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Cell-Mediated Immunological Processes in Leprosy*

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A large number of organisms such as viruses, protozoa, helminths, fungi and bacteria, especially mycobacteria, need cell-mediated immunological processes for their elimination. As well as being involved in protection, cell-mediated immunological processes are also involved in a number of allergic reactions to products derived from mycobacteria. Cell-mediated immunological processes can be demonstrated by a number of *in vitro* reactions. Leprosy can present with a wide range of different clinical patterns. The clinical spectrum of leprosy can be shown to depend on the degree of the cell-mediated immune response of the host against *Mycobacterium leprae*. Thus in tuberculoid leprosy there is a high degree of cell-mediated immune response whereas in lepromatous leprosy such a response is virtually absent. There appears to be a constitutional predisposition to lepromatous leprosy. In addition to a specific loss of cell-mediated immune response against *Myco. leprae*, there is also a non-specific drop in the ability of patients with lepromatous leprosy to show other aspects of cell-mediated immune response, e.g. contact sensitivity and skin homograft rejection. There is also a relative impairment of the ability of lymphocytes to react *in vitro*. Lymph nodes from patients with lepromatous leprosy show a deficiency in those areas associated with the development of cell-mediated immune responses. The article includes a discussion on the possible causes of deficiencies in cell-mediated immune responses in lepromatous leprosy.

THE NATURE OF THE IMMUNOLOGICAL RESPONSE

The body can respond to an antigenic stimulus in 2 fundamentally different ways. The end result of each is the production of a factor which will interact specifically with the antigen, setting off a train of events leading either to the elimination of the antigen from the body or the induction of an inflammatory reaction. In one case, the specific antigen-reacting factor is a serum protein, an immunoglobulin molecule, which we can refer to as a "humoral antibody". In the other, there is the production of lymphocytes which have a similar property of reacting specifically with the antigen. The production of these specifically sensitized lymphocytes is known as the "cell-mediated immune response" Although both humoral antibody and sensitized lymphocytes react specifically with the antigen, the subsequent train of events, which each leads to, is different. Thus, humoral antibodies may react with antigen on cell surfaces to produce anaphylactic phenomena (asthma, hay-fever, urticaria, etc.) or react with complement and be deposited in the walls of blood vessles, causing vasculitis, glomerulonephritis, arthritis, uveitis, etc. The interaction between humoral antibodies and the antigens of certain bacteria, such as pneumococci or *Salmonella*, may increase the phagocytosis and elimination of these organisms from the body. However, a large number of micro-organisms cannot be eliminated by the action of humoral antibodies alone. These organisms require the interaction of sensitized lymphocytes and macrophages for their elimination. It is becoming more apparent that cell-mediated immune processes are necessary for the protection of the body from viruses, protozoa, helminths, fungi and many bacteria, including organisms as different as the treponemata, *Brucella* and the mycobacteria.

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The need of cell-mediated immune processes for the elimination of mycobacteria has been known for many years. Much of the early work on this subject was performed on *Mycobacterium tuberculosis*, and started with the observation of Koch (1891), who demonstrated that if virulent tubercle bacilli were injected subcutaneously into a guinea-pig which was already infected, the animal reacted against the organisms differently from an uninfected animal. The rejection of the organisms was associated with a massive inflammation which developed at the site of injection. A similar type of inflammation could be induced if the guinea-pig, or a similarly infected human subject, was inoculated intradermally with a culture filtrate of the microorganisms (the tuberculin reaction). Subsequently, it has been shown that both the elimination of the organisms from the body and the inflammatory reaction produced by a sensitized person against the organisms or culture fluid are caused by the reaction with sensitized lymphocytes and macrophages, cell-mediated immunological reactions.

Both the cell mediated immune response and the humoral-antibody response are functions of the central lymphoid tissues of the body. An immunological response may be considered to have a number of phases. There is first contact with antigen and this is followed by a phase of cellular proliferation, lymphocytes in the case of cell-mediated immune responses, and plasma cells for humoral-antibody production. Cellular proliferation takes place mainly in the lymph nodes, spleen or the lymphoid tissue of the gut. As a result of this, either sensitized lymphocytes or humoral antibody are present in a sufficient concentration to react with the foreign substances (antigens) on subsequent contact.

Production of humoral antibody takes place in the plasma cells which are found at the cortico-medullary junction or in medullary cords of lymph nodes or in the red pulp of the spleen, and is usually associated with the formation of germinal centres in the cortex of the lymph nodes or the splenic white pulp. The proliferation of lymphocytes associated with cell-mediated immune reponses takes place in what is often referred to as the paracortical area of lymph nodes or in the area of the white pulp of the spleen around the central arteriole. The paracortical area of the lymph node is found situated between the true cortex and the medulla and between the lymph follicles and germinal centres (Oort and Turk, 1965).

Both this area and the areas of lymphocytes in the white pulp of the spleen round the central arteriole have been found to be dependent on thymus integrity in late foetal and neonatal life (Parrott, de Sousa and East, 1966). The differentiation of lymphocytes, probably sensitized by contact with antigen in the periphery (Medawar, 1958), into large pyroninophilic cells (immunoblasts), which can be shown to divide into other small lymphocytes takes place in the paracortical area of lymph nodes as part of a cell-mediated immune response (Oort and Turk, 1965). Removal of the thymus in neonatal life depletes these areas of lymphocytes and is associated with an inability to manifest such a response (Miller, 1961).

Babies born with congenital thymic hypoplasia are found to be unable to produce a cell-mediated immunological response and the paracortical areas of their lymph nodes and the area of the white pulp of the spleen round the central arteriole are deficient in lymphocytes and replaced by reticulo-histiocytes. A similar appearance is found in the lymphoid tissue of animals treated with anti-lymphocyte serum (Turk and Willoughby, 1967) which also prevents the development of cell-mediated immune responses. Neonatally thymectomized animals, babies with congenital thymic aplasia and animals treated with anti-lymphocyte sera are able to produce humoral antibodies, have normal levels of immunoglobulins in their serum and have normal numbers of plasma cells in their lymphoid tissue.

