*Lepr. Rev.* (1974) **45**, 244-272

# Reprinted Article

We are happy to include the following important reports
by the courtesy of the World Health Organization.

## Immunological Problems in Leprosy Research*: 1

This Memorandum reviews the present status of knowledge of the immunology of leprosy, with particular attention to developments since the publication of a similar review in 1970. The different types of lepromin reaction and their significance in healthy contacts and in patients with tuberculoid and lepromatous leprosy are discussed. The immunological responsiveness of patients with leprosy is also considered, with special attention to *in vitro* methods for evaluating this response. Part 2 of the Memorandum will cover possible mechanisms of altered immune response in leprosy (including a tentative scheme to explain the possible genesis of the lepromatous lesion); genetic, nutritional, and hormonal factors; the possibility of vaccination; attempts at immunotherapy; and areas in which further research is needed. A detailed protocol for evaluating the effect of transfer factor in leprosy will be included as an appendix.

Although leprosy is one of the principal public health problems of the world, the leprosy bacillus appears to be relatively innocuous and causes damage to host cells only after extensive proliferation. Immune responses protect the host against unlimited multiplication of bacteria and in most exposed individuals probably terminate the infection at a subclinical level (Godal *et al.*, 1973). Paradoxically, however, immune responses appear to play a major role in producing the various structural and functional disturbances that characterize leprosy. The analysis of immune mechanisms in persons infected with *Myco. leprae* is therefore complex and must range from the consideration of protective responses to intracellular microorganisms to the analysis of the mechanisms of immunologically-mediated tissue injury.

Leprosy presents a clinicopathological "spectrum" of disease between two polar forms (tuberculoid and lepromatous). Tuberculoid leprosy, the highly resistant form, presents with a few well defined skin lesions that histologically resemble typical delayed-type hypersensitivity granuloma. The cellular reaction consists of focal collections of epitheloid cells surrounded by large numbers of lymphocytes. Bacilli are not found by routine histological examinations or are very rare.

In lepromatous leprosy, the "low resistant" form, however, the lesions are multiple and diffuse and consist predominantly of macrophages with an undifferentiated (histiocytic) or "foamy" appearance. The number of

* This Memorandum was drafted by the signatories listed at the end of Part 2.

lymphocytes present is insignificant. Bacilli are present in large numbers in the macrophages.

Between these two forms a continuous range of clinical and histopathological features is seen. Outside this range, early leprosy may manifest itself as a single vague skin lesion, which has been called "indeterminate" leprosy. Histologically, loose collections of histiocytes and lymphocytes, often associated with dermal nerves, are seen. A few bacilli may sometimes be seen in histiocytes, dermal nerves, and erector pilae muscles.

An earlier paper on this subject (*Bull. Wld Hlth Org.*, 1970) emphasized the importance of a detailed clinical and histopathological classification of leprosy so that immunological data could be adequately compared. At that time the Ridley and Jopling (1966) scale* appeared to provide additional histological criteria that had assisted immunologists with the clinicopathological classification of patients for research purposes.

During the past two years the Ridley-Jopling classification has shown good correlation between the clinical-histological scale and the immunological status as assessed *in vitro* by lepromin sensitivity and lymphocyte reactivity to *Myco. leprae*.

An international collaborative study involving the exchange of tissue slides from patients whose immunological status has been determined by *in vivo* and *in vitro* studies would serve to resolve existing differences in the classification of patients with leprosy.†

## Physiological and Antigenic Properties of *Myco. leprae*

### *Bacteriology and antigenic composition*

*Myco. leprae* is an obligate intracellular parasite. It has not been cultivated on artificial media, and only irregular and limited growth has been reported in tissue culture. It has been grown in mice and other small rodents and in armadillos. Nearly all the bacteriological studies have been carried out in mice. The rate of growth during the logarithmic phase shows a doubling time of 12-13 days (Shepard and McRae, 1965), irrespective of the immunological competence of the mouse. The rate of growth has not changed on continued passage in mice during periods up to 14 years.

Isolates have been found to differ slightly in certain growth characteristics and have been classified as "fast" and "slow" (Shepard and McRae, 1971); "fast" strains have somewhat shorter incubation periods, faster average rates of growth between inoculation and harvest, and higher average bacterial populations at plateau. The distribution of various isolates is continuous between the two extremes of "fastness" and "slowness". The trait is stable on passage in mice and repeated isolations from the same patient have the same characteristic. There is no relation to drug resistance.

Isolates from different parts of the world and from patients in the range LL-BT have behaved similarly in mice. Studies of the tissue distribution of the bacilli in

* The Ridley-Jopling scale is as follows: TT, polar tuberculoid; BT, borderline tuberculoid; BB, borderline; BL, borderline lepromatous; LL, polar lepromatous.

† Since 1970, WHO has planned collaborative studies involving nine laboratories in connexion with the WHO International Reference Centre for Histological Identification and Classification in Leprosy (Instituto Nacional de Dermatologica, Caracas, Venezuela).

mice and in men at several constant ambient temperatures indicate that there is no optimum temperature for the growth of *Myco. leprae.*

Recent experience with mice in the tropics has shown that temperature control (e.g. air conditioning) is not necessary for experiments with *Myco. leprae,* although it is preferable for the health of the mice and for constant results at different seasons of the year.

Heat-killed suspensions of *Myco. leprae* fail to react as a skin-test antigen in patients with lepromatous disease. This property is not shown by any other mycobacterium. Immunodiffusion tests show that *Myco. leprae* shares many antigens with other mycobacteria. However, one specific protein antigen ("nodular extract"), which is inactivated by heat and trypsin, has been isolated so far (Abe, 1970; Abe *et al.,* 1973). The specificity of this antigen for *Myco. leprae* was demonstrated by immunodiffusion and immunofluorescence tests with rabbit antisera against nodular extract and a wide range of mycobacteria (Abe, 1971). It is present in the tissues of lepromatous nodules and in the bacterium. Observations suggest that it is a protoplasmic antigen. In a dose of 10 μg, it gave positive 48 h skin reactions in patients with tuberculoid leprosy but negative reactions in those with the lepromatous form of the disease (30 of each). It is recommended that the specificity of this antigen in human skin tests be fully investigated.

Another specific antigen, which is heat-stable, has been demonstrated by lymphocyte transformation, and its antigenic relationship to nodular extract should be investigated by means of the lymphocyte transformation test.

Detailed histological studies in man and in mice indicate that all leprosy infections are systemic. The tissues that contain the largest numbers of bacilli in lepromatous disease are: the skin (exclusive of the axillae, creases, etc.), the nasal mucous membrane, the peripheral nerves, and the reticuloendothelial system, including the bone marrow. There is a continuous bacteraemia in patients with untreated lepromatous disease. Tuberculoid disease shows few bacilli; they are largely concentrated in the dermal nerves and erector pilae muscles of the lesion and in the peripheral nerves. In the evolution of experimental disease, the invasion of nerves by bacilli is a late manifestation, and therefore in man may also be secondary to extraneural infection. Peripheral neuropathy in leprosy is important but the means by which the bacilli enter the nerves is unknown.

The route of transmission of leprosy to man is not established. The portal of exit has commonly been assumed to be the skin or the nasal mucosa. The number of bacilli excreted from the skin is small, whereas the output from the nose is large (comparable with that from the lungs in open cases of tuberculosis). The portal of entry also has usually been assumed to be the skin or the nasal mucosa, but is, in fact, unknown, and the bacillus might well enter the body in inhaled air.

### *Lepromin reaction**

*Type and standardization of lepromin.* The original lepromin of Mitsuda (1923) and Hayashi (1933) is a suspension of the whole autoclaved homogenized leproma, including some tissue elements. This is sometimes called "integral" lepromin. Purified bacillary suspensions have been made, more or less completely freed from tissue elements, and are sometimes called "bacillary" lepromins. Antigens that consist of the soluble proteins of the bacilli, with or without

* For reaction scale, see *Bull. Wld Hlth Org.* (1970).

proteins of the leproma not coagulated by heating, elicit only the early reaction. For distinction, such antigens should be called "leprolins". The "defatted" bacillary suspension devised by Dharmendra (1942) is used especially for testing the early reaction; it gives only a weak late reaction. Because this material is obtained by the chemical extraction of bacilli, it is neither a lepromin nor a leprolin as defined above, and therefore should be referred to as "Dharmendra antigen". The fundamental difference between lepromins and leprolins is that the latter elicit only the early (Fernandez) reaction after 48 h (Fernandez, 1940) and do not themselves sensitize; the former also elicit the early reaction to a variable extent and in addition induce the late (Mitsuda) reaction after four weeks. The test, being a form of "microvaccination", could affect the immunological status of the individual. One consequence is that persons negative to a first test may give positive reactions to a second or subsequent test.*

The injection of autoclaved normal skin or homogenates has given negative to weak reactions in healthy persons and in those with tuberculoid leprosy. From the practical point of view, therefore, there is a possibility that weak positive reactions may be caused by the excess of tissue components in the lepromin.

The Mitsuda reaction is of prime importance for prognosis, particularly in patients with indeterminate and borderline leprosy. Some investigators think it is useful for evaluating the relative resistance of contacts and populations. In order to obtain comparable data from different countries, the standardization of lepromin is necessary.

Recent research has been directed towards the production of an improved lepromin and the investigation of Mitsuda reactions to diluted lepromins. It is hoped to retain all significant reactions to the bacilli while decreasing the number of false positive (1+) reactions, which have been attributed to tissue components. An improved lepromin should possess three properties: (1) bacilli that have been subjected to minimal, controlled mechanical trauma, (2) a uniform range of bacterial clump sizes, and (3) freedom from visible, rapidly settling tissue particles. These qualities would increase the reliability of bacteria counts as a primary means of standardization and ensure the injection of uniform doses of bacteria with minimal tissue component.

*Healthy persons in endemic areas.* The lepromin test is usually negative in the first months after birth. Before the age of 1 year, approximately 50% of children may present a weak (1+) reaction. The proportion of positive reactions and the degree of positivity increase steadily with age, and at the age of 15 years and over most persons give a 2+ or 3+ reaction.

Maturation and/or exposure to *Myco. leprae, Myco. tuberculosis,* and possibly other mycobacteria may be the cause of such reactivity. The injection of lepromin can also act as a sensitizer. Some believe that prior sensitization with either *Myco. leprae* or *Myco. tuberculosis* does not offer an adequate explanation for the occurrence of natural reactivity to lepromin in some areas. The cause of such reactivity is in fact unknown.

