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Research in leprosy

A REPORT OF A COMMITTEE SET UP BY THE MEDICAL RESEARCH COUNCIL TO STUDY FUTURE PROSPECTS*

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SUMMARY

Recommendations for future research in leprosy include (i) cultivation of *M. leprae in vitro*; (ii) genetic control of susceptibility, including twin studies and HLA typing; (iii) precise antigenic analysis of *M. leprae*; (iv) mechanisms involved in the macrophage response to mycobacterial infections; (v) more use of experimental models such as normal mice infected with *M. lepraemurium*; (vi) reassessment of the protection afforded by BCG; (vii) assessment of protection afforded by killed (armadillo) *M. leprae* vaccine; (viii) pathogenesis of erythema nodosum leprosum including a study of the effect of thalidomide; and (ix) development of *in vitro* systems for drug sensitivity testing.

INTRODUCTION

The Committee was set up for a number of reasons. In the first place, the increasingly widespread recognition of dapsone resistance made it clear that the road to the effective control of leprosy was still undefined and certainly a long one. Secondly, the identification of the nine-banded armadillo as an animal host susceptible to *M. leprae* and capable of yielding hitherto unimaginable quantities of bacteria opened up a whole new range of research possibilities. Thirdly, in addition to these biological developments and perhaps in part because of them, the World Health Organization included leprosy in its Special Programme for Research and Training in Tropical Diseases.

Both the Scientific Working Groups on the Immunology (IMMLEP) and Therapy (THELEP) of Leprosy, set up under the Special Programme, have now made expert comprehensive reviews of their respective fields. This review has focused selectively on those areas for research which seem especially important or likely to be productive, and has tried to identify ways in which resources may be used most effectively to complement and support the WHO Special Programme.

BACTERIOLOGY

In vitro cultivation of M. leprae

Renewed attempts to cultivate *M. leprae in vitro* should constitute the most important aspect of research into leprosy at the bacteriological level. Fresh approaches should include modern cell culture techniques. This would give a steady source of bacteria without the need for an uncommon, expensive host such as the armadillo. If a vaccine against leprosy is developed, there will certainly not be enough armadillo-grown bacteria to satisfy world needs. Bacteria produced *in vitro* would also be more free from tissue components and micro-organisms likely to contaminate animal products, and thus more suitable for research into the antigenic structure of *M. leprae*, and for the development of a vaccine. *In vitro* cultivation would also greatly simplify the testing of chemotherapeutic agents for activity against *M. leprae*.

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The emergence of dapsone resistance means that new drugs must be developed and, if these are to be widely used in those parts of the world where they are most needed, they must be inexpensive. Unfortunately research into the development of new drugs against leprosy is at a low ebb. It is particularly important, therefore, that testing procedures for anti-leprosy drugs are not unduly complicated, time-consuming or expensive. An *in vitro* system for the growth of *M. leprae* would be the most attractive solution, but other tests for the viability of *M. leprae* could be developed and used to screen test substances for anti-bacterial activity. For instance, the incorporation of tritiated thymidine (or even tritiated DOPA) by freshly isolated dividing bacteria could give a measure of *in vitro* activity which might be adapted as a test system (Khanolkar *et al.*, 1978), and the same method can be used to check for the growth of *M. leprae* in cultivated human macrophages (Krishnaprasad *et al.*, 1977). Other and perhaps simpler tests of bacterial metabolism and viability could be explored.

There is a strong case for the continued study of mycobacteria other than *M. tuberculosis* and *M. leprae*. These other mycobacteria at times infect and sensitize man, either contributing to disease (as suggested for *M. vaccae* in Crohn's disease), or seriously affecting the response to mycobacterial vaccines such as BCG. Such studies seem likely to make an important and practical contribution to our understanding of immune responses to tuberculosis and leprosy.