As well as being involved in the protection of the body against a wide range of microorganisms, cell-mediated immune responses are involved in a number of allergic responses to substances produced by a number of microorganisms. These reactions, like the tuberculin reaction mentioned above, take 24 to 48 hr to

develop and include the "reaction of immunity" to vaccinia virus, the mumps skin test, skin reactions to streptococcal antigens such as those found in mixtures of streptokinase and streptodornase, to fungal antigens such as coccidiomycin and histoplasmin, and to protozoal antigens such as the Montenegro test for leishmaniasis. The reaction of rejection of a skin homograft is another example of a cell-mediated immune response, so also is contact sensitivity to simple chemical agents such as 2,4-dinitrochlorobenzene (DNCB) which attach readily to skin proteins.

Following contact between sensitized lymphocytes and antigen in the periphery, a soluble substance is released which has a direct effect on macrophages. The nature of the substances on or within the lymphocytes, with which the antigen reacts specifically, is not known. However, many people feel that, because the reaction resembles humoral antibody reactions so closely, the reacting molecule must be related in some way to an immunoglobulin molecule. Thus it has been suggested that it might be a polypeptide chain such as the Fd fragment of the immunoglobulin molecule, or some similar peptide chain carrying an amino-acid sequence specific to the particular antigen for which it is coded to react.

The soluble substances released by the incubation of specifically sensitized lymphocytes with specific antigen are in the molecular-weight range of approximately 70,000. These substances can be shown to have a direct effect on the cell surface of macrophages, decreasing the surface electrostatic charge, increasing their adhesibility both to each other and to similarly charged surfaces, and decreasing their permeability to certain organic chemicals with molecular weights from 600 to 900, such as diphosphopyridine nucleotide and triphosphopyridine nucleotide. The relationship of these changes to the development of an inflammation in the skin with erythema, induration and a mononuclear cell infiltrate is not yet completely clear. However, the soluble mediator can be isolated after culture of specifically sensitized lymphocytes with specific antigen and, when this is injected intradermally, it will produce an inflammatory reaction which has many of the characteristics of a tuberculin reaction (Bennett and Bloom, 1968; Pick *et al.*, 1969).

The effect of the interaction between specifically sensitized lymphocytes and antigen on the ability of macrophages to eliminate mycobacteria has also not been completely elucidated. However, Berthrong and Hamilton (1959) have observed the intracellular growth of these organisms in macrophages. Normally, tubercle bacilli multiply intracellularly and form cords which engorge the cells so that they eventually burst. Macrophages from immune animals do not allow the bacilli to grow intracellularly in this manner, and they survive longer than non-immune macrophages although they may have still ingested the organisms. At the same time it has been shown that macrophages from immune animals have a high rate of dehydrogenase enzyme activity (Allison, Zapposodi and Lurie, 1962), and an increase in lysosomal enzymes (acid phosphatase, *b*-glucuronidase and cathepsin). Lysosomes of macrophages from animals immune to mycobacteria are also probably more fragile than normal, as indicated by the release of considerable amounts of lysosomal enzymes, especially *b*-glucuronidase, into the plasma following the intravenous injection of an endotoxin (Saito and Suter, 1965*a*, *b*). The little work that has been published on the phagocytosis of *Myco. leprae* by macrophages suggests, as will be discussed later, that in tuberculoid leprosy, where there is a high degree of cell-mediated immunity, macrophages can readily lyse phagocytosed *Myco. leprae*, whereas in lepromatous leprosy, where cell-mediated immunity is deficient, the organisms are still phagocytosed but not lysed. Moreover, the lysis of *Myco. leprae* by macrophages from patients with lepromatous leprosy is immunologically specific (Beiguelman, 1967).

A few *in vitro* reactions have been studied extensively over the last few years in relation to cell-mediated immunity. One of these is related to the increased adhesiveness of macrophages following the release of a soluble substance after the interaction of specifically sensitized lympho-

cytes with specific antigen. The macrophages are grown in tissue culture in small capillary tubes. Under normal conditions, they migrate from these tubes in a fan-like manner. However, if they are in close contact with antigen and specifically sensitized lymphocytes, or incubated together with the soluble substances released from specifically sensitized lymphocytes incubated with antigen, they fail to migrate from the tubes. For this reason, the soluble substance released by incubation of specifically sensitized cells with antigen is referred to as the "migration inhibition factor" and the reaction is referred to as "inhibition of macrophage migration *in vitro*".

The other reaction performed in tissue culture is more related to the changes which occur to lymphocytes in lymph nodes following first contact with antigen, during the development of a cell-mediated immunological response. It will be remembered that after first contact with antigen, probably in the periphery, lymphocytes differentiate into large cells in the paracortical area of lymph nodes, prior to division into other lymphocytes. These large cells which are synthesizing DNA, and which can thus incorporate radioactively labelled thymidine, are referred to as "immunoblasts". Similar transformation of small lymphocytes into "blast" cells can be produced in tissue culture *in vitro*. However, this occurs most frequently if the person or animal has had a previous exposure to the antigen. Under these conditions, no more than 10% of the small lymphocytes are transformed. There are a number of substances which will regularly transform 80 to 90% of the small lymphocytes, without any known, previous exposure of the individual to the substance. The substances, or non-specific mitogens, include phytohaemagglutinin (PHA), a similar mitogen from pokeweed (*Phytolacca americana*), streptolysin S and the filtrate of *Staphylococcus aureus* cultures. Similar transformation can be obtained by treating the lymphocytes with an anti-immunoglobulin allotype serum. Although the relationship between lymphocyte transformation with specific antigen and cell-mediated immune response or humoral antibody production is highly controversial, there is a direct relationship between the ability of lymphocytes to be transformed by PHA and the ability of individuals to show cell-mediated immune phenomena. Thus an inability both to be sensitized to show contact sensitivity to DNCB and to have their lymphocytes transformed by PHA occurs in patients with congenital thymic aplasia, Hodgkin's disease and sarcoidosis.