The *in vitro* test of lymphocyte responsiveness to *Myco. leprae* appears to give a high proportion of positive results in the contacts of patients with leprosy, while

* Such studies are being carried out by the WHO regional reference centres for standardization of lepromin (Johns Hopkins University, Leonard Wood Memorial, Department of Pathobiology, Baltimore, USA, and the National Institute for Leprosy Research, Tokyo, Japan) and by the Instituto de Leprologia, Rio de Janeiro, Brazil. These laboratories are able to provide lepromin for research purposes.

most of those who are not contacts give negative results (Godal *et al.*, 1973). On the other hand, no association could be found between the intensity of the Mitsuda reaction and the *in vitro* lysing activity of the derived monocytes of healthy individuals.

*Healthy persons in nonendemic areas.* Several studies have shown that populations in areas where leprosy is not endemic (Europe and the USA) show a high proportion (about 80%) of positive Mitsuda reactions. The porportion of positive Fernandez reactions is low, even in patients with tuberculosis.

*Macroscopic and histological appearance.* It has been shown that positive late lepromin reactions are characterized histologically by a tuberculoid-type granuloma, while negative and doubtful reactions (0, ±) are associated with a nonspecific cellular response. Weak positive (+) reactions are associated with a nonspecific cellular response in approximately 15% of contacts, while in tuberculoid and indeterminate cases of leprosy they are usually associated with a tuberculoid-type granuloma.

Further histological studies are required to determine whether the greater refinement in the preparation of lepromin would reduce the frequency of nonspecific histological responses in + late lepromin reactions.

*Repeated reactions in healthy persons.* It is well established that repeated Mitsuda tests—often even a second test—can convey lepromin positivity. This type of conversion has been observed even in children who are tuberculin-negative. The reaction may also intensify (+) in a relatively high proportion of individuals. Thus lepromin may act as a sensitizer in persons who have the potentiality to react. Rees (1964) has reviewed the significance of the lepromin reaction in man.

## White Cell Responses to Infections

The main leucocytes involved in responses to chronic infections, including leprosy, are lymphocytes and macrophages, although polymorphonuclear infiltrations are found in acute exacerbations such as *erythema nodosum leprosum.* Collaboration between lymphocytes and macrophages appears to be the main defence mechanism against organisms, such as *Myco. leprae,* that multiply within cells.

### *Origin and activation of lymphocytes*

This subject has been discussed in detail by the WHO Scientific Group on Cell-Mediated Immunity and Resistance to Infection (1973). Lymphocytes are of two main types—thymus-derived (T cells) and bone-marrow-derived (B cells). T cells are generated within the thymus from precursor cells seeded from the bone marrow. Some of these T cells migrate to special regions of the lymph nodes and spleen (thymus-dependent areas) and proliferate following contact with the appropriate antigen. T cells are characterized by an extensive capacity for circulation from one lymphoid organ to another via the thoracic duct and blood and can freely penetrate most tissues, often returning to lymph nodes in the afferent lymph.

B lymphocytes also arise from precursors in the bone marrow and migrate to defined areas of the lymph nodes and spleen. These cells also proliferate following antigenic stimulation, and some B cells can circulate although most appear to remain localized at their site of production in the lymphoid tissues.

In the peripheral lymphoid organs, the main proliferative stimulus for T and B

cells is antigenic stimulation. This is a highly selective process since individual lymphocytes are stimulated only by one or a limited number of antigens. The individual, or restricted, responsiveness of lymphocytes is a property acquired by these cells in the thymus and bone marrow in a process involving selective gene derepression and synthesis of antigen receptors—which, in the case of B cells, are immunoglobulins that can be demonstrated on the plasma membrane.

While lymphocyte circulation ensures a regular distribution of lymphocytes to the tissues, this process is amplified when local deposits of antigens or microorganisms are present in a tissue. Reaction between antigen and the occasional circulating lymphocyte with specific receptors for that antigen causes lymphocyte activation and the local release of soluble factors, which may bring about the localization of additional leucocytes that are not necessarily specifically reactive. Since B and T cells responding to antigens become more adherent and less mobile than unstimulated cells, with the passage of time there may be a tendency towards selective accumulation of specifically reactive cells at local sites of antigen deposition and in the draining lymph nodes.

The clonally expanded populations of T and B lymphocytes that develop following antigenic stimulation have distinct immunological functions. B lymphocytes and their specialized variants, the plasma cells, secrete specific immunoglobulins (antibodies: Ab). T lymphocytes are the effector cells of cell-mediated immune responses and in humoral responses to most antigens T cells collaborate with B cells, helping the latter to proliferate and secrete antibody.

T lymphocytes responding to specific antigens undergo blast transformation and proliferate. Activated T cells can kill target mammalian cells with which they come into contact—e.g. foreign cells with antigens against which the T cells are specifically sensitized. This may possibly include target cells containing microorganisms if products of the latter reach the cell membrane. There is no evidence so far that T cells can kill microorganisms by such direct contact. However, activated lymphocytes can increase the capacity of macrophages to kill microorganisms (see below) and also release macrophage-immobilizing factors.

B lymphocytes responding to specific antigens secrete immunoglobulins, which, in conjunction with complement, are bacteriolytic for some bacteria and can opsonify many microorganisms, thereby facilitating their phagocytosis by leucocytes. No information yet exists as to whether antigen-stimulated B cells release other soluble products that affect the localization and functional activity of other leucocytes.

*Macrophage production, localization and activation*

Most tissue macrophages are derived from blood monocytes, which originate from precursor cells located mainly in the bone marrow, with smaller numbers in the spleen (Nelson, 1970; Metcalf and Moore, 1971).

Following localization in different organs and exposure to varying stimuli, macrophages can adopt a wide variety of morphological forms. The resulting pleomorphism is well exemplified in leprosy where macrophages may appear as epithelioid, multinucleate, histiocytic, or foamy cells. In culture, clonally-derived macrophages can assume a fibroblastic appearance, and this may also happen in tissues.

Macrophages and granulocytes are closely linked in origin and regulatory control. They share the same precursor cells (*in vitro* colony-forming cells) and their proliferative activity is controlled by the same regular system—the

glycoprotein colony-stimulating factor. Selective entry of the proliferating cells into the granulocytic or monocyte–macrophage pathway is determined by the concentration of this factor and by the operation of a complex of serum lipoproteins termed "colony-stimulating-factor inhibitors". Macrophage formation is favoured by relatively low or high levels of inhibitor.

Following viral or bacterial infections, or the injection of bacterial antigens, there is a rapid increase in tissue synthesis of stimulating factor and a subsequent rise in the levels of this factor in the serum. This response is radioresistant and not T-cell-mediated. Specifically preimmunized animals fail, on challenge, to exhibit this response and their depressed stimulating-factor reactivity is transferable to normal animals by immune sera. The rise in the levels of colony-stimulating factor following antigenic stimulation is followed by increased proliferative activity of granulocyte and monocyte-macrophage precursors, particularly in the spleen (Metcalf, 1972).

Under certain conditions of antigenic stimulation–e.g. after the use of complete adjuvants including mycobacteria–a limited degree of proliferation can occur in local tissue macrophages (see Nelson, 1970). Under normal conditions, a slow turnover of tissue macrophages results from the seeding of blood monocytes into the tissues and a limited degree of relocation of tissue macrophages among different organs. The latter process could result in the dissemination of organisms from one tissue to another–e.g. in leprosy, where the macrophages contain viable organisms. In the lepromatous form of the disease dissemination may be favoured by frank bacillaemia.

The localization of macrophages at sites of antigen deposition may be amplified by the release from reacting lymphoid cells of macrophage chemotactic and immobilizing factors.

Macrophages play a number of important roles in immune response (WHO Scientific Group on Cell-Mediated Immunity and Resistance to Infection, 1973). On the afferent side they allow (a) phagocytosis and modification of particulate antigens with the release of more antigenic determinants; and (b) long-term retention of antigen on the cell membrane in an accessible and correctly oriented form that is capable of activating potentially reactive lymphocytes. This latter process may be facilitated by the capacity of macrophages to allow attachment of the Fc portion of immunoglobulins at the cell membrane. Macrophage-associated antigen is especially efficient in eliciting cell-mediated immune responses.

On the efferent side of the immune response, the activation of macrophages–e.g. by exposure to products of interactions between antigens and T lymphocytes–increases the capacity of these cells to kill or inhibit the multiplication of certain pathogenic organisms. Macrophages so activated can kill *Listeria monocytogenes* and inhibit the multiplication of *Myco. microti* and *Myco. lepraemurium,* but their role in relation to *Myco. leprae* has not yet been investigated. The survival of some intracellular organisms (e.g. *Myco. tuberculosis*) may be favoured by the failure of lysosomes containing hydrolytic enzymes to fuse with phagocytic vacuoles containing living bacteria, but in macrophages infected with *Myco. leprae* and *Myco. lepraemurium* such fusion appears to take place. Whether *Myco. leprae* can be digested by macrophages is also unknown. If so, the process must be very slow since organisms remain demonstrable for years in the tissues of patients with lepromatous leprosy who are under treatment. Possibly the ultimate loss of organisms from lesions follows the relocation of macrophages containing indigestible cell walls.

Macrophages can also collaborate with fibroblasts in chronic inflammatory reactions. In silicosis, particles that are ingested by and damage macrophages stimulate collagen synthesis and extensive fibrosis. The presence of indigestible mycobacterial constituents or of immune complexes in certain types of leprosy may favour fibrogenesis by analagous mechanisms.

The functional efficiency of the extreme morphological variants of macrophages seen in leprosy lesions is unknown. The possibility that activated macrophages may release factors that modify the proliferative or functional activity of lymphoid or other cells has not yet been investigated, although macrophages exposed to mycobacterial and other adjuvants appear to increase lymphocyte proliferation in immune responses (WHO Scientific Group on Cell-Mediated Immunity and Resistance to Infection, 1973).