EPIDEMIOLOGY

Genetic control of susceptibility

There have been a number of reports suggesting a genetic determinant of susceptibility to *M. leprae*. Especially important have been studies of the relative susceptibilities of monozygotic twins. The best study is that of Chakravarti & Vogel (1973) in India, in which among sixty-two monozygotic pairs, thirty-seven (59·7%) were concordant for leprosy and thirty-two were also concordant for leprosy type.

Another approach to the search for a genetic factor has been to survey patients for HLA type. The best study so far has been from the Leiden group (de Vries *et al.*, 1976). Initial studies in Surinam were with HLA-A and HLA-B types. Siblings with the same type of leprosy were found to have a significant excess of identical HLA haplotypes, whereas siblings affected with different types of leprosy shared a haplotype less often than expected. This was taken to indicate that susceptibility both to leprosy and the type of disease are controlled by at least two HLA-controlled genes. Recent studies by the same group in Wardha (India) suggest the association of a particular HLA-determinant (DW2) with susceptibility genes for tuberculoid leprosy. In a small study in Ethiopia of HLA-D identity using mixed lymphocyte culture, Stoner *et al.* (1978) compared seven patients with lepromatous leprosy with their nine non-infected siblings. Four of the nine were HLA-D identical with their lepromatous siblings, suggesting that susceptibility and the development of lepromatous disease are not under HLA-D control.

Studies of transmission

The route by which *M. leprae* enters and infects the body has not yet been established. The likely possibilities are through the skin or nasal mucosa, or by inhalation into the lungs. New evidence is accumulating in support of airborne transmission. Of particular importance is the observation that the average yield of *M. leprae* from a nose blow in a lepromatous patient is as high as $2{\cdot}8 \times 10^8$ bacilli. These bacilli have been shown to be viable by mouse footpad inoculations: they may remain viable for 1–2 days, and occasionally for as long as 7 days (Davey & Rees, 1974). Evidence for infection through the nasal mucosa has been provided by biopsy studies. In some patients nasal mucosa biopsy has provided the only evidence of leprosy. *M. leprae* has been found in the nasal mucosa of household contacts; some biopsies have shown evidence of nerve inflammation suggesting local infection with *M. leprae* (Chacko *et al.*, 1977).

Further research on transmission is needed. Immunologically deprived mice could be a useful animal model for the study of infection by inhalation (Rees & McDougal, 1977). Understanding the mode of infection in man is so important to all control programmes that the possibility of primary infection through the nasal mucosa is clearly a field for intensive investigation.

IMMUNOLOGY

Background of immunological spectrum

A major breakthrough was achieved by Ridley & Jopling (1966) in the classification of leprosy according to a clinico-pathological spectrum. This classification, which correlated lymphocytic infiltration, the appearance of cells of the mononuclear phagocyte series and bacillary infiltration with Mitsuda skin test reactivity, indicated that the spectrum of disease ranged from a high resistance (tuberculoid: TT), through a number of borderline points (borderline tuberculoid: BT, borderline: BB and borderline lepromatous: BL), to a low resistance (lepromatous: LL). It suggested that the basis for the spectrum was determined by the immunological status of the individual. Moreover, as host resistance to mycobacteria was manifestly due to cell-mediated immunity, it was logical to suppose that the variations in the clinical status of the patient could be correlated with other parameters of cell-mediated immunity. These included delayed hypersensitivity skin tests to extracts of *M. leprae* and the lymphocyte transformation test.

Early results from Godal and his collaborators, working with relatively small patient samples, appear to confirm the spectrum using these tests (Myrvang *et al.*, 1973). However, a number of observations (Bjune *et al.*, 1976) have indicated that although delayed hypersensitivity skin tests and lymphocyte transformation tests may correlate with the allergic reactivity of the patient, they correlate only broadly with host resistance. At two particular points on the spectrum, TT and BT, correlation is with inflammatory response rather than with resistance. Much stronger reactions are found in BT than in TT. This would indicate a dissociation in the antigens responsible for host resistance and those responsible for allergic reactivity. A number of points follow on from this. Firstly, the use of a soluble skin test antigen, like PPD-tuberculin derived from *M. leprae*, is not a good reagent for assessing host resistance in leprosy. In practice it has been found that even with an armadillo-grown antigen, the best skin test reagent to date is one containing all the antigens of *M. leprae* that would give a nodular granuloma in the skin and be read 2–4 weeks after intradermal injection. Secondly, there is at present no suitable *in vitro* test that can be used to assess host resistance to *M. leprae*. This is especially so as the other widely used parameter of cell-mediated immunity, the leucocyte migration inhibition test, is poorly reproducible and difficult to quantify.