THE SPECTRUM OF CLINICAL LEPROSY

It has been recognized for a considerable time that leprosy may present clinically in a wide variety of forms. The pleomorphism of the presentation of the disease was first rationalized by the separation of those forms of the disease where the presentation was one of peripheral nerve damage from the form where the lesions mainly took the form of nodular deposits in the skin. The association of anaesthetic patches in the skin with a surrounding raised erythematous edge or with a large erythematous plaque led to the description of these lesions as "tuberculoid" owing to their occasionally resembling the lesions of lupus vulgaris. The nodular lesions are generally referred to as "lepromatous" as they are typical of this form of disease. Thus leprosy has become divided into 2 polar forms, "tuberculoid" leprosy and "lepromatous" leprosy. However, more recently many descriptions have been made of patients who may show, at one and the same time, manifestations of both polar forms of the disease. This form of leprosy has been referred to as "dimorphous" or "borderline" leprosy. Occasionally, patients may present with just one or more hypopigmented macules. These cases may be difficult to classify into one or other of the more florid forms of the disease, and are referred to as "indeterminate" leprosy. However, the disease rarely stays in this form and may develop with the production of more typical tuberculoid or lepromatous lesions. It is thought that the indeterminate state is probably the earliest form of the disease and that many patients pass through it before they develop the typical manifestions of tuberculoid or lepromatous leprosy.

Thus, clinically, leprosy forms a spectrum between the 2 polar forms—tuberculoid and lepromatous leprosy—and patients may present with varying lesions depending on the degree to which tuberculoid or the lepromatous elements contribute to the disease. Apart from those with the 2 polar forms, patients may vary from time to time in the degree to which the 2 elements contribute to the disease.

Thus the disease may often be considered to be "mobile". A patient with leprosy at the tuberculoid end of the spectrum may move to a more borderline condition, or a patient with borderline leprosy may move to the lepromatous end of the spectrum as a result of an exacerbation of the disease. Conversely, on sulfone therapy a patient may move from the lepromatous end of the leprosy scale towards a more borderline position, or a patient with borderline leprosy may move towards a tuberculoid position.

The lesions in the 2 polar forms of leprosy show marked histological differences. In tuberculoid leprosy, the granuloma is invariably infiltrated with a dense collection of small lymphocytes and, despite well-developed collections of histiocytic cells (macrophages), *Myco. leprae* are not found on standard methods of examination. This is in marked contrast to the appearance in lepromatous leprosy where small lymphocytes are absent or scanty and the macrophages are packed with leprosy bacilli which may form globi. The tissues throughout the body may be infiltrated with macrophages containing proliferating bacilli which, it appears, cannot be eliminated. As with the clinical state, the histological appearance shows a broad spectrum between the 2 polar forms, the main variable being the proportion of small lymphocytes present in the lesion, and the intensity of parasitism of the macrophages by mycobacteria. Also, in parallel with the trend towards the lepromatous end of the scale as the disease worsens, lesions may show a decrease in the number of small lymphocytes and an increase in the number of bacilli present. Similarly with treatment, as the clinical condition swings back towards the tuberculoid end of the spectrum there will be an increased infiltration with small lymphocytes as the bacterial load gradually decreases.

In an attempt to provide a scheme whereby the patient can be assigned at any time to the correct place on the spectrum, Ridley and Jopling (1966) attempted to characterize a number of points on the leprosy spectrum, understanding, however, that the disease in a number of patients was mobile and that the patients could only be assigned to one place on the scale at a particular point in time. By closely correlating the clinical state of the patient with the histological appearance of the lesions, Ridley and Jopling were able to define 5 points on the spectrum. The 2 polar forms of the disease at the tuberculoid and lepromatous ends of the spectrum are referred to as TT and LL, respectively, while the central position, dimorphous or borderline leprosy, is referred to as BB. They added 2 other points, BT between TT and BB, and BL between BB and LL. It was recognized that patients with single-lesion TT rarely, if ever, swing forward across the spectrum, and patients with true polar LL also rarely swing back across the spectrum. However, within the range BT–BL there could be considerable variation from year to year. In south-east Asia, lepromatous leprosy appears to be more labile than it is in other parts of the world and patients have a form of lepromatous leprosy which is far less stable than that found in LL patients in other parts of the world, presenting histologically with a slightly different picture in which the granuloma consists of undifferentiated histocytes, often with a fibrocytic appearance. These patients, intermediate between BL and LL with a potentiality to revert to BL on treatment, are sometimes referred to as LI (Ridley and Waters, personal communication). It must, however, be emphasized that the points TT, BT, BB, BL, LI and LL are arbitary points on what is a spectrum of disease. Patients can be at any particular time at points intermediate between the 5 marker points, i.e. TT/BT, BT/BB, BB/BL, BL/LI or LI/LL, depending on the state of their disease at that time.

THE IMPORTANCE OF THE SPECTRUM CONCEPT IN ASSESSING THE STATE OF RESISTANCE OF THE PATIENT TO HIS DISEASE

The correlation between the lepromin test and the state of clinical leprosy has been accepted for many years. Thus patients with tuberculoid leprosy are well known to be strongly lepromin-positive, while those at the lepromatous end of the scale are lepromin-negative. The presence of a positive lepromin test in a patient with indeterminate leprosy would indicate that he is more likely to develop tuberculoid leprosy, whereas a negative lepromin test would indicate a considerable risk of lepromatous leprosy developing.

At this stage it is profitable to digress slightly and discuss the nature and importance of the lepromin test. The antigen used is an extract of lepromatous tissue containing particulate material, especially particulate mycobacteria, as well as antigens derived from the human tissue from which the extract is derived. The reaction appears in 2 phases: a typical, delayed hypersensitivity reaction—the Fernandez reaction—maximal 48 to 72 hr after intradermal inoculation, and a papular granulomatous reaction—the Mitsuda reaction—which develops 3 weeks after inoculation. It is the Mitsuda reaction which is used by most leprologists to indicate whether a patient with leprosy is at the tuberculoid end of the scale. Although this reaction takes place as long as 3 weeks after inoculation, and is not a typical delayed hypersensitivity reaction, it is probably evidence of a high degree of cell-mediated immunity directed against the mycobacterial antigens. As the inoculated material is particulate, it is retained in the tissues for a long period and does not diffuse always as rapidly as does tuberculin. However, it may be that in the form in which it is injected the antigen is not readily available to react with the sensitized lymphocytes of a cell-mediated immune process and possibly the 3-week latent period is the time taken for the mycobacterial antigens to be exposed and made available, perhaps by tissue enzymes.