*Lymphocyte function tests*

*Distinguishing lymphocyte subpopulations.* Principal lymphocyte subpopulations can be identified and distinguished by virtue of their surface markers and by their response *in vitro* to certain nonspecific stimuli. B lymphocytes are known to have surface immunoglobulins that can be identified by direct or indirect immunofluorescence using polyvalent antisera to human immunoglobulins. In addition, B lymphocytes have a complement receptor that can be detected by rosette formation (termed the "EAC rosette test") with sheep erythrocytes coated with a rabbit anti-Forssman antibody (i.e. amboceptor) plus mouse complement (or human complement in sublytic concentrations). They also have a receptor for the Fc fragment of human immunoglobulin that has been either bound to antigen in the form of a soluble immune complex or heated at 60° C. Because this last method requires the use of isotopically labelled immunoglobulins or antigen, the EAC rosette test is more generally applicable. There is much evidence that T lymphocytes form spontaneous rosettes (termed "E rosettes") with washed sheep erythrocytes. Clearly, T lymphocytes do not have surface receptors for immunoglobulin or for complement; nor do they have a receptor for the Fc fragment of human immunoglobulins. It has been possible to separate T and B subpopulations of human peripheral blood lymphocytes by physical methods using these properties. In addition, T lymphocytes can be recognized by the blast-cell transformation test using phytohaemagglutinin (PHA test) or allogeneic lymphocytes (MLC test); here lymphocyte stimulation is detected by morphological enumeration of blast cells or by their ability to incorporate isotopically labelled thymidine.

*Specific immunological reactivity of cultured lymphocytes.* A number of *in vitro* tests have been and are currently being developed to detect specific immunological reactivity of T and B lymphocyte subpopulations. This discussion is limited to the two tests that have been most extensively applied to man. Antigen-induced blast-cell transformation is thought to detect primarily T-cell sensitization, although there are situations in which B cells are thought to respond. In order to assess the significance of lymphocyte transformation responses it is desirable to test reactivity to a panel of common antigens with which most individuals in the population may have had contact (e.g. tuberculin PPD, streptokinase-streptodornase, *Candida,* mumps). A positive lymphocyte transformation test in inferred when the number of blast cells in antigen-stimulated cultures exceeds by 3% the number in the control cultures or when there is a twofold (or greater) increase in thymidine incorporation relative

to the control cultures. The second widely used test of lymphocyte reactivity is that in which antigen inhibits the outward migration of leucocytes from cell suspensions packed in capillary tubes. In this test it is thought that specific antigen stimulates the lymphocytes to generate a migration inhibition factor whose action in this sytem is recognized by the inhibition of polymorph migration. A positive leucocyte migration test in inferred when specific antigen reduces leucocyte migration areas by 20% (or more) with respect to control cultures. In this test it is important to delineate toxic (i.e. nonimmunological) effects of antigens on the migration of leucocytes; accordingly, antigen preparations should be used at concentrations below those that inhibit leucocyte migration from individuals known not to be sensitized. As with lymphocyte transformation, the significance of leucocyte migration inhibition is best assessed by reference to other common antigens to which individuals may be expected to have developed sensitivity.

*Technical considerations.* For distinguishing T cells and B cells it is desirable to work with purified washed lymphocytes. Peripheral blood lymphocytes are best obtained by centrifugation through a solution containing "Ficoll" (a sucrose polymer) and sodium metrizoate (or sodium diatrizoate or diodone) at a specific gravity of 1.078 (Böyum, 1968); cells purified in this way can also be used for lymphocyte transformation studies. In the assessment of lymphocyte transformation it is desirable to employ a range of antigen concentrations and to determine the time course of response, and dose-response relationships should similarly be obtained in leucocyte migration. In lymphocyte transformation tests, the measurement of radiolabelled thymidine uptake is preferable owing to its greater precision and sensitivity; however, morphological assessment of blast cells correlates well with thymidine uptake if the cells are counted by an experienced observer. It is recommended that individual tests of lymphocyte transformation be carried out in duplicate or triplicate, and that in leucocyte migration six replicate capillaries be used at each antigen concentration. Both tests are now available in a miniaturized form that permits the use of smaller quantities of blood, making replicate tests and paediatric applications possible.

## Immunological Responsiveness in Leprosy

While the humoral immune responses, as gauged by serum immunoglobulin levels and the ability to form antibodies to bacterial vaccines such as TAB, are unimpaired in patients with leprosy, there is increasing evidence of a depression of cell-mediated immunity in certain categories of patients suffering from leprosy. The degree of depression varies from the TT to the LL end of the spectrum in relation to the clinical, bacteriological, and histopathological status of the subject. The maximum impairment is noticeable in untreated patients with the polar form of lepromatous leprosy. As will be specified below, it appears that the depression of cell-mediated immune response is both specific and nonspecific. Peripheral leucocytes from untreated cases of lepromatous leprosy show poor transformation with PHA and *Myco. leprae* antigens. Upon treatment, the mitogenic response to PHA is improved, while anergy to *Myco. leprae* antigens is maintained over many years of treatment with sulfones. The status of immunological responses in various categories of patients with leprosy as shown by *in vivo* and *in vitro* tests is considered below.

In vivo *studies on cell-mediated immunity*

*In vivo* studies on cell mediated immune processes in leprosy have included the following tests: (1) reactivity to killed (autoclaved) suspensions of whole *Myco. leprae* bacilli (bacillary lepromin) and Dharmendra lepromin; (2) delayed hypersensitivity to other bacterial antigens, such as tuberculin (PPD); (3) active sensitization to 1-chloro-2,4-dinitrobenzene and 2-chloro-1,3,5-trinitrobenzene ("picryl chloride"); and (4) skin allograft rejection. The findings may be summarized as follows.

*Lepromin testing.* Patients with tuberculoid leprosy give uniformly strong reactions of the late type (Mitsuda reaction) but a variable degree of reactivity of the early type (Fernandez reaction). Patients with polar lepromatous disease give uniformly negative results in both tests. Patients with borderline leprosy show a variable degree of reactivity, depending on their place in the pathological spectrum—i.e. those with the BT and TT forms react similarly, while untreated patients with the BL form usually reveal a negative response (Myrvang, 1972).

*Delayed hypersensitivity to PPD and other microbial antigens.* In contrast to late lepromin, the reactivity to PPD does not bear a consistent relationship to the spectrum of disease in patients with leprosy. Variable proportions of positive and negative reactors are found in patients with tuberculoid, borderline, and lepromatous disease (Myrvang, 1972). Similar observations have been made with other microbial antigens (Turk and Bryceson, 1971).

*Active sensitization.* A high proportion of patients with lepromatous disease have been found not to respond to 1-chloro-2,4-dinitrobenzene, in contrast to healthy persons and those with the tuberculoid form of the disease (Turk and Bryceson, 1971). Similar findings have been noted with 2-chloro-1,3,5-trinitrobenzene (Bullock, 1968).

*Skin allograft rejection.* Patients with the lepromatous and, to a lesser degree, the tuberculoid form of the disease have revealed a delayed rejection of allogeneic skin grafts as compared with healthy individuals. The degree of impairment in patients with lepromatous leprosy was found to be correlated to bacillary concentration of the skin at the site of transplantation (Han *et al.*, 1971).

*Assessment of lymphocyte function* in vitro

The following methods have been used to assess cell-mediated immune response in patients with leprosy: (1) blast transformation of peripheral leucocytes in the presence of whole *Myco. leprae,* leprolin, other mycobacteria, and PHA; (2) mixed leucocyte culture, and (3) tests of the ability of sensitized lymphocytes to produce biologically active products.

*Blast transformation.* The method of lymphocyte transformation has recently been established for the study of lymphocyte reactivity to *Myco. leprae in vitro* (Bullock and Fasal, 1971; Godal *et al.*, 1971). While patients with TT leprosy respond quite strongly, those with the LL form of the disease regularly give negative results (Bullock and Fasal, 1971; Godal *et al.*, 1971). Those with BT leprosy show a variable degree of responsiveness, while those with untreated BL disease are usually negative (Myrvang, 1972). The failure of lepromatous lymphocytes to respond to *Myco. leprae* appears not to be reversed even after many years of chemotherapy (Godal *et al.*, 1972*a*).

Although lepromatous leucocytes fail to transform in the presence of *Myco. leprae,* they respond to a varying degree of other mycobacterial antigens, such as whole BCG and PPD (Myrvang, 1972). The level of reactivity appears to depend

on the status of treatment—i.e. untreated patients with lepromatous leprosy have revealed lower reactivity than those who received prolonged chemotherapy (Godal *et al.*, 1972).

Phytohaemagglutinin in solution induces the blast transformation of T cells and can thus be used as a measure of T cell responsiveness. The test can be performed on a micro scale with 50 μl of blood. Well-standardized methods that permit sensitive and repeatable measurements have been described (Mehra *et al.*, 1972). The mitogenic response of peripheral leucocytes to PHA is observed to be depressed in untreated cases of lepromatous leprosy, while tuberculoid and DDS-treated lepromatous cases show a normal response (Talwar *et al.*, 1972).

Peripheral leucocytes from untreated cases of lepromatous leprosy manifest low transformation when cultured in autologous as well as in AB serum (Mehra *et al.*, 1972). Furthermore, serum from patients with lepromatous leprosy fails to inhibit *Myco. leprae*-induced transformation of leucocytes from patients with the tuberculoid form of the disease, suggesting that transformation inhibitory factors are absent from these sera (Godal *et al.*, 1972).

*Mixed leucocyte culture.* The blast-cell responsiveness of lepromatous leucocytes (L x L) appear to be of similar degree to that of tuberculoid (T x T) leucocytes in mixed leucocyte cultures (Godal *et al.*, 1971). The response to phytomitogens, as compared with allogeneic cells, has so far not been studied in individual patients.

*Production of lymphocyte factors.* The supernatant fluid from cultures of *Myco. leprae* with lymphocytes from patients with untreated lepromatous leprosy does not aggregate guineapig macrophages (MAF) (Talwar *et al.*, 1972) or affect their migration (MIF) (Katz *et al.*, 1971). Moreover, the migration of lepromatous leucocytes is not inhibited when they are cultured in the presence of *Myco. leprae* (Myrvang, 1972). Patients with tuberculoid leprosy give a positive response in all these tests.

*Humoral responses in leprosy*

*Immunogloublins.* Increased levels of IgG, IgA, and IgM are frequently found in cases of lepromatous leprosy and *erythema nodosum leprosum,* although many patients have values within normal limits. IgD levels are within normal limits but IgE levels are sometimes higher than those in control samples from the same areas. The levels are somewhat higher in lepromatous leprosy than in the tuberculoid form of the disease, although there is considerable overlap. The production of antibodies to unrelated antigens such as typhoid/paratyphoid vaccines appears to be normal in patients with tuberculoid and lepromatous leprosy (Sheagren *et al.*, 1969; Jha *et al.*, 1971).