Antigenic analysis of M. leprae

M. leprae is poorly antigenic compared with other mycobacteria. Immunization of rabbits with *M. leprae* derived from armadillos results in antibodies against seven antigens only, as compared with BCG which stimulates production of antibodies against seventy components, and *M. lepraemurium* which produces antibodies against thirty to forty antigens. However, if the rabbit serum is concentrated, antibodies can be detected against twenty *M. leprae* antigens. All seven components that are reasonably strong antigens cross-react with the antigens of other mycobacteria, especially BCG. Antibodies against *M. leprae* antigen 7, which is equivalent to BCG antigen 60, are found in the sera of tuberculoid as well as lepromatous patients. So far antigenic analysis of *M. leprae*, which began with striking results from Harboe and his group in Oslo (Harboe *et al.*, 1977), has failed to demonstrate any specific antigen of *M. leprae* that might be associated with host resistance.

There is no doubt that a more precise antigenic analysis of *M. leprae* is of critical importance in the investigation of the clinical status of patients with leprosy. It is particularly important to determine which antigens are responsible for the allergic manifestations of tuberculoid types of leprosy and which are associated with the development of host resistance. Moreover, it is possible that host resistance depends on the development of an immune response against some of the weakest antigens, which might in turn explain why many patients develop the low resistance lepromatous form of the disease.

Experimental models

Much of the earlier experimental work in leprosy has been with the thymectomized and irradiated mouse, in which host resistance to *M. leprae* has been artificially reduced. Currently, a number of

laboratories are working on mouse infection with *M. lepraemurium*, which is a more natural infection in mice and provides a model where immunological mechanisms are intact. Inbred strains of mice can be divided into two groups, high resistance strains (such as C57Bl) and low resistance strains (such as BALB/c). In these, there is direct evidence that host resistance is under genetic control. Although *M. lepraemurium* causes systemic rather than cutaneous disease, the pattern of infection in the different strains directly parallels the spectrum in human leprosy.

M. lepraemurium infection of mice has a number of other advantages over *M. leprae* infection. (1) In a natural infection, it is possible to follow the development and loss of cell-mediated immunity using both the delayed hypersensitivity and lymphocyte transformation tests. Different types of delayed hypersensitivity, e.g. Jones–Mote reactivity, can also be studied in this model. (2) The mechanism of failure of cell-mediated immunity and host resistance in a mycobacterial infection can be studied with particular reference to the role of suppressor cells and immunoregulatory mechanisms. (3) Owing to the greater potency of *M. lepraemurium* antigens, it should be easier to characterize them and determine which are responsible for the development of host resistance and which are involved in cell-mediated hypersensitivity mechanisms.

Erythema nodosum leprosum (ENL)

Whereas it has been considered that the hypersensitivity mechanisms underlying the cutaneous and nerve lesions of tuberculoid and borderline tuberculoid leprosy and reversal reactions are cell-mediated, it has been suggested that ENL could be an immune complex-mediated reaction.

ENL may in fact be two disease states occurring frequently at the same time and developing about 6 months after the onset of chemotherapy. Cutaneous ENL, which incidentally bears no resemblance to erythema nodosum, has features suggestive of an Arthus reaction. These include cutaneous vasculitis, massive infiltration with polymorphonuclear leucocytes and the demonstration of granular deposits of immunoglobulin and complement (C3) in the tissues. Systemic manifestations associated with ENL consist of fever, arthritis, uveitis and a transient proteinuria which is distinct from the massive proteinuria of amyloid disease found in advanced cases of leprosy, especially those with recurrent ENL. The uveitis of ENL, and indeed the ocular manifestations of leprosy in general, are important lesions which have been relatively neglected. All these systemic manifestations of ENL may occasionally be dissociated from the cutaneous manifestations.