The lepromin test is not in itself an indication of infection with *Myco. leprae*, but indicates that the patient has had previous contact and has been immunized with these antigens which are probably common to a number of mycobacteria. Normal subjects can be immunized to produce positive lepromin tests by repeated intradermal injections of the killed antigen. However, it does indicate that the patients are capable of mounting a strong cell-mediated immune reaction against *Myco. leprae* associated with marked lymphocytic infiltration.

A positive lepromin test and the extensive lymphocytic infiltration in the lesions of patients with tuberculoid leprosy of the TT type indicate that these patients have a high degree of cell-mediated immunity directed against *Myco. leprae*. This means that they have in their circulation sensitized lymphocytes capable of activating macrophages and interacting with *Myco. leprae* antigens. This would account for their lesions having the appearance of typical cell-mediated allergic reactions (i.e. prolonged erythema, induration and infiltration with small lymphocytes). It would also account for the fact that mycobacteria are absent from the lesions. Thus they also have a high degree of cell-mediated *immunity* and are capable of eliminating *Myco. leprae* from the body, although they over-react with an intense allergic reaction to the antigens at the same time.

In tuberculoid leprosy of the BT type lymphocytic infiltration is of the same degree as that in TT leprosy and the extent of the allergic reaction in the skin is as intense. However, more than one lesion is present and mycobacteria can be found in the lesion, indicating that the degree of cell-mediated immunity is less, so that not all organisms are immediately eliminated. The lepromin test is also not so intensely positive as in the polar form (TT). Throughout the BB and BL forms, cell-mediated immunity appears to be increasingly less as indicated by the negative lepromin test, decreasing lymphocytic infiltration in the lesions and increasing numbers of mycobacteria that can be found.

This loss of cell-mediated immunity increases

so that in the LI or polar LL forms of leprosy there may be no lymphocytic infiltration and the mycobacteria proliferate throughout the body as though the body possessed no defence mechanisms towards the organisms at all. Mycobacteria are found growing in colonies and forming globi within the macrophages in an apparently undisturbed manner.

Thus the spectrum of clinical leprosy from TT to LL appears to reflect a massive cell-mediated immune response at the one extreme to a complete absence of cell-mediated immunity at the other. The main problems to be discussed in understanding the various manifestations of leprosy are why in some patients there is this high degree of cell-mediated immunity and in others cell-mediated immunity is absent, why some patients starting with lesions indicating a high degree of immunity can develop lesions indicating a progressive loss of immunity, and why some patients with little or no immunity can, on chemotherapeutic treatment, develop lesions of a different type, indicating that they are regaining a certain degree of immunity against the organisms. This latter state may be particularly distressing since, at the same time as they begin to eliminate organisms from their body, they will start to develop allergic reactions, affecting not only the skin but also the nerves, of a cell-mediated immune type directed against residual antigen. The resulting disease may not only be temporarily more severe than their original lepromatous state, but also may result in permanent scarring or nerve damage.

A CONSTITUTIONAL PREDISPOSITION TO LEPROMATOUS LEPROSY

Lepromatous leprosy seems not to occur in a fixed proportion of leprosy infections. In a population in which the rate of leprosy infection is decreasing there is not a parallel fall in the incidence of lepromatous leprosy. It has therefore been suggested by Newell (1966) that the development of lepromatous leprosy in an infected person is a host-determined characteristic that is present in a fixed proportion of all people everywhere.

Evidence that leprosy in its various forms occurred more frequently in certain families in endemic areas has been cited by a number of workers since it was first demonstrated by Danielsen and Boeck in 1848. Jamison and Vollum (1968) have recently cited evidence which suggests that leprosy occurs in families in which there is a naturally occurring weakness in their ability to develop a cell-mediated immune response. When tuberculin-negative children with a family history of leprosy were vaccinated with an avirulent strain of mycobacterium containing antigens which cross-reacted with *Myco. tuberculosis* (vole tuberculosis vaccine) only 18% were converted to tuberculin positivity, whereas 90% of children who came from families where there was no history of leprosy were converted to tuberculin positivity by the vaccine. This would indicate that children from families in which leprosy occurs are less able to develop a strong cell-mediated immune response, than children from families in which leprosy does not occur.

A similar relation between weakness of an ability to show a cell-mediated immune response and the development of leprosy can be shown in experimental animals. Under normal conditions it is only possible for *Myco. leprae* to proliferate locally and for a limited number of generations when inoculated into mice and other rodents, and after a period of 8 months, the organisms are rejected by the body. However, if adolescent mice are thymectomized and irradiated with 900 R, a process known to suppress the ability of the body to develop a cell-mediated immune response, the mycobacteria are able to spread throughout the body and produce a disease resembling leprosy in man (Rees *et al.*, 1967). The signs include nodular swellings of the skin similar to those seen in patients with lepromatous leprosy. If the mice received lymphoid tissue from animals with a normal state of cell-mediated immunity, the infection was far less severe than in those animals which had been thymectomized and irradiated but had not received lymphoid cell replacement. If animals were allowed to develop an infection like lepromatous leprosy and were then given an

inoculation of lymphocytes the nodular swellings became inflamed and subsided, after which the lesions developed a condition resembling that seen in borderline leprosy with nerve damage (Rees and Weddell, 1968). This would indicate that in experimental animals, a condition resembling lepromatous leprosy can be produced by eliminating cell-mediated immunity, and that the lepromatous state can swing towards the borderline state if cell-mediated immunity is restored partially by a transfusion of viable lymphocytes capable of mounting an immune reaction against *Myco. leprae.*

This suggests also that the constitutional defects predisposing to the development of lepromatous leprosy might be a weakness in thymus function in the young person, or an equivalent defect in the adult.