*Antimycobacterial antibodies.* Circulating antibodies against the polysaccharide antigen shared by *Myco. leprae* and other mycobacteria have been demonstrated by immunodiffusion, immunofluorescence, and indirect haemagglutination tests. A very high proportion of patients with lepromatous leprosy and a minority of those with the tuberculoid form of the disease have these antimycobacterial antibodies, and the antibody levels are higher in the former than in the latter. Specific antibodies against *Myco. leprae* have also been demonstrated by indirect immunfluorescence with sera from patients with lepromatous disease absorbed with cardiolipin and mycobacterial polysaccharide (Abe *et al.*, 1972*b*). These antibodies seem to react with protein antigens of *Myco leprae,* because the reaction is greatly reduced by autoclaving or trypsin digestion of bacilli. The

antibodies compete with rabbit antibodies against "nodular extract" antigen as judged by immunofluorescence reactions.

The antibodies specifically reacting with *Myco. leprae* are found in IgG and IgM classes, but none has yet been found in the IgA (Abe *et al.*, 1972). It would be of special interest to know whether IgA antibodies to *Myco. leprae* are present in secretory fluids such as nasal fluid.

Although irradiated or ALS-treated thymectomized mice can produce antibodies against heterologous proteins, the specific antibody response of these immunosuppressed mice against *Myco. leprae* and its relationship with the development of disease are not yet fully clarified. Antibodies against *Myco. leprae* have not yet been demonstrable in the immunosuppressed mice.

It would be interesting to investigate, using the model disease in mice, the possibility that a humoral response against *Myco. leprae* may inhibit the development of cell-mediated immune responses. Some intact mice infected with *Myco. leprae* develop a lepromatous type of disease after a long incubation period, and antibody against mycobacterial polysaccharide antigens has been observed by immunodiffusion. The contrast with T-cell-deprived mice suggests that a T-cell helper effect may be required for the formation of antibody against mycobacterial antigens. The presence of antimycobacterial antibodies in patients with lepromatous leprosy suggests the presence of T-cell function.

*Autoimmunity.* Since mycobacteria exert adjuvant effects, they might be expected to stimulate the formation of many autoantibodies. There have been many reports of increased autoantibodies in patients with lepromatous leprosy, but the findings have not been uniform. Cryoglobulins are common in India and Italy but are rare in Japan and Malaysia. The incidence of antinuclear factors, rheumatoid factors, antithyroglobulin antibodies, and cold autoantibodies is raised in patients with lepromatous leprosy in some countries but not in others. False positive results of serological tests for syphilis are common, and may be related to the presence in *Myco. leprae* of a cardiolipin antigen. Cytotoxic antibodies that react with lymphocytes from a panel of normal subjects at 15°C and lyse these cells in the presence of complement have been found in patients with lepromatous and, to a lesser extent, tuberculoid leprosy. Further comparative observations on autoimmune reactions in leprosy would be of interest, especially the evaluation of the possible role of autoantibodies against lymphocytes in the generalized depression of T-cell function often found in the lepromatous form of the disease. Such antibodies should be tested against lymphocytes from the same patient rather than from other subjects. It would also be of interest to know whether autoimmune reactions against nervous tissue contribute to peripheral nerve injury in a manner analagous to experimental allergic neuritis.

*Allergic reactions and nerve damage in leprosy*

*Allergic reactions.* Two types of allergic reaction in leprosy were described in *Bull. Wld Hlth Org.* (1970)—*erythema nodosum leprosum* and reversal reactions.

*Erythema nodosum leprosum* occurs in highly bacilliferous patients (BL-LL) and both the local and systemic manifestations observed suggest that immune complexes are involved in the pathogenesis of these lesions. Moreover, IgG and $\beta$1c/$\beta$1a (components of C3) have been demonstrated in the lesions by immunofluorescence (Wemambu *et al.*, 1969). Further support for this concept has recently been provided by the finding of immune complexes in the serum of

such patients as demonstrated by precipitation with Clq (Moran *et al.,* 1972; Estrada-Parra, 1973). However, as a proportion of patients with lepromatous leprosy but without *erythema nodosum leprosum* also show complexes by this technique, further studies–including more detailed characterization of the antigen and the antibody in these complexes–are needed. There appears to be no difference in the immunoglobulin levels in patients with *erythema nodosum leprosum* and in those without this condition but with lepromatous leprosy; however, complement (C2 and C3) is raised (*Bull. Wld Hlth Org.,* 1970).

Reversal reactions may occur throughout the spectrum of leprosy, with the exception of the two polar groups as defined by the Ridley-Jopling classification. Such reactions often appear "spontaneously" in untreated patients, but may also be precipitated by chemotherapy against *Myco. leprae.* Some observations suggest that BCG vaccination or exposure to *Myco. tuberculosis* may precipitate such reactions.

Present evidence indicates that reversal reactions are due to a rapid increase of cell-mediated immune response to *Myco. leprae* for the following reasons: (1) reversal reactions are associated with a shift in histological classification, both in lesions and in lymph nodes, towards the tuberculoid end of the scale (Souza Lima and Cerqueira, 1946; Souza Lima and Rath de Souza, 1949; Souza Lima, 1953; Ridley, 1969; Turk and Waters, 1971); (2) patients with reversal reactions reveal strong responses to *Myco. leprae in vitro* as measured by lymphocyte transformation and leucocyte migration inhibition (Godal, 1972); and (3) mice with high bacillary loads develop, following injections of syngeneic lymphoid cells, changes in their lesions that resemble the reversal reaction in man (Rees, 1970; Gaugas *et al.,* 1971).

*Nerve damage.* Peripheral nerves are affected in all forms of leprosy and during allergic reactions. In tuberculoid leprosy the histological lesion in the nerve is the same as in the skin, but can also sometimes produce caseation ("abscess"), both resulting in destruction of nerve fibres with subsequent fibrosis. In lepromatous leprosy nerves are heavily infected with bacilli, mainly in Schwann and perineurial cells. Although the infected nerves are not damaged initially in lepromatous leprosy, neuritis eventually develops in a high proportion of such patients, and the perineurium is mainly affected. In patients developing reversal reactions or *erythema nodosum leprosum,* cellular changes corresponding to those in the skin occur in nerves, leading to severe damage to nerve fibres.

Experimental leprosy in the mouse also results ultimately (8-30 months) in nerve involvement and has provided a model for studying in detail the evolution of leprosy neuritis and the effect of "reversal reactions" on infected nerves (Rees and Weddell, 1968; Weddell *et al.,* 1971). Thus, in reversal reactions, oedema formation within the nerves is an important cause of nerve fibre destruction. In mice with leprosy the perineurial cells are frequently damaged and later there is also increased permeability of the capillaries in the nerves (Boddingius *et al.,* 1972).

The observations in the mouse suggest that leprosy neuritis may be due to a change in the endoneural environment following a defect in the blood and/or perineurial nerve barrier. Further comparative observations on human leprosy neuritis would be of interest, especially evidence of such a defect in man and for an immunological origin of the defect.

# Immunological Problems in Leprosy Research: 2

Part 2 of this Memorandum covers possible mechanisms of altered immune response in leprosy (including a tentative scheme to explain the possible genesis of the lepromatous lesion); genetic, nutritional, and hormonal factors; the possibility of vaccination; attempts at immunotherapy; and areas in which further research is needed. A detailed protocol for evaluating the effect of transfer factor in leprosy is included as an appendix.

## Possible Mechanisms of Altered Immune Responsiveness in Leprosy

*Depression of specific immunological responses*

*Humoral and cell-mediated immunity.* In complete immunological tolerance there is a failure to mount immune responses to a particular antigenic determinant together with a normal ability to respond to other antigens. This can be induced by exposure to a wide variety of antigens in high dosage or, in the case of soluble protein antigens, by the repeated administration of low doses. High-dose tolerance appears to involve the elimination or inactivation of both B and T cells with specificity for the specific antigen. In contrast, low-dose tolerance appears to involve the selective inactivation of specifically-reactive T cells leaving B-cell function intact. Immunological tolerance may not always be absolute, and various degrees of suppression of specific cell-mediated immune responses and specific antibody formation have been described. The duration of tolerance depends on the dose of antigen used to induce the unresponsive state.

Certain individual animals are genetically incapable of mounting an effective immune response to particular antigenic determinants. Genetic unresponsiveness is controlled by "immune response" (Ir) genes, which may be linked to genes controlling major histocompatibility antigens or to genes controlling immunoglobulin structure (e.g. allotypy of immunoglobulins). Other types of similar unresponsiveness may be under multifactorial genetic control.

*Selective suppression of cell-mediated immunity.* A form of partial immunological unresponsiveness that primarily affects T-lumphocyte function can result from the administration of protein or microbial antigens by a route or in a physical form that does not normally produce cell-mediated immunity. This phenomenon of antigen-mediated depression has been referred to as "immune deviation". While the mechanisms underlying this phenomenon have not been fully established, it is possible either that the T cells are selectively rendered tolerant or that prior induction of antibody deviates antigen away from engaging T cells. In contrast, "immunological enhancement" refers to the situation in which sensitized lymphocytes present in an individual are unable to effect cell-mediated immunity because of blocking by antibodies or antigen-antibody complexes. The simplest mechanism is that in which antibody or antigen-antibody complexes cover antigens on target cells, thereby rendering them inaccessible to sensitized T cells. These forms of immuno-depression can be differentiated by ascertaining the extent of T-cell desensitization and the ability of serum from immunized donors to block specific T-cell function *in vivo* or *in vitro*.

Desensitization of cell-mediated immunity can be achieved by the injection of antigen in large or repeated doses. It is believed that sensitized T cells are activated in the circulation, exhausted of their capacity to produce mediators, and

unable to respond when they encounter antigen in the tissues. Unless antigen is administered repeatedly, sensitization usually reappears.

In one type of immunodeficiency, and in some collagen autoimmune diseases, depression of T-cell function has been related to the presence of circulating lymphocytotoxic autoantibodies. In this situation, cell-mediated immune responses to a wide variety of antigens are suppressed.

*Selective suppression of antibody.* Under physiological conditions, the formation of specific antibody is under feedback control. This is mediated by IgG antibodies that selectively suppress the formation of IgG and IgM antibody directed against the same antigenic determinants. The immunization of animals with low doses of antigen can lead to the formation of small amounts of high-affinity antibody that can suppress antibody formation on subsequent exposure to larger amounts of the same antigen.

T cells not only exert a "helper" function in antibody formation but also exert selective feedback inhibition of specific antibody formation (e.g. allotype and autoantibody suppression).

*Antigenic competition.* There is experimental evidence that cell-mediated and humoral responses to a given antigen may be suppressed by the prior or simultaneous injection of unrelated antigens. This phenomenon is referred to as "antigenic competition" and is thought to result from the preemption of T and B cells or from competition for pathways of antigen processing in macrophages. Experiments have been described in which antigenic competition may take place between different determinants on the same antigenic macromolecule.

