Field Workers' Forum

IMMUNOLOGY OF LEPROSY

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This article is taken from the second Edition of "Leprosy for Students of Medicine" by A. D. M. Bryceson and R. E. Pfaltzgraff to be published by Churchill Livingstone in 1977, who have kindly consented to its publication in *Leprosy Review*.

Introduction

The response of the body to invading micro-organisms involves both non-specific and specific mechanisms of defence. Non-specific mechanisms include the barriers of the skin and mucosae, antiseptics of sebum and mucosal secretions and simple phagocytosis by polymorphonuclear leucocytes and macrophages. Specific mechanisms are those elaborated by the immune response. They contribute to healing and immunity, hypersensitivity and pathology.

Basic Immunology Relevant to Leprosy

INDUCTION OF THE IMMUNE RESPONSE

A micro-organism consists of and produces many different chemical substances which are antigenic. These antigens reach the lymphoid organs (lymph nodes or spleen) where each is potentially capable of inducing an immune response. The type of response induced is determined by a number of factors.

Types of response

No response. This is the rule to 'self' substances, as in autografting of skin for burns, or rarely to foreign antigens.

Immune response. This is the commonest response to most microorganisms and has two components, cellular and humoral. Cell-mediated immunity is mediated by lymphocytes that are processed in foetal life by the *thymus*, and are often referred to as T-lymphocytes. Humoral immunity is mediated by B-lymphocytes and their progeny, plasma cells. In birds B-lymphocytes are processed by the *bursa* of Fabricius, an organ associated with the hind gut and having no anatomical equivalent in mammals.

Tolerance. The antigen kills the receptor cell in the lymphoid tissue or paralyses it in such a way that it fails to initiate an immune response to this and to any subsequent challenge with the same antigen. Both the humoral and cellular

responses are paralysed and the host 'tolerates' the foreign antigen. Occasionally only T-lymphocytes are paralysed.

Immune deviation. Antibody 'deviates' the antigen away from T-lymphocytes (see below) in such a way that cell-mediated immunity is not induced. The humoral response is normal. The situation in lepromatous leprosy (LL) resembles this, in that the individual seems able to produce antibodies but no cell-mediated response to *M. leprae*.

Factors affecting the type of response

Antigen determined

Chemical nature. Some antigens especially polysaccharides and simple chemicals (haptens) with a poor affinity to host proteins are only weakly antigenic.

Molecular size. Substances, including some proteins, of low molecular weight (below about 10,000) are often poorly antigenic, and are liable, under certain circumstances, to induce tolerance. Polymers of these substances and molecules of high molecular weight are more immunogenic.

Concentration. A very high concentration of antigen may induce tolerance rather than immunity. In an already immunized host such a concentration may suppress the expression of cellular immunity (desensitization), producing a situation similar to immune deviation. This mechanism may underlie the process of downgrading in borderline leprosy. A very low concentration of antigen may also be tolergenic.

Solubility. A highly soluble antigen is more likely to achieve a high circulating concentration and be less immunogenic than a poorly soluble antigen.

Route of presentation to lymphoid tissue. Antigens may reach the lymphoid tissue through the blood stream or along the lymphatics. They may be free or taken up by macrophages. Poorly soluble antigens, inoculated intradermally, phagocytosed by macrophages and taken by the lymphatics to the regional lymph node are highly immunogenic, especially with regard to cellular immunity. This is usually the case with *M. leprae*. Highly soluble antigens circulating free in the blood stream are much less efficient in inducing immunity, especially cellular immunity, and may be liable to induce tolerance. In lepromatous leprosy soluble antigens of *M. leprae* are found in the circulation. Their chemical nature and size and effect on the immune response are unknown.

Adjuvants. Certain substances, notably Wax D, a component of walls of *Mycobacteria*, have the property of augmenting both the humoral and cellular immune response to a wide range of antigens inoculated in emulsion with the adjuvants. Cell walls of *M. leprae* probably act as efficient adjuvants.

Host determined

Genetic. Congenital immunological deficiencies of either the cellular or humoral components of the immune response are rare and usually lead to an early death from infection. Genetically determined inability to respond normally to one particular antigen may be a factor in determining the clinical picture and outcome of a particular infection in that individual. Such a mechanism could contribute to the pathogenesis of lepromatous leprosy. Males are most susceptible to some infections than are females. This susceptibility may be a factor influencing the sex pattern of leprosy.

Integrity of lymphoid tissue. For the induction of a normal response the draining lymphoid organ must be intact and not grossly damaged by fibrosis or distorted by infiltration with abnormal substances such as amyloid, abnormal cells as in Hodgkin's disease, or normal cells as with the excessive ingress of macrophages from a large persisting depot of antigen in advanced lepromatous leprosy. If lymphoid tissue is preoccupied by the presence of one antigen it may respond poorly to the presence of another, a condition known as an antigenic competition. These mechanisms could contribute to the partial suppression of cellular immunity to unrelated antigens which is found in some cases of lepromatous leprosy.