THE ABILITY TO SHOW OTHER ASPECTS OF CELL-MEDIATED IMMUNITY IN LEPROMATOUS LEPROSY

A generalized depression of the ability to mount a cell-mediated immune response has been demonstrated recently, by a number of authors, in patients with lepromatous leprosy (Waldorf *et al.*, 1966; Bullock, 1968). The general disability to show delayed hypersensitivity reactions was demonstrated by an inability of patients with lepromatous leprosy to become sensitized with DNCB (Waldorf *et al.*, 1966). Altogether, 75% of patients with lepromatous leprosy without erythema nodosum leprosum failed to be sensitized to DNCB whereas only 1 out of 5 patients with dimorphous (borderline) leprosy failed to be sensitized in this way; the technique used to assess whether sensitivity had developed was to skin-test the patients with 100 and 50 μg of DNCB in acetone. The result is curious since it has been found that the application of 100 μg of DNCB in acetone can produce a non-specific inflammatory reaction in unsensitized individuals (Turk and Waters, 1969). Moreover, 6 out of the 17 patients who could not be sensitized with DNCB were shown at the same time to be able to mount a cell-mediated immune reaction to tuberculin. As no record is given of what proportion of patients reacted to the 100-μg dose only and what proportion to both the 100- and 50-μg doses, this report shows some unsatisfactory features. The authors also found it difficult to explain why a similar state of anergy or inability to be sensitized with DNCB did not occur in patients in the same part of the lepromatous spectrum but who were having attacks of erythema nodosum leprosum. Also, it is difficult to interpret these results because the patients were only classified broadly into lepromatous or dimorphous leprosy without any assessment as to whether they were moving in the clinical spectrum of leprosy. We do not know whether the patients classified as lepromatous had the polar LL form of the disease or a form between LL and BB.

In another report by Bullock (1968), the Ridley-Jopling scale (TT-LL) was used to place the patients more accurately according to the clinical and histological state of their disease. As well as pre-existing sensitivity to lepromin, tuberculin, *Candida* and trichophytin, the ability of patients to become actively sensitized was assessed by their response to picryl chloride. Picryl chloride is a much weaker sensitizer than DNCB in experimental animals. It is known that DNCB will sensitize some 90% of normal individuals, and Bullock (1968) was able to sensitize 28 out of 30 normal people with this agent. Although patients in this series were originally classified according to the Ridley-Jopling scale, patients with TT and BT leprosy are referred to as tuberculoid and patients with BL and LL as lepromatous. In the patients with lepromatous leprosy who had been under treatment for less than 18 months, 70% could not be sensitized with picryl chloride; however a discrepancy again exists because only 50% of these patients were tuberculin-negative. Moreover, 10% of the patients were lepromin-positive, suggesting that they were closer to the borderline or tuberculoid end of the spectrum than the other patients in this group.

In a second group of lepromatous patients who had been treated for longer than 18 months, only 47% could not be sensitized with picryl

chloride. In this group, 40% were lepromin positive. A further problem in interpreting this result is that as many as 55% of the patients with tuberculoid leprosy failed to be sensitized with the picryl chloride. There appears to be no logical explanation why patients with tuberculoid leprosy fail to be sensitized to picryl chloride when 90% of controls can be sensitized easily. One possible thought is that the 2 populations are not identical and that the controls come from a population which is more readily sensitized to picryl chloride than the group from which the patients with tuberculoid leprosy are derived. One explanation that Bullock (1968) himself has suggested is that in tuberculoid leprosy cell-mediated immunity is deviated so strongly to produce the reaction against *Myco. leprae* that no reserves are available to react with other antigens. The report of increased immunological activity following therapy in patients with lepromatous leprosy is interesting and has been observed in other groups of patients (Waters, personal communication). However, difficulty is obtained in interpreting these results as BL and LL patients are grouped together and it would be important to know whether increased ability to be sensitized to picryl chloride was associated with clinical evidence of an increase in the immune state, such as a movement across the immunological spectrum of the disease from LL to BL.

The finding of patients who were tuberculin-sensitive but unable to be sensitized to DNCB, poses an interesting problem. These patients could have a degree of cell-mediated immunity high enough to respond to antigens to which they were exposed before they developed leprosy, but insufficient to respond while they had their leprosy. Another possibility is that contact-sensitizing agents provide a weaker antigenic stimulus than bacterial antigens. In recent experiments (Turk and Waters, 1969) it has been found that patients with leprosy who could not be sensitized to DNCB could, however, be sensitized to develop delayed hypersensitivity to keyhole-limpet haemocyanin (KLH). KLH provides a more powerful antigenic stimulus than DNCB which acts by modifying the body's own proteins. The inability to respond to DNCB does not therefore indicate a complete anergy in respect to cell-mediated immunity, but suggests that this property is only somewhat weakened.

IN VITRO REACTIVITY OF LYMPHOCYTES FROM LEPROMATOUS PATIENTS

The failure of lymphocytes from patients with impaired cell-mediated immunity, by non-specific mitogens such as PHA, has been described in a number of diseases. These include primary thymic dysplasia, Hodgkin's disease, sarcoidosis, Sjögren's disease and primary biliary cirrhosis. It is possible that in these conditions the lymphocytes which are capable of being stimulated are those that are under the control of the thymus in neonatal life and are thus the same class of lymphocytes that can be stimulated to proliferate into "sensitized lymphocytes" in a cell-mediated immune response. As has been discussed above, considerable evidence is accumulating that there is an impaired ability of patients with lepromatous leprosy to manifest a wide range of cell-mediated immune reactions. These include contact sensitivity to simple chemical sensitizers and delayed hypersensitivity to antigens from *Candida* and trichophytin. Moreover, experiments by Job and Karat (personal communication, cited by Hart and Rees, 1967) in 4 patients with lepromatous leprosy indicate that these patients show prolonged survival of allogeneic skin grafts. In normal cultures of lymphocytes, approximately 80% of the cells can be transformed into lymphoblastoid type cells by the addition of PHA. Dierks and Shepard (1968) found that the cells from 8 active lepromatous patients had a low response to PHA (only 10% of the cells being transformed by this mitogen). Of these patients, 4 had erythema nodosum leprosum and this did not affect the response of the patients. In another series, 3 patients with active lepromatous leprosy were found to have a markedly diminished response. One surprising result in this series

was that 3 patients with tuberculoid leprosy also had a diminished response to PHA. The reason why patients with lepromatous leprosy fail to be stimulated with PHA and show a diminished ability to manifest delayed hypersensitivity reactions, could be explained as being due to a general failure to manifest cell-mediated immune reactions. This might be genetic as suggested by Jamison and Vollum (1968). However, other causes should be considered, such as the changes which are seen in the lymphoid organs of such patients which will be discussed in another section. Whether these changes themselves are also hereditary and due to thymus dysfunction will also be discussed.