*Mechanisms by which specific immune responses are increased*

*Adjuvants.* The administration of antigen with adjuvants may increase antibody formation, cell-mediated immune responses, or both. Some adjuvants, such as alum or vitamin A, selectively increase antibody formation but not cell-mediated immunity. Killed mycobacteria in oil (Freund's complete adjuvant) or, under certain circumstances, BCG increase cell-mediated immunity to a greater extent than antibody formation, although this effect is not always predictable. Recent studies suggest that bacterial and other adjuvants such as poly-A: poly-U exert effects directly on macrophages and indirectly through the proliferation of T lymphocytes (Allison, 1973). Such adjuvant effect disappear in animals deprived of T lymphocytes. Certain adjuvants such as poly-A: poly-U added *in vitro* to leucocyte cultures increase reactions carried out by T cells, such as mixed lymphocyte reactions and responses to tuberculin.

While it would be of great importance to have adjuvants that could increase cell-mediated immunity in patients with lepromatous leprosy, the possible hazards involved in the use of adjuvants should be recognized. Among these are the danger of producing *erythema nodosum leprosum*, increasing antibody formation rather than cell-mediated immunity, and the possible precipitation of autoimmune complications, such as allergic neuritis.

*Composition and chemical modification of antigens.* The antigenic determinants recognized by T and B lymphocytes are often different. Certain antigens, such as chemical contact sensitizers, certain basic proteins of membranes, and schistosome egg antigens, preferentially stimulate cell-mediated immune responses. The chemical modification of antigens—e.g. the aceto-acetylation of proteins (Parish and Lieuw, 1972) or coupling with fatty acids—selectively stimulates cell-mediated immune responses rather than antibody

formation. Analogous modification of *Myco. leprae* or cross-reacting antigens might be used to stimulate cell-mediated immune responses in patients with leprosy.

The administration of very small doses of antigen, or of antigen-antibody complexes in the correct proportions, can also favour cell-mediated immunity rather than antibody formation. The route of administration may also affect the outcome. Chemical sensitizers induce cell-mediated immune responses when applied to the skin, whereas administration by the oral or intravenous route can prevent the eliciting of contact sensitivity on subsequent application to the skin.

*Lymphoid cell transfer.* The injection of viable lymphocytes from specifically sensitized donors into nonsensitized recipients of the same species can confer both specific cell-mediated immunity and enhanced specific antibody production on the recipient. The extent and duration of these responses is usually greater if donor and recipient are syngeneic. The ability of such recipients to manifest cell-mediated immunity (e.g. delayed hypersensitivity) is thought to depend on adequate survival of the donor's T cells; thus cell transfer of cell-mediated immunity is ineffective between animals of different species. The injection of both sensitized lymphoid cells and the specific sensitizing antigen may confer on syngeneic recipients on ability to give enhanced cellular and humoral responses to an unrelated antigen. This can also occur if sensitized or non-sensitized lymphoid cells are injected into allogeneic recipients; this phenomenon, termed the "allogeneic cell effect", is thought to be mediated by activation of the recipient's T cells and possibly macrophages also.

*Injection of sensitized lymphoid cell extracts.* Cell-free extracts of peripheral blood leucocytes from human subjects with delayed hypersensitivity to bacterial antigens can apparently confer a state of specific delayed hypersensitivity when injected into nonsensitized human subjects. Transferred sensitivity may persist for some weeks or months, and its duration is thought to be extended by the repeated injection of leucocyte extract. The active principle, termed "transfer factor", is found in RNA-rich dialysate fractions of human leucocyte extracts; dialysable human transfer factor is not active when injected into experimental animals, nor can such transfer factor be generated by species other than man. However, resistance to certain experimental tumours has been stimulated by the injection of macromolecular RNA derived from cells from specifically immunized donor animals. There is some doubt as to the specificity with which cell-mediated immunity is stimulated; thus an adjuvant-like effect of transfer factor (in man) or RNA preparations (in animals) has not been excluded.

### *Tentative scheme for the possible genesis of leprosy lesions*

Studies in mice suggest that an infection with *Myco. leprae* can be established with about five viable bacilli. After a long period these grow exponentially with a doubling time of 12-13 days for a limited period, after which the rate of growth declines, even in immunosuppressed mice. It is likely that the undetected early stages of the human infection are similar. Many infections probably show spontaneous cure before the number of organisms is sufficiently large to produce a clinically detectable lesion. Since tuberculoid lesions can show spontaneous cure, it seems reasonable to postulate that a microtuberculoid reaction is involved in the recovery from infection at the subclinical level, and the demonstration of specific lymphocyte transformation with *Myco. leprae* antigens in contacts of leprosy cases supports this view.

One possiblity is that in the absence of any immunological reaction the number of organisms can ultimately become sufficiently large to produce clinically detectable lesions. These would be the indeterminate cases, in which it may be postulated that lesions result from simple infiltration with macrophages, in a manner analogous to a foreign-body reaction without any immunological component. Infiltrating macrophages become infected and there may be local depigmentation.

Immune responses to *Myco. leprae* antigens somehow occur later in the course of bacterial multiplication. If there is effective cell-mediated immunity, a clinically apparent tuberculoid lesion develops while the number of organisms is relatively small. The predominant feature of such lesions is cell-mediated immunopathology.

There are two possible explanations for the evolution of a polar lepromatous lesion from an indeterminate one: (1) that as the number of infected macrophages increases the character of the lesion changes without any local allergic reactions; and (2) that there is a chronic antibody-mediated immunopathological reaction somewhat analogous to extrinsic allergic alveolitis in the lung. The generation of small amounts of immune complexes over a long period might facilitate the recruitment of macrophages to produce a pure macrophage granuloma with mild fibrogenesis. Experience with the lung shows that in chronic antibody-mediated immunopathology, bound immunoglobulin and complement are not usually detectable in lesions, so that it may be difficult to ascertain directly whether there is any immunopathological component in the lepromatous lesions.

Irrespective of its role in lepromatous lesions, antibody-mediated immunopathology probably makes an important contribution to *erythema nodosum leprosum*. Various possible explanations exist for the susceptibility of some but not other patients with lepromatous leprosy to this complication. One is that the quantity or nature of the antibodies against *Myco. leprae* antigens is different in patients who develop *erythema nodosum leprosum*. For instance, they might have antibodies belonging to a complement-fixing subclass of immunoglobulin, whereas insusceptible patients do not. Alternatively, susceptible patients might have IgE as well as IgG antibodies. Reactions of *Myco. leprae* antigen with IgE might increase vascular permeability, thereby promoting local accumulation of IgG or IgM antibodies, which on reaction with antigen might induce polymorph infiltration, degranulation, and other signs of Arthus-type hypersensitivity.

In borderline cases of leprosy, both cell-mediated and antibody-mediated immunopathological reactions may be present in varying degrees, depending on the position of the case in the range between the polar forms of the disease.

Immunopathological reactions are of special interest in relation to the mechanisms of nerve damage in leprosy. Cell-mediated immunopathological reactions are of major importance in the tuberculoid forms of the disease and during the course of reversal reactions. Chronic antibody-mediated immunopathology may play a role in stimulating fibrogenesis in peripheral nerves in chronic lepromatous disease. Antibody-mediated allergic reactions in a patient with *erythema nodosum leprosum* may cause acute aggravation of nerve damage.

It is useful to make a distinction between the presence of systemic immune responses, humoral and cell-mediated, and localizing factors that ensure that these are manifested at the sites of bacterial multiplication. Little is known about initial localizing factors in cell-mediated immunity, although once a local reaction occurs

it may be increased through autocatalytic events by which more specifically sensitized T cells enter reaction sites. Perhaps reactions to BCG or other antigens might be used to facilitate localization of sensitized T cells in lesions. The possibility should also be considered that antibody-mediated reactions may help to localize T cells that are reactive against *Myco. leprae* antigens. These concepts are illustrated in Fig. 1.

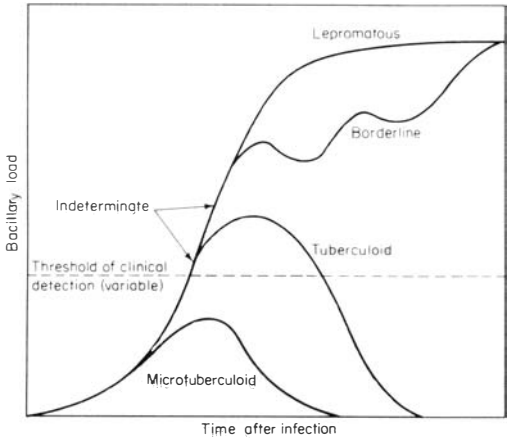


Fig. 1. Tentative scheme of the possible genesis of leprosy lesions.

*Evaluation of immunological mechanisms in lepromatous leprosy*

Of the possible mechanisms of selective unresponsiveness in lepromatous leprosy, complete tolerance of B and T cells to all antigens of *Myco. leprae* can be excluded, since high levels of antibodies to some antigens of *Myco. leprae* are frequently detectable in the sera of patients with lepromatous leprosy. However, it is still possible that complete tolerance is induced to a small number of *Myco. leprae* antigens that are important with respect to cell-mediated immunity.

Immunological enhancement appears unlikely since (1) serum from patients with lepromatous leprosy fails to inhibit the *in vitro* response of lymphocytes from patients with tuberculoid disease to *Myco. leprae*, and (2) lymphocytes from patients with lepromatous leprosy do not respond to *Myco. leprae* in normal human serum. The desensitization of cell-mediated immunity also appears unlikely because patients with lepromatous leprosy fail to respond to *Myco. leprae*, even when the antigenic load has been reduced or eliminated by treatment.

The available data do not permit a distinction between selective T-cell tolerance or antibody-induced deviation of T-cell sensitization.

## Genetic, Nutritional and Hormonal Factors

A recent study on 102 twin pairs indicated that among monozygotic twins there was a high concordant incidence (59.7%, 37/62) of leprosy in both siblings—i.e. both twins were affected. In the dizygotic pairs, the concordant incidence of leprosy was lower (20%, 8/40). These observations suggest that genetic constitution is a predisposing factor to leprosy.

These studies also showed that in 40% of monozygotic twins one member of the twin pair remained free of any clinical manifestation of the disease. Moreover, the type of leprosy in five identical twins was different. These results indicate that factors other than genetic composition—e.g. intensity and type of infection, nutritional status, and other systemic factors—may be important. Some recent studies have pointed to a notable effect of protein-calorie malnutrition on immune responses (WHO Scientific Group on Cell-Mediated Immunity and Resistance to Infection, 1973). Other systematic factors, such as hormones, have also been observed to affect lymphoid organs (Pandian and Talwar, 1971; Talwar, 1972).

