Lep. Rev. (1967) **38**, 2, 97-101

Advances in Immunology and Biochemistry in Leprosy in the Next Decade

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In an era in which man is concerned with sub-atomic particles and colonisation of the moon and the planet Mars, to speculate on advances that might occur in the realm of scientific knowledge in the next 100 years is not a gainful employment of time, especially since I do not possess the prophetic perception of H. G. Wells. I shall, therefore, confine myself to what seems probable in the next 10 years in the fields of immunology, metabolism and biochemistry in leprosy, in the light of our present experience and knowledge.

IMMUNOLOGY

Immunology of leprosy is an area of scientific work where there is more confusion than clarity of thought. On the one hand we speak of 'high resistance' to infection in the tuberculoid group of leprosy where there is a paucity of anti-bodies against *M. leprae* in the serum and no significant rise in gamma globulin. On the other hand, in lepromatous leprosy one finds a large rise in circulating antibodies against *M. leprae* in the serum and a marked rise in gamma globulin and yet this group of leprosy represents the 'no resistance' end of the spectrum in the host-parasite relationship. This is an enigma which may find a solution in the elucidation of the nature of symbiosis that exists between the intracellular parasite, *M. leprae* and the cells in which they proliferate. The mycobacterial capsular characteristics may be such as to protect it from the effect of the antibodies as well as from the hydrolytic enzymes—'phagolysosomes'. A study of the relationship between lysosomes in the cells parasitised by *Mycobacterium leprae* and the mycobacterium themselves may yield fruitful results. It has been suggested that DDS acts through the lysosomes which release the hydrolytic enzymes in the cells and that the enzymes kill the bacillus and help in the removal of the dead bacillus[1]. The study of lysosomes may not only give some clue regarding the pathogenesis of the reactive phases in leprosy, but also help in devising effective and rapid means of cure of leprosy.

The exacerbated phases in leprosy designated by the familiar term 'reaction' are a mystery. We know very little at the moment about the etiological background and pathogenesis of this distressing complication of leprosy which not uncommonly renders the patient incapable of being treated with the potent antileprosy drugs that are currently available. It is fairly well established that these reactive phases of leprosy are not directly related to mycobacterial activity. Current opinion favours an immunological phenomenon as the basic mechanism of production of these reactive phases.[2 3 4] Whether the reactive phase is a hypersensitivity phenomenon or whether it is fundamentally an autoimmune phenomenon is not yet clear.

No specific antigen-antibody reaction has been demonstrated in reactive phase. The presence of some of the 'markers of auto-immunity' in the sera of leprosy patients such as hypergammaglobulinaemia, anti-thyroid antibodies, anti-nuclear factor, rheumatoid-arthritis factor, lupus erythematous cell, have been well documented.[5 6] What is still open to debate is whether these play a causative role in the pathogenesis of reactive phases or whether they are the result of the tissue damage that occurs during reactive phase.

Paper read at the Scientific Session on the occasion of the Centenary Celebration of the Scudder Memorial Hospital, Ranipet, South India, on 10th December, 1966.

The recent work on lysosomes raises several hitherto unanticipated questions in this regard. Lysosomes are ultra-structural intracellular particles bounded by a membrane and contain hydrolytic and other enzymes, primarily concerned with phagocytosis and destruction of foreign particles.[7] It is not beyond reason to suggest that the hydrolytic and degradative enzymes released from lysosomes, may denature the native constituents of cells or connective tissue. Such denatured protein products would be expected to behave like antigens inducing the formation of circulating antibodies against themselves and the parent cell as part of the normal immune response. Thus the so-called 'auto-antibodies'—at least those encountered in the circulation—may represent a normal reaction to tissue damage and inflammation.[8] The 'auto-antibodies' in these diseases have not been shown to induce tissue damage. On the other hand, the release of lysosomal products into the cell-substance or surrounding tissues has been shown to initiate inflammation and tissue destruction.

In clinical practice, the disruption of lysosomes in living cells by ultra-violet light in patients with systemic lupus causes exacerbation of the disease. On the other hand, cortisone and its derivatives as well as Chloroquin have the property of stabilising the lysosomal membrane and retarding the release of enzymes from lysosomes. This can be demonstrated by pre-treatment with these drugs and exposure to ultra-violet light. Thus these therapeutically potent anti-inflammatory substances may, at least in part, exert their anti-inflammatory action by preventing or retarding release of lysosomes.[8]

The fundamental search, if the above thesis is correct, must shift from search for auto-immune antibodies and auto-immune mechanisms to a study of the characteristics and behaviour of lysosomes. In the next decade, then, I would venture to suggest that lysosomes will merit more attention in the study of immunity in leprosy and in other currently named auto-immune diseases, and may provide the key to unravel the mystery of what we now designate 'auto-immune phenomenon'.