More difficult to understand is the diminished responsiveness of the lymphocytes of patients with tuberculoid leprosy to respond to PHA. This may be associated with the diminished ability of these patients to develop delayed hypersensitivity to picryl chloride. One suggestion that has been put forward (Bullock, personal communication) is that the lymphoid tissue of such patients is responding so strongly to antigens from *Myco. leprae* that there is no residual activity to respond to other antigens. This would explain an inability to respond to an antigen, but it is difficult to see how this effect could cause an inability of the lymphocytes to respond to non-specific mitogenic stimulation with PHA.

Extremely interesting experiments have been described by Bullock and Fasal (1968). In these experiments, a depression of DNA synthesis, indicative of the transformation of lymphocytes into blast cells was found in lymphocytes from patients with lepromatous leprosy when exposed to PHA and streptolysin O (SLO) which also acts as a mitogen probably through its antigenic properties. A similar depression in the transformation of lymphocytes of patients with tuberculoid leprosy was found, as was described by Dierks and Shepard (1968). However, one of the most fascinating aspects of this investigation was that the depression of response to SLO was found only when the lymphocytes from lepromatous patients were suspended in autologous plasma. When the cells were suspended in homologous plasma from normal subjects no depression of activity was found. When the cells of normal subjects were suspended in the plasma of some lepromatous subjects depression in the ability of these cells to be transformed by SLO was observed. However, plasma from lepromatous patients did not suppress the response of lymphocytes from normal subjects to PHA. Thus the plasma from some patients with lepromatous leprosy contains a factor which can inhibit the response of normal lymphocytes to SLO. Much might be learned about the depression of cell-mediated immunity in leprosy if the nature of this factor could be discovered.

A similar phenomenon has been described by Melli *et al.* (1968) in patients treated with heterologous anti-lymphocyte serum. After 14 to 21 days of treatment, there was a severe depression of the response of the patients' lymphocytes to PHA. However, this only occurred if the cells were cultured in autologous plasma. In the presence of homologous plasma the response of the cells was normal. Correspondingly, the plasma of patients treated in this way would inhibit the ability of PHA to transform lymphocytes from normal people. Melli *et al.* (1968) considered, reasonably, that the factor present in the plasma of patients treated with anti-lymphocytic serum, which inhibited the transformation of lymphocytes by PHA, was in fact an anti-lymphocyte antibody. It would thus appear that a factor is present in the plasma of both patients with lepromatous leprosy and those treated with anti-lymphocytic serum which could inhibit the response of normal lymphocytes by non-specific mitogens. In both these cases it should be easy to find out whether the factor involved is an immunoglobulin, and thus an antibody. If an anti-lymphocytic antibody is present in the circulation of patients with lepromatous leprosy, this could have considerable implications. It is now well known that anti-lymphocytic antibodies act specifically by suppressing the circulating pool of long-lived small lymphocytes, influenced by the thymus in neonatal life, which are necessary for the

normal manifestation of a cell-mediated immunological reaction. Treatment of animals with anti-lymphocytic antibody has an effect very similar to thymectomy in neonatal life, in depleting the paracortical area of lymph nodes and the corresponding area of the spleen of these small lymphocytes, without affecting the lymphocytes in the lymph follicles, thought to be of direct bone-marrow origin, the germinal centres and the plasma cells in the medulla, involved in humoral antibodies production. Thus the presence of an anti-lymphocytic antibody in the circulation selectively inhibits cell-mediated immune processes without affecting the production of most types of circulating antibody. If an anti-lymphocytic antibody was present in the circulation, this could account for the anergy in respect of cell-mediated immunity in certain patients with lepromatous leprosy. How could such an antibody develop? Such an antibody would be an "autoantibody". Autoantibodies of a wide variety have been found in patients with lepromatous leprosy; they include the rheumatoid factor, thyroglobulin autoantibodies, antinuclear factor and the Wassermann antibody which is an autoantibody directed against heart muscle (Bonomo and Dammacco, 1968). The presence of these different autoantibodies may be due to the exposure of hidden antigenic groups as a result of tissue damage caused by the presence of such large amounts of microorganisms throughout the body. A similar wide spectrum of autoantibodies is found in a number of other chronic infective diseases such as tuberculosis and syphilis. In experimental animals the production of autoantibodies can be stimulated by the injection of an emulsion of homologous tissues in oil containing mycobacteria, thus reproducing the antigenic stimulus which the body receives from its own tissues in certain chronic infective diseases.

Thus an important step forward in understanding the mechanism of the loss of cell-mediated immunity in patients with lepromatous leprosy will be the characterization of the factor which inhibits the transformation of lymphocytes by SLO in the plasma of some of these patients.

IN VITRO EXAMINATION OF MACROPHAGE ACTIVITY IN LEPROSY

Although much work has been done on the behaviour of macrophages in experimental animals with tuberculosis, little study has been made of macrophage activity in patients with leprosy. Beiguelman (1967) has developed a technique for testing the lysogenic ability of macrophages for *Myco. leprae*. He observed the behaviour of macrophages from leprosy patients maintained in a tissue-culture medium after the addition of the dead leprosy bacilli. The macrophages from both lepromatous and tuberculoid patients actively phagocytosed the dead leprosy bacilli; the macrophages from the tuberculoid patients completely lysed the ingested bacilli, so that they became free of lipids. However, the macrophages from the lepromatous patients were unable to lyse the organisms and became transformed into typical lepra cells. Their cytoplasm was found to contain numerous bacilli and droplets of lipid material which stained readily with Sudan Black. A similar study was reported by Hanks (1947, pp. 21, 31, 38) who cultured phagocytic cells morphologically resembling fibroblasts from patients with leprosy. Those from patients with tuberculoid leprosy were able to lyse the organisms, whereas the cells from patients with lepromatous leprosy degenerated and released the organisms free into the medium. Although unable to lyse *Myco. leprae*, cultured macrophages from patients with lepromatous leprosy have been reported to be able to lyse *Myco. lepraemurium* and *Myco. tuberculosis* (Beiguelman, 1967).

