15] Dailey, M. O. and Hunter, R. L. The role of lipid in the induction of haptene-specific delayed hypersensitivity and contact sensitivity. J. Immunol. **112** (1974) 1526-1534.

[14] *Op. cit.*

[16] Dean, M. F., Muir, H., Benson, P. F., Button, L. R., Boylston, A. and Mowbray, J. Enzyme replacement therapy by fibroblast transplantation in a case of Hunter syndrome. Nature **261** (1976) 323-325.

[17] Watanabe, K. and Masubuchi, S. Histochemistry in inflammation. *In:* Histochemistry in Diseases, T. Takeuchi, K. Ogawa and K. Uno, eds., Tokyo: Asakura Shoten, 1972, pp 99-122 (in Japanese).

[18] Lim, S. D., Fusaro, R. and Good, R. Leprosy VI. The treatment of leprosy patients with intravenous infusions of leukocytes from normal persons. Clin. Immunol. Immunopathol. **1** (1972) 122-139.

as that long sought for tuberculosis. This would be a preparation capable of stimulating CMI in those persons who have the capacity for such response. The tuberculosis experience would suggest that this type of vaccine would be most likely with a live, attenuated nonvirulent strain of *M. leprae*. The Uganda study involving BCG as a vaccine would have a limited applicable effect. It is unlikely that such a vaccine would be any more effective than BCG is for tuberculosis, and perhaps not much more effective than the BCG results for leprosy in the Uganda trial. There the somewhat favorable results seem largely to have been the consequence of the use of BCG in a population genetically more prone to the tuberculoid type of leprosy. The Burma trial on a population more prone to lepromatous leprosy supports the concept that vaccination has no significant effect on the depressed macrophage.

If the primary defect lies in T cell clones in lepromatous leprosy and if the lepromatous macrophages do, indeed, process the ingested bacilli into adequately stimulating antigens, then it is difficult to see how either an attenuated live vaccine or a vaccine derived from fractionation of *M. leprae* can be effective. If the defect is of the nature of a complex deficiency in lymphocytes and macrophages and their interaction, then the probability of an effective classical vaccine also seems remote. No good hypothesis for such an approach has come to attention.

For the immediate future, it would appear that the availability of *M. leprae,* either from armadillo or *in vitro* culture, is more likely to be productive of studies into an understanding of these mechanisms than in crash programs to develop the vaccine so imminently expected by press and public now that availability of bacilli has been announced.

In fact, a far more urgent need than the classical concept of a vaccine is the development of an understanding of the lepromatous defect which may lead to a determination of how to enable the defense mechanisms of the lepromatous patient to assist the chemotherapeutic agents now available.[19]

No doubt, as has been found, some will regard these considerations as "unsophisticated" relative to some hypotheses in immunology. Perhaps so. Nevertheless, true sophistication in science consists of an examination of all aspects of the problem and sometimes the most mundane of technics may be the most sophisticated if it unlocks the problem.

A. Szentgyorgy is credited with the statement,[20] "Research is to see what everybody has seen and to think what nobody has thought." And, Confucius wrote, "To study without thinking is useless; to think without studying is dangerous."

—OLAF K. SKINSNES

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[19] Skinsnes, O. K. Is leprosy treatment ineffective? Int. J. Lepr. **39** (1971) 890-891.

[20] Kato, Laszlo. Personal communication.