IMMUNOLOGICAL METHODS FOR EARLY DETECTION AND DIAGNOSIS OF LEPROSY

One of the major problems in epidemiology and control of leprosy is of early diagnosis of leprosy, even before clinically detectable skin or nerve lesions are noticed, i.e., in the asymptomatic phase. At the moment there is no means of diagnosing latent or asymptomatic leprosy nor is there a satisfactory means of definitive diagnosis of leprosy when only a hypopigmented macule without sensory loss is found on routine examination. One can arrive at a definite diagnosis in patients with single 'patch' without sensory loss by means of careful and expert histopathological examination of skin biopsy. This can be done in a very few centres in the world.

Lepromin is not a diagnostic test in that it does not signify previous sensitisation with *Mycobacterium leprae*. Lepromin reaction only denotes the cellular response that may be elicited when the individual is challenged by *Mycobacterium leprae*. Thus, lepromin test, as we know it, is a useful prognostic tool but is of no use whatsoever in making a definite diagnosis of leprosy. Much work is in progress on the production of an antigen which is sufficiently specific for *M. leprae* to be capable of being used as a diagnostic tool in the study of asymptomatic, latent leprosy as well as early leprosy. One fondly hopes that such a specific antigen may emerge in the next 10 years and be of immense help to the epidemiologist as well as the field worker who is trying to detect and treat very early cases of leprosy.

Serological test for diagnosis of leprosy may be another important development. As I have already pointed out, in non-lepromatous leprosy there is no significant rise in serum antibody against *M. leprae*. On the other hand, in lepromatous leprosy which can be easily diagnosed by clinical examination and skin smears, there is plenty of antibody against *M. leprae* which can be demonstrated by fluorescence microscopy as well as immuno-

fluorescent techniques. A great deal of work is in progress to find a ready method of serological diagnosis of leprosy like in typhoid, brucella, etc. The standardisation of such a test is a likely advance during the next decade.

PROPHYLAXIS BY MASS VACCINATION

During the last decade striking progress has been made in the attempts to grow *M. leprae* in laboratory animals, in tissue culture and in artificial media. It is now authenticated beyond doubt that one can obtain limited multiplication of *M. leprae* in the footpads of mice by the technique described by Shepard and Rees indepdently.[9,10] Binford also has shown similar findings in the ears of golden hamsters.[11] Chang and Garbutt have demonstrated the possibility of multiplication of *M. leprae* in cell cultures.[12,13] B. R. Chatterjee has tentatively shown that *M. leprae* may be cultured in artificial medium.[14]

It appears very likely that during the next decade one may expect successful transmission of leprosy to laboratory animals and successful culture of *M. leprae* in the laboratory in artificial culture medium and in tissue cultures. If such an advance should result in '*in vitro*' growth of *M. leprae*, then it is not too much to expect the production of a vaccine which would protect against leprosy.

The limited success with BCG both in the footpad work[15] and in the field in protecting against leprosy[16] establishes the hope of the feasibility of the production of a vaccine which could be successfully used in prophylaxis against leprosy. This may well become the most significant advance in the epidemiology and control of leprosy in the next decade.

METABOLIC AND BIOCHEMICAL STUDIES IN LEPROSY

These have so far received step-motherly treatment in leprosy work. But for the early work of Sister Ross,[17] there is precious little done in biochemical and metabolic study in leprosy.

During the last 2 years I have been particularly intrigued by the metabolic and biochemical inter-relationship in leprosy. Shuster in Newcastle has recently demonstrated a whole range of metabolic and other systemic disturbances which are apparently caused by skin diseases themselves.[18] These major syndromes he has described in patients with skin diseases are the association of small intestinal malabsorption of fat, d-xylose, iron, folic acid and calcium; protein losing enteropathy as measured by faecal loss of radio-iodinated (I^{131}) polyvinyl pyrrolidine; villous atrophy in the jejunum in parallel with the chemical evidence of malabsorption; hyperoestrogenism and gynaecomastia.

In my study of the small bowel functions in patients with leprosy, nearly 30% of them are found to have malabsorption syndrome compared to an incidence of 10% in the control group. Nearly half the patients are folic deficient. To my knowledge, no such study has been undertaken among leprosy patients so far and our findings need to be carefully analysed and confirmed. I would suggest that there is much food for thought here.

The role of folic acid in the metabolism of *Mycobacterium leprae* is another aspect that is likely to lead to better understanding of the manifestations of the disease and perhaps to newer methods of treatment of the patient who is refractory to conventional treatment either because of resistance to the drug or because of recurrent attacks of erythema nodosum leprosum. A preliminary trial of one of the less known folic acid antagonists which I have conducted during the last few months has given encouraging results in my patients. This needs to be carefully authenticated before we can come to firm conclusions.