Numerous attempts have been made to demonstrate changes in serum complement levels in ENL, including total CH50, C3 and Clq binding tests. Most have shown little correlation. Recently, however, Bjortavn *et al.* (1976) have demonstrated increased levels of the C3 breakdown product, C3d, in the plasma of 70% of patients with ENL and in only 18% of patients with lepromatous disease without ENL. This has suggested that in cutaneous ENL the breakdown of C3 is an extravascular event. No other studies of the different parameters of immune complex formation have looked at the cutaneous and the systemic disease separately. One possibility is that changes in circulating complement and the increase in Clq binding activity only occur in systemic forms of the disease. Moreover, there is no evidence that the cutaneous manifestations are due to immune complexes that are known to deposit in areas where the blood vessels are damaged from other causes. It could be that the primary cutaneous lesion is due to the activation of C3 through the alternative pathway, which results from the release of mycobacterial polysaccharides when organisms are killed too rapidly by chemotherapy.

A further line of research into ENL that needs consideration is the role of thalidomide in suppressing this reaction. So far, thalidomide has not been shown to affect any allergic reaction in experimental animals, nor does it appear to affect any other allergic type of reactions in man. Further thought should be given to the action of thalidomide *in vivo*, as elucidation of its action could throw light on some of the pathological and immunological mechanisms underlying ENL.

Armadillo M. leprae vaccine

A protocol has been developed by IMMLEP for isolating *M. leprae* from the tissues of infected armadillos by means of enzyme digestion using collagenase, trypsin and chymotrypsin. Proteolytic

enzymes are known to destroy protein antigenicity; the present method of extracting *M. leprae* may therefore be reducing the potency of the preparation and have to be modified.

A vaccine may be ineffective in patients with lepromatous leprosy in whom there is an underlying specific immunological defect and whose tissues are loaded with large numbers of live *M. leprae*. However, such a vaccine will be particularly useful in children at special risk, for example those who are considered uninfected but who live in a leprosy endemic area, and especially those resident in a leprosy household. As there is every indication that armadillo *M. leprae* are not attenuated and would be highly infective for man, these organisms could be used for a vaccine only if rendered uninfective. The relative efficiencies of dead mycobacteria and attenuated live mycobacteria in increasing host resistance have been intensively studied over 50 years by workers such as Arnold Rich and Sidney Raffel (Turk, 1975). Whereas a live attenuated vaccine like BCG markedly increases host resistance, dead mycobacterial preparations, even in adjuvant, do not appear to be able to increase host resistance although they produce strong delayed hypersensitivity reactions to tuberculin. The production of an attenuated live armadillo *M. leprae* vaccine comparable to BCG could well take many years. A dead vaccine might produce strong allergic reactivity, possibly without protection. Experiments now in progress on the inhibitory effect of a killed armadillo *M. leprae* vaccine on the limited growth of *M. leprae* in the footpads of normal mice are, therefore, of particular interest (Shephard, Walker & Van Landingham, 1978). Another approach to this problem is to look at the effect of irradiation in abolishing the infectivity of *M. leprae* without reducing its immunizing potential (R.J.W. Rees, personal communication). A suitable protocol for such a study might be developed using *M. lepraemurium* in mice.

BCG and leprosy: a re-appraisal

There is considerable cross-reaction between the antigens of *M. leprae* and those of other mycobacteria. So far, it appears that an antigen specific for *M. leprae* has not been characterized. It is thus logical to look more carefully at the possible usefulness of the most widely available live attenuated mycobacterial preparation, BCG vaccine.

Stanford (1977) has compared the protection conferred by BCG against leprosy in Uganda and Burma with the protection against tuberculosis in the UK and the USA (Table 1). He considered that the differences observed could have been due to differences in the mycobacteria in the environment. He then looked at protection against leprosy in Burma and Uganda as a function of the age at which the children had been vaccinated (Table 2).