Studies on the interaction of genetic, hormonal, nutritional, and other predisposing factors should be extended with a view to defining their role in the susceptibility of a subject to leprosy.

## Vaccination

*Vaccination in man*

The development of a specific vaccine for the prevention of leprosy in handicapped by the present inability to culture *Myco. leprae in vitro*, and vaccination efforts have been restricted to trials with BCG.

The United Kingdom Medical Research Council trial in Uganda (September 1960-September 1970) (Brown and Stone, 1966; Brown *et al.*, 1968*a*, *b*, 1969) was confined to child contacts and relatives of known cases of leprosy (BCG group, 8085; control group, 8065). By the end of the fourth follow-up examination (1970), 201 cases of leprosy had been diagnosed in the unvaccinated children of the primary (main) intake, and 41 cases in the BCG-vaccinated children. This represents a reduction in incidence of 80% attributable to the BCG vaccination. The efficacy of BCG does not appear to be associated with any of the following factors: sex, age at intake, and type of leprosy (lepromatous or non-lepromatous) in the index case. Findings also showed that there is a slight falling-off in the efficacy after the second follow-up among the children of the main intake.

In Karimui (New Guinea), a study has been carried out on about 5000 persons of all ages. The findings (Scott *et al.*, 1966; Russell *et al.*, 1968) indicated, with regard to leprosy incidence (1963-1969), that the efficacy of BCG vaccination was 46% for the total population, 47% for males (all ages), 47% for females (all ages), 44% for children aged 0-9 years, 56% for children aged 5-14 years, 56% for the age group 20-29 years and 25% for persons aged over 30 years.

The WHO trial in Burma, started in August 1964, is concerned with a child population, mainly not exposed at home (BCG group, 14,108; control group, 14,112) (Bechelli *et al.*, 1970). The findings up to the end of June 1971, which related to six annual follow-up examinations, were 285 cases of leprosy in the BCG group and 325 cases in the control group. The incidence rates in the age group 5-9 and 10-14 years were similar in the vaccinated and unvaccinated groups, irrespective of tuberculin reaction. However, in the group aged 0-4 years at intake, BCG-vaccinated children had a lower rate than those of the control group—a protection rate of 44.2%. The proportions of indeterminate, tuberculoid, and tuberculoid in reaction cases were similar in both trial groups.

The findings concerning the incidence in the three trials are strikingly different and relate only to early cases of leprosy. The interpretation of these results

requires further data on: forms of leprosy, bacterial status, lepromin reactivity, evolution of cases, and level of endemicity. Once this information is available, it should be possible to appraise the preventive effect of BCG in leprosy and the impact the vaccine may have on the trend of the disease.

*Experimental immunity in mice*

Protection is observed as a reduction in the number of *Myco. leprae* that grow in vaccinated mice. Heat-killed vaccines prepared from various mycobacterial cultures have been compared and *Myco. tuberculosis* has been found to confer the greatest degree of protection. Living BCG was at least as effective, and the effect was not much reduced when the BCG organisms were heat-killed. The route of administration has been studied with living BCG, the intravenous route being most effective, although the interdermal and intraperitoneal routes were not much inferior; a low dose given in the foot was sometimes highly effective. The degree of protection was dose-dependent and was still detectable with $1 \times 10^6$ BCG bacilli given intradermally. Vaccine given after challenge was effective.

Reinfection experiments with *Myco. leprae* given in a new site (the other footpad) have usually failed to indicate any immunity, and even though the first infection showed a plateau, the mice usually developed a second infection that was fully comparable to a primary one.

Mice experimentally inoculated with *Toxoplasma gondii* develop chronic infection with persisting antigen and delayed hypersensitivity. Their macrophages are activated, as demonstrated morphologically and by the ability of the macrophages to kill *Listeria.* Such mice have been found to be partially resistant to *Myco. leprae.* The immunity is increased if *Toxoplasma* antigen is given locally in the footpad.

## Attempts at Immunotherapy

Attempts at immunotherapy have consisted chiefly in the injection of peripheral leucocyte suspensions and of leucocyte extracts in an attempt to increase cell-mediated immunity in cases of lepromatous leprosy.

*Injection of allogeneic lymphocytes*

Three groups of workers have reported the intravenous injection of allogeneic lymphocytes into patients with lepromatous leprosy. Paradisi *et al.* (1969) observed the acquisition of Fernandez sensitivity in four of 13 patients who received leucocytes from normal healthy subjects with intense positive tuberculin reactions. Saha *et al.* (1971) injected, on three successive occasions, peripheral lymphocytes from healthy donors with strong Mitsuda sensitivity into four patients who showed dapsone intolerance; the patients showed clinical, histological, and bacteriological improvement. Improvement in clinical status was maintained for 2½ years. One patient developed Fernandez sensitivity after five months but none developed Mitsuda reactions (Saha *et al.*, 1972). In a third study Lim, Fusaro and Good (personal communication) treated 15 patients with lepromatous, mixed, or tuberculoid leprosy with 8-10 successive weekly injections of leucocytes from different healthy donors either presumed to be lepromin positive or shown to be lepromin negative and tuberculin negative. Each leucocyte infusion was given from a separate donor, and donors and recipients were deliberately mismatched to facilitate reactions with donor cells and to avoid early

immune elimination of the cells. Patients of all three groups showed clinical, histological, and bacterial improvement. The improved clinical status was maintained for four months. With respect to the prolonged response to leucocyte injections, the second and third of the reports noted above are similar.

*Infection of leucocyte extracts (transfer factor)*

Bullock *et al.* (1972) prepared dialysable leucocyte extracts from lepromin-sensitive healthy subjects and injected the preparations subcutaneously into nine patients with lepromatous disease on one occasion. Within seven days, six of the patients developed weak Fernandez reactions and experienced transient inflammation of leprous skin infiltrates. Lymphocyte transformation to *Myco. leprae* antigen remained negative and there was no long-term improvement in clinical status. Mittal *et al.* (unpublished data, 1972) prepared nondialysed leucocyte extracts from healthy Indian subjects and injected the extracts intravenously into four patients with lepromatous leprosy on three occasions at monthly intervals. During a 5-month period, two patients developed positive Mitsuda tests, but no clinical, histological, or bacteriological improvement was obtained.

## Areas for Further Research

(1) Animal experiments should be undertaken using killed mycobacteria and isolated mycobacterial antigens—e.g. "nodular extract" and cell walls—antigen being administered (e.g. intravenously, in low doses, by repeated feeding, and intranasally) so as to bring about preferential stimulation of antibody formation. Challenge with infectious mycobacteria and skin-test antigens would indicate whether prior antibody formation can suppress cell-mediated immune responses to these antigens as predicted by an immune deviation model.

(2) The transfer of lymphoid cells to non-immuno-suppressed "lepromatous" mice with high levels of *Myco. leprae* antibodies would indicate whether T cells can function in this situation. The occurrence of "reversal reactions" induced by the transferred cells would suggest a state of T-cell tolerance; the failure of the cells to induce such reactions would also be consistent with immune deviation.

(3) The class and subclass of antibodies to *Myco. leprae* in the serum, infected tissue, and nasal secretions of patients with all forms of the disease should be determined. This information may help to predict which lepromatous patients are more likely to develop *erythema nodosum leprosum*—e.g. whether only patients with antibodies in complement-fixing classes or subclasses are susceptible to this complication. Regional differences in susceptibility to the condition may be explicable on this basis. These studies may also help to clarify the role of local antibody formation in the immunopathology of leprosy. A search should be made for reaginic antibodies to *Myco. leprae* antigens. Likewise, the presence of such antibodies may contribute to susceptibility to *erythema nodosum leprosum*.

(4) It would be of interest to determine whether the failure of some patients with indeterminate leprosy to become lepromin positive following treatment is related to the level and class or subclass of antibodies before and following treatment. A correlation between the presence of such antibodies before or during treatment and failure to develop cell-mediated immunity would be most consistent with the immune deviation model.

(5) It is important to use modern techniques to characterize more fully the nature of the cellular defect in lepromatous leprosy. The number of lymphocytes,

the proportion of T cells (E rosettes) and B cells (EAC rosettes), and responsiveness in the lymphocyte transformation test to a battery of common antigen should be determined.

(6) Immunological techniques recently applied to leprosy (particularly the specific lymphocyte transformation test) should be widely employed (a) in field studies on the reactivity of contacts and non-contacts of patients with leprosy in a geographically-defined endemic leprosy area; and (b) in population samples in the three BCG trial areas.

(7) Further *in vitro* studies are needed on the interaction between lymphocytes and macrophages in relation to host resistance to *Myco leprae.* The studies should be designed so that the role of macrophages, lymphocytes, and products of activated lymphocytes in both the induction and expression of immunity may be separately analysed. It is recommended that recently established *in vitro* methods for studying lymphocyte-mediated modification of antibacterial activity of macrophages be used.

(8) It would be of interest to establish whether *in vitro* transformation of human lymphocytes by *Myco. leprae* antigens is due to T lymphocytes. Selective enrichment or depletion of T cells by E-rosette formation, antisera reacting specifically with T cells, or removal of B cells by immunoabsorbants would help to decide the point.

(9) A possible role for T-cell inhibition of T-cell reactivity in the lepromatous form of the disease might be evaluated in mixed lymphocyte cultures. Positive suppression would be expected if the leucocytes of an identical twin with tuberculoid leprosy were cultured in the presence of antigen together with leucocytes of the other twin with lepromatous leprosy. Sufficient numbers of identical twins who are discordant for leprosy type are available to allow this distinction to be made.

(10) The availability of concordant and discordant leprosy cases amongst identical twins should also provide a useful opportunity to test the hypothesis relating to specific and non-specific depression of cell-mediated immunity.

(11) The regulation of macrophage proliferation should be investigated in patients with various types of leprosy by measuring serum levels of colony-stimulating factor and its inhibitor. The agar-culture technique should be used to determine the incidence and proliferative activity of macrophage precursors in the bone marrow, blood, and local lesions of patients with leprosy.

(12) Further studies are needed on the biochemical mechanisms by which macrophages kill and degrade intracellular *Myco. leprae.* Attempts should be made to establish whether human macrophages activated by products of activated lymphocytes or colony-stimulating factor are able to inhibit the multiplication of *Myco. leprae.* The use of colony-stimulating factor and newly-developed media may facilitate the establishment of long-term human mononuclear phagocyte cultures which are required for this purpose. Such studies may help to explain the mechanism by which the body eliminates mycobacteria in tuberculoid leprosy, although not lepromatous leprosy.