The inhibition of migration of macrophages *in vitro* in the presence of sensitized lymphocytes and antigen is now a technique regularly used in the study of cell-mediated immunity. This technique, developed originally in experimental animals by Rich and Lewis in 1932 using tissue explants, has been adapted to free-cell suspensions by George and Vaughan (1962). More recently, methods have been described where this technique can be adapted for use with human tissues. In one of these methods (Thor, 1967; Thor and Dray, 1967) human lymph-node cells are cultured as a free suspension for 72 hr

prior to being placed in the capillary tubes. The lymph-node cells then grow out in a fan-like manner, similar to that seen in cultures of guinea-pig macrophages. In the presence of specific antigen they are inhibited from migrating.

Another modification is that described by Søberg and Bendixen (1967). In this modification, peripheral blood leucocytes are used in the capillary tubes and inhibition of the migration by specific antigen. Both adaptations of the leucocyte migration technique appear to correlate well with the presence of delayed hypersensitivity. The first technique has the disadvantage that it uses lymph-node cells and needs biopsy, whereas the second technique uses peripheral leucocytes. Moreover, the second technique has been performed with particulate organisms, especially *Brucella abortus*. The development of the cells' ability to be inhibited from migrating in the presence of specific antigen correlates well with the known course in time of the development of cellular, rather than humoral, immunity (Søberg, 1968).

The technique of Søberg and Bendixen (1967) lends itself as an *in vitro* approach which could be used to investigate cellular immunity against *Myco. leprae* in humans. The antigen could be killed *Myco. leprae*, similar to that used by Beiguelman (1967) to investigate macrophage activity. The technique of Thor (1967) is more difficult in that it uses lymph-node cells which are more difficult to obtain than peripheral blood leucocytes and also entails a 72 hr culture of the cells as a free-cell suspension, with the associated risk of any long-term tissue culture.

In further experiments, Thor *et al.* (1968) incubated peripheral blood lymphocytes with soluble antigen for 24 to 48 hr, then added the supernatant to a suspension of guinea-pig peritoneal macrophages in capillary tubes. The incubation of specifically sensitized human lymphocytes with the specific antigen causes the release of a migration inhibition factor similar to that described by Bennett and Bloom (1968) which inhibits the migration of the guinea-pig macrophages. This technique, although also using peripheral blood cells, is difficult and involves cultivation of the lymphocytes prior to the release of the factor; being a 2-stage cultivation procedure, it is not an easy technique but could be adapted to leprosy research under carefully controlled experimental conditions.

Extensive research on cell-mediated immunity *in vitro* is thus recommended along 2 lines. The first, using the leucocyte-inhibition technique of Søberg and Bendixen (1967) or Thor *et al.* (1968), should give information on the presence of sensitized lymphocytes capable of secreting a migration inhibition factor after reaction with *Myco. leprae* or soluble antigens derived from the organisms. This substance can be presumed to act on leucocyte cell surfaces causing increased stickiness of these cells. The second approach should be an extension of the work of Beiguelman (1967) to investigate what it is that causes the macrophages of tuberculoid leprosy patients to lyse *Myco. leprae* after phagocytosis, whereas the macrophages from lepromatous patients cannot lyse the organisms. It could perhaps be shown that there was inhibition of leucocyte migration by *Myco. leprae* using the blood from tuberculoid leprosy patients and that *Myco. leprae* fails to inhibit the migration of leucocytes from lepromatous leprosy patients. Moreover, a soluble substance could be obtained from the culture supernatants of leucocytes from tuberculoid leprosy patients with *Myco. leprae*, which might be able to stimulate the macrophages from patients with lepromatous leprosy to lyse *Myco. leprae*.

THE HISTOLOGICAL APPEARANCE OF LYMPHOID TISSUE FROM PATIENTS WITH LEPROSY

As has been mentioned above, the lymph nodes from experimental animals treated in various ways to suppress cell-mediated immunity show a particular histological appearance. Neonatal thymectomy or treatment with antilymphocyte serum causes a marked depletion of lymphocytes from the paracortical areas of the lymph nodes and these cells are replaced by pale-staining reticulo-histiocytes. The germinal centres and

their marginal cuff of small lymphocytes are unaffected by this process, as also are the plasma cells at the cortico-medullary junction and in the medullary cords. A similar picture is found in the lymphoid tissue of children with congenital thymic aplasia or with the Wiskott-Aldrich syndrome which is also associated with a failure on the part of the patient to show cell-mediated immune reponses. However, lymph follicles, germinal centre formation and plasma cell proliferation in the medulla, which are associated with humoral antibody formation are unaffected by this process.

In a recent study, a similar histological pattern has been found in the lymph nodes of patients with lepromatous leprosy (Turk and Waters, 1968). The lymphocytes in the paracortical area of the lymph nodes were almost completely replaced by reticulo-histiocytes. These cells were morphologically similar to the cells seen replacing the lymphocytes in the paracortical area of lymph nodes from the guinea-pigs treated with anti-lymphocyte serum (Turk and Willoughby, 1967). These cells also had striking phagocytic activity and in advanced cases they were seen to have ingested large numbers of *Myco. leprae*. However, this appears to be a secondary phenomenon as in cases that were nearer the borderline part of the spectrum, often there were no bacilli within these cells. The infiltration was confined exclusively to the paracortical areas of the lymph nodes and can be considered as due to drainage of these cells from areas of histiocytic infiltration in the periphery. The germinal centres were normal and their marginal zone of small lymphocytes was not decreased. The medullary cords of the lymph nodes were unaffected and contained large numbers of plasma cells. In borderline leprosy there was some infiltration of the paracortical areas with these histiocytic cells but there were considerable numbers of small lymphocytes still present. In one case, where the biopsy specimen had been taken after treatment for 7 months (during which the patient had moved clinically from the lepromatous end of the spectrum to borderline leprosy, indicating that he had regained a certain degree of cell-mediated immunity) there was evidence of the beginning of repopulation of the paracortical areas with small lymphocytes. Some of the capillaries in the paracortical area were dilated and were surrounded by a cuff of small lymphocytes. At the tuberculoid end of the spectrum a completely different picture is seen. The paracortical areas are well developed and packed full of small lymphocytes; an occasional immunoblast can also be found. Germinal centres are not seen in the epitrochlear nodes and only an occasional plasma cell is seen. This is consistent with the observation of Hanks (1961) that no significant levels of antibody are found in those forms of the disease (tuberculoid) that are characterized by very small numbers of bacilli, strong lepromin reactions and a frank tendency to self-healing. On the other hand, patients with lepromatous leprosy, who have no immunity to infection, possess large amounts of circulating antibody to mycobacterial antigens. A similar finding was made by Rees *et al.* (1965) who found precipitating antibody in the sera of all patients with lepromatous leprosy directed against a number of different mycobacterial antigens and crude homogenates of leprosy tissue. In contrast, antibodies directed against mycobacterial antigens were only rarely found in the sera of patients at the tuberculoid end of the leprosy spectrum.