TABLE 1.

	Protection from tuberculosis (%)		Protection from leprosy (%)
UK	78	Uganda	80
USA	14	Burma	17

TABLE 2.

Age vaccinated (years)	Protected (%)	
	Burma	Uganda
0–4	66	78
5–14	25	77

Analysed in this way, the data suggested something had happened to children between the ages of 4 and 15 in Burma to change their response to BCG vaccination. He found that *M. marianum* was more common in Burma than in Uganda and that skin test positivity to *M. marianum* antigens reached 30% at the age of 10 years. He therefore suggests that *M. marianum* is one of the environmental mycobacteria immunizing children in Burma in a way which blocks their ability to respond to BCG, with the increase in host resistance observed elsewhere. In the laboratory it was found that skin testing of mice by the footpad swelling technique with reagents of high specificity, following challenge with various species of mycobacteria, showed that there were two patterns of response, one for species not pathogenic for mice and another for pathogens. Moreover, he has shown that pre-feeding mice with *M. marianum* converts the response to BCG from the non-pathogenic to the pathogenic. *M. leprae* has been found to have a similar effect to *M. marianum*, so the possibility exists that *M. leprae* vaccination, far from increasing host resistance to *M. leprae*, might actually decrease resistance.

It was considered that more attention should be given to the results of BCG vaccination in leprosy and that further trials should be undertaken in which the BCG is administered shortly after birth.

CHEMOTHERAPY

The early hope that dapsone would be an all-sufficient drug for the treatment of leprosy has not been fulfilled for two reasons, drug-resistance and microbial persistence.

Secondary resistance to dapsone has now been recognised clinically in most countries where leprosy is endemic. Although low dosage and irregularity of treatment appear to facilitate the emergence of resistance, this still develops where treatment is regular and dosage adequate (Meade *et al.*, 1973). The duration of treatment before resistance becomes evident, has varied between 5 and 24 years in one series (Pearson, Rees & Waters, 1975). Resistance has been encountered only in lepromatous (LL) and borderline lepromatous (BL) cases. These clinical observations have been confirmed in the laboratory by mouse footpad studies. In these, *M. leprae* has been found fully resistant to concentrations of 0·01% in the mouse diet, compared with levels of 0·0001% to which all strains of *M. leprae* tested in the past have been fully sensitive (Ellard *et al.*, 1971).

In this context of a world-wide emergent secondary dapsone resistance, primary infections with resistant *M. leprae* are inevitable, and have now been identified in at least two centres where a careful search has been made (Pearson, Haile & Rees, 1977; Jacobson & Hastings, 1978).

Quite separate and distinct from the problem presented by dapsone resistance is the phenomenon of microbial persistence. In cases of lepromatous leprosy, *M. leprae* may remain viable in tissues over long periods of time in spite of apparently effective therapy. In one study, fresh tissue biopsies from skin, nerve, striated muscle and smooth muscle (dartos) from lepromatous patients who had received continuous chemotherapy, principally with dapsone, for 10–12·5 years yielded bacilli which multiplied in mouse footpads, and on successful passage were found to be fully sensitive to 0·0001% dapsone in the mouse diet (Waters *et al.*, 1974). Persisters have also been isolated from sulphone-resistant patients treated for long periods with clofazimine or with rifampicin, either alone or in combination with thiambutosine (Rees *et al.*, 1976). Thus, there is as yet no indication that any of the newer mycobactericidal drugs are more effective than dapsone in preventing persistence. Monitoring lepromatous patients for persistence will be an essential feature of all future chemotherapy trial protocols.

Persistence of bacteria seems to occur largely or entirely in macrophages. Research into the macrophage response has focused on the lysosomal system. Pathogenic mycobacteria, in a way not well understood, often inhibit lysosomal fusion or the fusion of lysosomes with phagosomes. Because none of the drugs so far tried appears to prevent persistence, it is obviously essential that this line of research should continue to be followed.

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