(13) It would be of interest to determine whether a primary nasal infection is associated with a greater risk of developing lepromatous leprosy than a primary skin infection. In practice, nasal infection could be sought in contacts of patients with lepromatous leprosy and in early indeterminate cases. The incidence of persisting lepromin negativity in such cases as compared with those lacking evidence of nasal infection could be determined. Such studies may indicate

whether routes of infection other than through the skin predispose to lower cell-mediated immunity.

(14) It would be of interest to follow the lepromin reactivity *in vivo* and *in vitro* of babies born of mothers with lepromatous leprosy where *Myco. leprae* had been found in the placenta or cord blood. While little is known about the induction of immunological tolerance in neonate humans, the failure of such children to become Mitsuda positive would be consistent with the immunological tolerance model.

(15) While assessing the role of BCG in protection against leprosy it would be of interest to compare the effect of BCG inoculated directly into skin lesions with that of inoculation at other sites. Intra-lesion inoculation may help to localize T-cell effects at the sites of lesions.

(16) Further studies on autoimmunity in patients with leprosy from different populations should be undertaken, with special reference to evaluating the possible role of autoimmune reactions in nerve damage.

(17) The possible presence of autoantibodies that are cytotoxic for lymphocytes should be explored, particularly by comparison between patients with lepromatous leprosy showing unimpaired cell-mediated immunity to unrelated antigens and those with a generalized nonspecific depression of cell-mediated immunity.

* *
*

Abe, M. *National Institute of Leprosy Research, Tokyo, Japan.*
Allison, A. C. *Clinical Research Centre, Northwick Park Hospital, Harrow, England.*
Bechelli, L. M. *Ribeirão Prête Faculty of Medicine, University of São Paulo, Brazil.*
Bloom, B. R. *Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, N.Y., U.S.A.*
Dumonde, D. C. *The Mathilda and Terence Kennedy Institute of Rheumatology, London, England.*
Godal, T. *Armauer Hansen Research Institute, Addis Ababa, Ethiopia.*
Goodman, H. C. *World Health Organization, Geneva, Switzerland.*
Hopwood, B. E. C. *The Wellcome Trust, London, England.*
Metcalf, D. *Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Parkville, Victoria, Australia.*
Olakowski, T. *WHO Regional Office for South-East Asia, New Delhi, India.*
Pervuhin, J. V. *Research Institute for the Study of Leprosy, Astrakhan, U.S.S.R.*
Rees, R. J. W. *National Institute for Medical Research, London, England.*
Saha, K. *Department of Bacteriology, G. B. Pant Hospital, New Delhi, India.*
Sansarricq, H. *World Health Organization, Geneva, Switzerland.*
Shepard, C. C. *Leprosy and Rickettsial Diseases Unit, Centre for Disease Control, Atlanta, Ga., U.S.A.*
Talwar, G. P. *Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India.*
Torrigiani, G. *World Health Organization, Geneva, Switzerland.*

## Résumé

### Problèmes d'Immunologie dans la Recherche sur la Lèpre

Le présent document fait le point des connaissances actuelles en matière d'immunologie de la lèpre et expose les progrès accomplis dans ce domaine depuis la publication d'une revue similaire en 1970.

L'épreuve la plus anciennement utilisée pour évaluer l'état immunitaire de l'organisme à

l'égard de *Myco. leprae* est la réaction tardive à la lépromine. Les renseignements les plus significatifs donnés par cette réaction sont indiqués.

Des méthodes plus récentes permettant d'analyser l'état de l'immunité à support cellulaire, telles la transformation lymphoblastique on l'inhibition de la migration des leucocytes, ont été appliquées à l'étude de la lèpre. Il est de plus en plus démontré que l'immunité à support cellulaire est déprimée dans certaines formes de lèpre. Le niveau de l'immunité à support cellulaire baisse du type polaire tuberculoïde au type polaire lépromateux selon une gradation continue correspondant aux divers degrés d'atteinte clinique et bactériologique. La dépression est à son maximum chez les malades lépromateux non traités. Cette dépression de l'immunité à support cellulaire possède deux composantes, l'une spécifique de *Myco. leprae*, l'autre non spécifique. En ce qui concerne la composante spécifique, il est probable qu'elle se développe par un mécanisme de "déviation immunitaire" mais il n'est pas possible pour l'instant de préciser si la tolérance à *Myco. leprae* est sélectivement induite au niveau des lymphocytes T ou si des anticorps circulants prélablement formés réagissent avec les antigènes, les empêchant ainsi d'entrer en rapport avec ces lymphocytes T.

On a pu montrer l'existence d'anticorps circulants dirigés contre un antigène polysaccharidique commun à *Myco. leprae* et à d'autres mycobactéries. Ces anticorps sont trouvés chez la plupart des lépromateux et chez quelques cas tuberculoïdes. Des anticorps spécifiques de *Myco. leprae* appartenant aux IgG et aux IgM ont été également mis en évidence dans les sérums de lépromateux.

La présence d'autoanticorps chez les malades lépromateux a été maintes fois signalée, mais les différents autoanticorps ont une fréquence variable suivant les pays.

Des crises d'érythème noueux lépreux apparaissent chez certains malades lépromateux très bacillifères. L'hypothèse selon laquelle elles sont déclenchées par la formation d'immunocomplexes, suggérée par les manifestations cliniques, est étayées par la mise en évidence d'IgG et de certains composants du complément au niveau des lésions, ainsi que d'immunocomplexes dans le sérum. Toutefois, on observe aussi des immunocomplexes dans le sérum de lépromateux ne présentant pas d'érythème noueux lépreux. De nouvelles recherches sont de ce fait nécessaires pour déterminer l'origine précise et le mécanisme exact des crises d'érythème noueux lépreux.

Quant à la réaction d'inversion (*reversal reaction*), elle est liée à une rapide augmentation de l'immunité à support cellulaire comme le montrent les modifications histologiques observées au niveau des lésions et des ganglions lymphatiques, l'apparition de tests positifs pour ce type d'immunité et certains faits observés chez la souris d'expérience.

Il est possible que des mécanismes immunologiques jouent, dans le développement des lésions nerveuses de la lèpre, un rôle beaucoup plus large que celui avanché jusqu'ici.

Certains faits suggèrent que des facteurs génétiques prédisposent à contracter la lèpre, tandis que le rôle possible de facteurs nutritionnels et hormonaux demande à être étudié.

Alors que les résultats de trois essais contrôlés sur le terrain du vaccin BCG dans la prévention de la lèpre sont jusqu'ici nettement différents entre eux, chez la souris le vaccin BCG et d'autres vaccins mycobactériens confèrent un certain degré de protection contre *Myco. leprae* inoculé dans le coussinet plantaire.

Le dernier chapitre formule des recommandations touchant aux nouvelles recherches qui devraient être effectuées. La majorité des problèmes proposés à l'attention des chercheurs ont trait aux rôles respectifs des différents facteurs intervenant pour créer les situations immunologiques diverses rencontrées dans le cadre de la lèpre. D'autres sont des problèmes d'épidémiologie, de pathogénie, ou même de bactériologie auxquelles certaines techniques immunologiques modernes pourront apporter des solutions.

## References

Abe, M. (1970). *Int. J. Lepr.* **38**, 113.
Abe, M. (1971). *Int. J. Lepr.* **39**, 87.
Abe, M. *et al.* (1972). *Report of the 7th U.S.-Japan Cooperative Medical Science Program*, Tokyo.
Abe, M. *et al.* (1973). *Int. J. Lepr.* In press.
Allison, A. C. (1973). In *Ciba Foundation Symposium on Immunopotentiation*. London: Churchill.
Bechelli, L. M. *et al.* (1970). *Bull. Wld Hlth Org.* **42**, 235.

Bodingius, J. *et al.* (1972). *Nature, Lond.* **237**, 190.
Böyum, A. (1968). *Scand. J. clin. lab. Invest.* **21**, Suppl.
Brown, J. A. K. and Stone, M. M. (1966). *Brit. Med. J.* **1**, 7.
Brown, J. A. K. *et al.* (1968*a*). *Brit. Med. J.* **1**, 24.
Brown, J. A. K. *et al.* (1968*b*). In *Abstracts of papers of the Ninth Int. Leprosy Congress,* London, p. 58.
Brown, J. A. K. *et al.* (1969). *Lepr. Rev.* **40**, 3.
Bullock, W. E. (1968). *New Engl. J. Med.* **278**, 298.
Bullock, W. E. and Fasal, P. (1971). *J. Immunol.* **106**, 888.
Bullock, W. E. *et al.* (1972). *New Engl. J. Med.* **287**, 1053.
*Bull. Wld Hlth Org.* (1970). **43**, 879.
Dharmendra (1942). *Leprosy in India* **14**, 122.
Estrada-Parra, S. (1973). In *Proceedings of the Indian Medical Research Council Symposium on Immunology of Leprosy* (in press).
Fernandez, J. M. M. (1940). *Int. J. Lepr.* **8**, 1.
Gaugas, J. M. *et al.* (1971). *Int. J. Lepr.* **39**, 388.
Godal, T. (1973). In *Proceedings of the Indian Medical Research Council Symposium on Immunology of Leprosy* (in press).
Godal, T. *et al.* (1971). *Clin. exp. Immunol.* **9**, 821.
Godal, T. *et al.* (1972). *Scand. J. Immunol.* **1**, 311.
Godal, T. *et al.* (1973). *Int. J. Lepr.* (in press).
Han, S. H. *et al.* (1971). *Int. J. Lepr.* **39**, 1.
Hayashi, F. (1933). *Int. J. Lepr.* **1**, 31.
Jha, P. *et al.* (1971). *Int. J. Lepr.* **39**, 1.
Katz, S. I. *et al.* (1971). *Arch. Derm.* **103**, 358.
Mehra, V. L. *et al.* (1972). *Clin. exp. Immunol.* **12**, 205.
Metcalf, D. (1972). *Aust. J. exp. Biol. med. Sci.* **50**, 547.
Metcalf, D. and Moore, M. A. S. (1971). *Haemopoietic Cells.* Amsterdam: North Holland.
Mitsuda, K. (1923). *III Conf. Int. Lèpre, Strasbourg,* p. 219.
Moran, C. J. *et al.* (1972). *Lancet* **2**, 572.
Myrvang, B. (1973). In *Proceedings of the Indian Medical Research Council Symposium on Immunology of Leprosy* (in press).
Nelson, D. S. (1970). *Macrophages in Immunity.* Amsterdam: North Holland.
Pandian, M. R. and Talwar, G. P. (1971). *J. exp. Med.* **134**, 1095.
Paradisi, E. R. *et al.* (1969). *New Engl. J. Med.* **280**, 859.
Parish, C. R. and Lieuw, F. Y. (1972). *J. exp. Med.* **135**, 298.
Rees, R. J. W. (1964). *Prog. Allergy* 8, 224.
Rees, R. J. W. (1970). *Proc. roy. Soc. Med.* **63**, 1060.
Rees, R. J. W. and Weddell, A. G. M. (1968). *Ann. N.Y. Acad. Sci.* **154**, 214.
Ridley, D. S. (1969). *Lepr. Rev.* **40**, 77.
Ridley, D. S. and Jopling, W. H. (1966). *Int. J. Lepr.* **34**, 225.
Russell, D. A. *et al.* (1968). In *Abstracts of Papers of the Ninth Int. Leprosy Congress*, London, p. 58.
Saha, K. *et al.* (1971). *Aspects of Allergy and Applied Immunology* **5**, 83.
Saha, K. *et al.* (1972). In *Proceedings of the Indian Council of Medical Research Symposium on Immonology of Leprosy* (in press).
Scott, G. C. *et al.* (1966). *Int. J. Lepr.* **34**, 139.
Sheagren, J. N. *et al.* (1969). *Ann. intern. Med.* **70**, 295.
Shepard, C. C. and McRae, D. H. (1965). *J. Bacteriol.* **89**, 365.
Shepard, C. C. and McRae, D. H. (1971). *Infec. Immun.* **3**, 121.
Souza Lima, L. de (1953). *Estado atual da terapêutica da Lepra.* São Paulo: Servico Nacional de Lepra.
Souza Lima, L. de and Cerqueira, G. C. (1946). *IInd Cong. Panamericana de Lepra, 1946, Rio de Janeiro,* **1**, 9.
Souza Lima, L. de and Rath de Souza, P. (1949). *Int. J. Lepr.* **17**, 19.
Talwar, G. P. (1972). *Int. J. Biochem.* **3**, 39.
Talwar, G. P. *et al.* (1972). *Clin. exp. Immunol.* **12**, 195.
Turk, J. L. and Bryceson, A. D. M. (1971). *Adv. Immunol.* **13**, 209.
Turk, J. L. and Waters, M. F. R. (1971). *Clin. exp. Immunol.* 8, 363.
Weddell, A. C. M. *et al.* (1971). *J. Path. Bact.* **104**, 77.