ERYTHEMA NODOSUM LEPROSUM

Although the antibodies against mycobacterial antigens do not contribute to the elimination of the mycobacteria, they are in a position to react with mycobacterial antigens *in vivo*. It is probable that the interactions between these antibodies and mycobacterial antigens exposed as a result of chemotherapy underlie the condition known as erythema nodosum leprosum. This condition takes the form of an acute vasculitis in the skin associated with polymorphonuclear leucocytic infiltration. It may be associated with iridocyclitis, arthritis and even glomerulonephritis. This symptom complex is reminiscent of that found in serum sickness and is then associated with the circulation of antigen-antibody complexes formed in slight

antigen excess which circulate and become deposited in small blood vessels, producing in the skin lesions which resemble the Arthus phenomenon. There is no doubt that the skin lesions of erythema nodosum leprosum have many features in common with the Arthus reaction as seen in experimental animals and may even have a haemorrhagic centre.

Erythema nodosum leprosum always occurs in that part of the leprosy spectrum where cell-mediated immune processes against the mycobacteria are absent or minimal and under conditions where there are large amounts of circulating antibodies against mycobacterial antigens, so high that they can be detected by techniques as crude as precipitation in agar (Rees *et al.*, 1965).

POSSIBLE CAUSES OF DEFICIENCES OF CELL-MEDIATED IMMUNITY IN LEPROSY

Depression of cell-mediated immunity in lepromatous leprosy is probably due to an interaction of a number of different factors and cannot be attributed to a single mechanism.

In the first place, there is no doubt that in patients with lepromatous leprosy a fundamental constitutional defect contributes considerably to the eventual deficiency. This is indicated by the observation that lepromatous disease is a host-determined characteristic that is possessed by a fixed proportion of all people everywhere (Newell, 1966). This observation is supported by the finding that the children of parents with leprosy showed a marked reduction in their ability to become converted to tuberculin positivity when vaccinated with vole tuberculosis vaccine, as compared with normal children (Jamison and Vollum, 1968).

The appearance of lymph nodes from patients with lepromatous leprosy is very similar to that seen in children with congenital thymic aplasia (Turk and Waters, 1968) and suggests that the defect might be due to an inherent deficiency in thymus control of cell-mediated immunity. The suggestion is supported by the observation of Rees *et al.* (1967) that a disease resembling lepromatous leprosy can be produced by infection in mice following a combination of adolescent thymectomy and deep X-irradiation.

However, it is more likely that this is due to drainage of histiocytes from peripheral lesions. Such cells passing down the afferent lymphatics will tend to accumulate in the paracortical areas. Although the appearance of lymph nodes resembles those seen under conditions where there is thymus deficiency, there is no evidence that this plays any role in these appearances in leprosy. There is a suggestion from the work of Bullock and Fasal (1968) that an anti-lymphocytic antibody could be present in the plasma of such patients. Moreover, Gaugas (1968) has found that a combination of thymectomy at 6 weeks of age and treatment with antilymphocytic antibody will allow *Myco. leprae* to proliferate to the extent of reproducing many of the features of lepromatous leprosy. As has been discussed above, such an antilymphocytic antibody could be developed as an autoimmune phenomenon as a result of extensive tissue damage during infection with *Myco. leprae.*

Another non-specific factor that could be relevant to the depression of cell-mediated immunity in leprosy is that the ability to develop delayed hypersensitivity is suppressed in guinea-pigs pretreated with Freund's adjuvant (Jankovic, 1962). The infiltration of tissues with Freund's adjuvant containing mycobacteria, might produce conditions similar to those found in leprosy. Guinea-pigs treated in this way are not only not able to produce cell-mediated immune reactions when actively stimulated with antigen but also have a reduced ability to manifest passively transferred reactivity (Asherson and Allwood, 1969).

All these factors will contribute to a non-specific depression of cell-mediated immunity. However, except in the polar form of lepromatous leprosy, the inability to show cell-mediated immunity is specific to *Myco. leprae* and a non-specific depression does not occur in other parts of the spectrum where bacillary growth may be little controlled. Thus a specific immunological tolerance must play a certain role in the ability to respond to the organism.

Moreover, this tolerance must be in respect of cell-mediated immunity only, as humoral antibody production, far from being depressed, is actually increased. Such "split tolerance" or immune deviation is found in experimental animals following the injection of alum-precipitated antigens (Asherson and Stone, 1965) or by the intraveous injection of soluble antigen (Dvorak *et al.*, 1965) prior to immunization. A similar state of split tolerance involving chemical-contact-sensitivity has been produced in already highly sensitive guinea-pigs (Polák and Turk, 1968). Thus, a cell-mediated immunity can be suppressed after it has developed. This may explain certain aspects of loss of immunity during the exacerbation of leprosy, which may occur when a patient with borderline leprosy swings towards lepromatous leprosy, or a patient at the tuberculoid end of the spectrum develops a disease with certain lepromatous aspects (borderline leprosy).

Therefore, it is probable that the loss of cell-mediated immunity in leprosy could originally be the result of the development of a state of specific immunological tolerance in an individual who already has a basic constitutional defect in cell-mediated immunity. With progressive infiltration of the tissues with *Myco. leprae*, the basic constitutional defect is aggravated so that there occurs a generalized non-specific failure to show certain aspects of cell-mediated immunity such as contact sensibility and homograft rejection. However, this in itself is never complete. Patients with lepromatous leprosy do not die within 6 months from virus and parasitic infections, as do babies with congenital thymic aplasia. A certain degree of cell-mediated immunity is always retained and patients who cannot be sensitized with DNCB can be sensitized with a stronger antigen such as KLH. Moreover, there is no increased incidence in the development of cancer by these patients, as might be expected if there was no ability retained to show cell-mediated immunity. If anything, leprologists have the impression that cancer is somewhat less prevalent in patients with leprosy than it is in the general population.

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