Another aspect of folic acid metabolism has attracted my attention. So far we have taken for granted that all neurological deficit in leprosy is a result of damage to peripheral nerves caused by the activity of *M. leprae*. I am aware that any view expressed to the contrary may raise a hornet's nest around my ears! Yet I cannot help putting it before you—I have often wondered whether the peripheral neuritis of leprosy may not in part be due to, or precipitated by, conditioned deficiency of folic

acid and/or B12. I suggest that much is to be learned by a careful study of these metabolic changes in leprosy patients. We should not glibly accept what appears to be obvious as the whole TRUTH.

Finally, I would like to bring to your attention *Mucopolysaccharide Metabolism in leprosy.* This is a fascinating facet that embraces a wide variety of changes. I shall confine myself to 2 features only:—

First—*C-reactive Protein* in the sera of leprosy patients is seen in some of them during reactive phases of the disease, quite independent of any rise in anti-streptolysin titre and the presence or absence of intercurrent infection.

C-reactive protein is a mucopolysaccharide one usually associates with tissue breakdown. What seems rather fascinating is the tentative conclusion that first, those patients in whom C-reactive protein can be demonstrated during the reactive phase tend to be the patients who are liable to get recurrent episodes of reaction; secondly, those patients who have C-reactive protein in the serum tend to take longer to clear the leprosy bacillus and hence take longer to become negative. These observations are tentative and need to be further studied. If true, this test may be prognostically significant and therapeutically helpful in that such patients may be considered unsuitable for treatment with DDS, at least intially.

The last observation I wish to make is in relation to 17-*Hydroxyproline*, which is a non-essential aminoacid that has been found to occur almost exclusively in collagen where it accounts for 13 to 14% of total aminoacids. A small amount is also found in elastin. With the development of specific methods for analysis, it was found that urinary hydroxyproline is excreted in a free form (3%) and in a polypeptide form (97%). The urinary excretion has been reported to be increased in a variety of clinical conditions ranging from cancer to collagen disease.[19]

We do not yet know of tests that would help the clinician to detect a patient who is likely to develop 'reaction' and pass into the subacute and chronic phases of this complication. During reaction, there is an accelerated breakdown of collagen as judged by the histological appearance of erythema nodosum leprosum. I wondered whether a quantative measurement of a product of collagen breakdown might tell us something about the metabolic make-up of a given patient. We began estimating 17-hydroxyproline, urocanic acid and other fractions of mucopolysaccharides in the urine of healthy controls and of leprosy patients. The preliminary observations suggest that the 'reactors' among our patients have a significantly higher urinary excretion of these metabolites even when they are free of clinically recognisable exacerbation and show further peaks in urinary excretion during phases of clinically obvious 'reaction'.

Metabolic survey of a patient may thus enable us to pick out the potentially difficult patient even before starting on treatment and by a careful choice of appropriate drug help to avoid or reduce the morbidity and mortality of reactions.

I am only bringing these data to your attention in the hope that you will see the wide spectrum of research possibilities and fascination in leprosy work. I am certain you will agree with me when I say that '*what the mind does not know, the eye cannot see*'.

From what has gone before I hope I have convinced you that 'Leprosy is Medicine' and leprosy work calls for the best that the medical profession can give to solve its mysteries which have baffled, frightened and fascinated man from time immemorial. All the newer knowledge and every new research tool in medicine must be harnessed if we are to solve the problem of leprosy. Leprosy is very much with us—should we not try and save the unborn child from a heritage of loneliness, shame, disfigurement and desolation ? Such an objective can only be attained when the modern Medicine Man finds it worth his time and talents to study leprosy and treat leprosy patients.

ACKNOWLEDGEMENT

I am grateful to Mrs. L. Furness for secretarial assistance.

SUMMARY

Advances in the realms of immunology, metabolism and biochemistry in leprosy that are likely to occur during the next decade are discussed in the light of our present experience and knowledge.

It is suggested that a more intensive study of the role of lysosomes in the pathogenesis of 'reaction' (exacerbated phases of leprosy) in leprosy may not only elucidate the mechanism of production of these complications but also help in devising effective and rapid means of treatment.

In the next decade one may look forward to the production of a specific antigen for the diagnosis of leprosy by skin tests. A specific serological test may also emerge.

Advances in prophylaxis against leprosy—chemotherapeutic and immunological (BCG)—can be expected.

A few advances in metabolic and biochemical studies in leprosy and their significance in the diagnosis and treatment of leprosy are discussed.

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