Wemambu, S. N. C. *et al.* (1969). *Lancet* 2, 933.
WHO Scientific Group on Cell-Mediated Immunity and Resistance to Infection (1973). *Wld Hlth Org. techn. Rep. Ser.* No. 519.

## Appendix

### Protocol for Evaluating Transfer Factor in Leprosy

*Purpose*

(1) To ascertain whether transfer factor engenders immune reactivity to *Myco. leprae* antigens *in vivo* and *in vitro* and, concomitantly, whether it changes the pathological classification and/or increases the rate of clearance of bacteria.

(2) To determine whether transfer factor prepared from donors with a high degree of hypersensitivity to *Myco. leprae* is more efficacious than that prepared from non-sensitive donors. Once this determination has been made, it would not be necessary to include transfer factor from both sensitized and non-sensitized donors in future protocols.

*Rationale.* (a) The ultimate goal of immunotherapy in patients with lepromatous leprosy is both to terminate their infection and to prevent recurrence. However, evaluation of the effects of transfer factor in terms of marked clinical change would require a large study with a long period of observation. A more limited trial to assess the effects of transfer factor on the immunological and histopathological status of these patients is therefore recommended.

(b) While most clinical trials of transfer factor have used material prepared from highly sensitized donors, the possibilities that it acts non-specifically and that when prepared from normal donors may be equally effective have not been explored. There are a number of instances in which transfer factor from a donor positive to several antigens has conveyed only some reactivities to recipients, particularly to those antigens with which they have had contact. There are other instances in which recipients have shown conversion in response to antigens to which the donor was negative. These findings justify testing the possibility that transfer factor acts primarily as a non-specific immunological adjuvant. Were this demonstrated to be the case—i.e. that transfer factor from non-sensitive donors acted as effectively as that from positive donors—then the availability of transfer factor would be vastly increased since blood-bank blood from normal donors could be used in many situations. This might also lead to the development of synthetic adjuvants for use in man.

*Patient groups*

(1) *Clinical-histopathological status.* It is recommended that previously untreated patients with lepromatous leprosy be studied, and that transfer factor from sensitized donors and chemotherapy be given at the same time. The patients should be in the BL/LL and LL categories.

Controls would be matched for classification, and for locale, age, and sex where possible. There would be two control groups, one receiving non-sensitive transfer factor and the other no transfer factor but a slightly irritating placebo. All patients would receive chemotherapy. Patient groups could be established from an initial large pool, or consecutive patients could be assigned to each group. Between 12 and 20 patients should be included in each group.

Histopathological and bacteriological specimens and pretreatment sera must be saved for later reference, and the morphological and bacteriological indices should be determined.

(2) *Immunological status.* Recipients should be negative in the lepromin skin test (Fernandez and Mitsuda) and in the lymphocyte transformation test. They should also give normal phyto haemagglutinin (PHA) responses, or at least two-thirds of the average normal stimulation, and should be reactive to at least one other common antigen—e.g. PPD, streptokinase-streptodornase, *Candida*, or mumps, *in vivo* or *in vitro*.

The IgG levels should be less than 20 g/l.

*Rationale.* (a) For this protocol the untreated BL/LL (LI) group has been selected. There is a relatively low incidence of these patients spontaneously moving up the scale within the test period and *erythema nodosum leprosum* reactions would possibly be lower in this group. While patients with indeterminate leprosy (I) have a sufficiently low bacterial load and immune response to be considered for such a study, since only a minority convert to the lepromatous form, large numbers of recipients would have to be included in order to obtain a statistically significant result. Further, it would not be ethical to withhold chemotherapy in this group for any considerable period, and chemotherapy alone would terminate the disease and eliminate bacilli in the skin in such patients. The disadvantages of using patients in the borderline lepromatous group for study are that the frequency of reversal reactions with chemotherapy is greater and that too high a percentage may move up the scale with chemotherapy alone.

(b) It is essential that patients selected for this trial have adequate T-lymphocyte function—i.e. react well to PHA and unrelated antigens. In addition, they must be specifically nonresponsive to *Myco leprae*.

(c) Normal serum levels of IgG are preferable to minimize possible inhibitory effects of antibodies to mycobacteria on development of cell-mediated immunity and to diminish the likelihood of *erythema nodosum leprosum*.

*Transfer factor donors*

(1) *Sensitized donors* must have strong positive skin tests (Fernandez) and show lymphocyte transformation *in vitro*, preferably to a single pool of *Myco. leprae* bacilli. Ideally, they should all be negative for a known control antigen—e.g. histoplasmin, coccidioiodin.

(2) *Nonsensitized donors* must be negative *in vitro* to a single test pool of *Myco. leprae* and *in vivo* to BCG, and to PPD (second strength). Where possible, Fernandez tests can be given to these donors retrospectively, i.e. after the blood leucocytes have been collected. Ideally they should be strongly positive to the second test antigen, e.g. histoplasmin, coccidioiodin.

(3) All donors should be in good physical condition and negative for hepatitis B antigen.

(4) The transfer factor should be prepared in a limited number of centres using either fresh or frozen leucocytes sent by mail. The final pools of transfer factor must be tested for sterility and must be negative for hepatitis B antigen in the most sensitive test available. In addition biochemical analysis should be made. Pools should be coded before being sent to the appropriate clinical centres for testing.

*Rationale.* (a) The protocol is designed using sensitivity to two antigens so that donors sensitive to *Myco. leprae* would be unexposed and negative to one other

antigen, and control donors would be negative for reactivity to *Myco leprae* but positive to the second antigen. The recipients should be negative to both antigens. In this way the specificity of transfer factor for two antigens can be tested simultaneously.

(b) The study should be double blind—i.e. the transfer factor preparations must be coded and the code not broken until the end of the study.

(c) The lepromin-positive donors should preferably be recovered tuberculoid patients (TT or BT/TT) since they have demonstrated adequate levels of true cell-mediated immunity, although highly-positive contacts could be employed.

*Treatment of recipients*

(1) An essential prerequisite for such a study is the availability of close medical supervision and hospital facilities, so that the necessary treatment can be provided should reactions occur.

(2) Coded transfer factor should be administered subcutaneously at monthly intervals for 12 months. The code should not be broken until the end of the study.

(3) Recipients should receive the equivalent of $400 \times 10^6$ leucocytes (obtained from approximately 500 ml of blood) but the dose may be increased if reactions are not encountered, or lowered if reactions are severe.

All patients, whether in hospital or out-patients, must be seen at least weekly. If any patients respond to several injections with substantial clinical deterioration, they must be dropped from the study.

*Rationale.* In previous studies some lepromatous leprosy recipients of transfer factor showed "flare" reversal reactions, and, in at least one case, *erythema nodosum leprosum.* Since in studies in patients with other conditions the effect of transfer factor has been transient, it seems likely that in patients with lepromatous leprosy repeated doses would be required for histopathological changes and clearance of bacteria from the tissues to be observed.

*Evaluation of the results*

(1) All patients should be examined weekly for clinical status, special attention being given to neurological and ophthalmological symptoms.*

(2) At least three skin tests to lepromin and to the unrelated antigen should be made approximately 8-10 days after the first administration of transfer factor and 6 and 12 months. Biopsies should be taken from these sites.

(3) The lymphocyte transformation test or other *in vitro* tests for cell-mediated immunity should be performed at monthly intervals.

(4) At least two skin biopsies should be taken after 6 and 12 months. In addition biopsies should be obtained from affected areas whenever reactions are observed. Several centres should be available for expert histopathological evaluation of tissue samples. In addition serum samples must be obtained at the beginning of the study, and at 6 and 12 months, as well as during any periods of *erythema nodosum leprosum* reaction that may occur.

(5) Bacteriological indices and morphological indices should be taken from 6-12 areas on a monthly basis. Again, several centres should be available for expert evaluation of slides.

* The necessary clinical protocol can be obtained, on request, from Immunology, World Health Organization, 1211 Geneva 27, Switzerland.

(6) After one year, the results, including clinical, histopathological, and immunological data, should be reviewed and evaluated by experts convened by WHO.

(7) The clinical status of all patients should be followed up for at least one additional year.