Acquired immunosuppression. Some diseases may impair the induction of an immune response to certain antigens (malaria to tetanus toxoid, malnutrition to contact sensitizing agents); others may impair the expression of an immune response (measles to tuberculin, leukaemia to a variety of infections). Some drugs, the commonest of which are the corticosteroids, are immunosuppressive. This knowledge is made use of in the treatment of reactions. The partial, generalised immunosuppression acquired in lepromatous leprosy may render some patients more susceptible to intercurrent infection.

Enhancing antibodies. In situations where cellular immunity provides the effector arm of the immune response, circulating antibody may coat antigen molecules on the surface of cells without damaging that cell, and in this way block the recognition of that antigen by cytotoxic lymphocytes. This phenomenon, known as enhancement, contributes to the establishment and growth of certain tumours.

T-lymphocyte modulation of the immune response. T-lymphocytes help regulate the immune response in several ways. Two important ways are:

1. B-lymphocytes require the cooperation of specifically sensitized T-lymphocytes for the production of antibody to a wide range of antigens (so called thymus dependant antigens).
2. Some T-lymphocytes, generated during the induction of the immune response, regulate the production of effector T-lymphocytes. This is probably a normal physiological feed-back process. On occasion these regulatory cells, which are sometimes called suppressor T-cells, totally inhibit the production of effector cells and so create a situation which resembles immune deviation or selective T-lymphocyte tolerance.

Enhancing antibodies and suppressor T-cells have been little studied in infectious diseases. Their presence, if that could be demonstrated, might provide mechanisms whereby specific cell-mediated immunity was suppressed in lepromatous leprosy.

Changes in lymphoid tissue

During the course of an immune response certain changes take place in the lymph node which receives the antigen, or the spleen in the case of circulating antigens. Antigen from an intradermal site is presented by macrophages which enter through the marginal sinus and pass between the germinal centres into the paracortex. After this the parts of the node which develop depend upon whether humoral or cellular immunity is induced.

Humoral immunity. The cortex expands and germinal centres increase greatly in number and size, often bulging into and distorting the rest of the node. Plasma

cells appear in the medullary cords which become thickened. In a pure antibody response the paracortex becomes thinned. Histological staining with pyronin, which stains ribonucleic acid red, shows the intense activity of germinal centres and medullary cords. These changes are seen in lepromatous leprosy.

Cellular immunity. The paracortical area of the node, whose development is dependent upon the integrity of the thymus in foetal life, expands. It becomes populated by small lymphocytes, which enter through the postcapillary venules, and start to divide. These large pale dividing cells are also pyroninophilic on staining. Small non-pyroninophilic lymphocytes are produced and leave the node through the intermediary sinuses, which become greatly distended. In a pure cellular response the cortex and medulla are thinned. These changes are seen in tuberculoid leprosy (TT).

Most micro-organisms contain antigens which between them elicit both types of response.

Cellular events leading to antibody production and cell-mediated immunity

These events are complex and still imperfectly understood. A simplified concept involves the following stages:

1. Antigen brought to lymphoid tissue sensitizes T- and B-lymphocytes of the specific clone of cells that have the appropriate receptor site on their surface. The sensitized cells divide.
2. T-lymphocytes, on division, give rise to:
 - (a) a population of effector cells (cytotoxic or "killer" lymphocytes which are able directly to attack cells coated with the specific antigen. The cells leave the lymphoid tissue *via* the intermediary sinuses, enter the blood stream and recirculate continuously until they reach their "target", where further division may take place.
 - (b) a population of memory cells which take part in the secondary immune response.
 - (c) helper and suppressor cells which, respectively modulate B- and T-lymphocyte function. It is possible that helper and suppressor effects are simply different physiological functions of effector cells, and do not represent distinct cell populations.
 - (d) the secretion of a number of soluble factors (lymphokines) which increase the digestive power of macrophages and mediate the phenomena of delayed hypersensitivity.
3. B-lymphocytes divide several times and give rise to plasma cells which secrete specific antibody. Most plasma cells remain in the lymphoid organs. B-memory cells are produced.

EXPRESSION OF IMMUNE RESPONSE

Humoral immunity (antibody mediated)

There are several ways by which antibody can help the body to get rid of micro-organisms. At the same time 'unwanted' responses to many of the antigens induce a state of hypersensitivity which contributes to the pathology of the disease.

The most important of these mechanisms are:

1. Antibody combines with and neutralizes toxins, as in diphtheria or typhoid, but probably not in leprosy.

2. Circulating antibody (opsonizing or complement fixing) reacts with antigen on the wall of the organism. In the first case rapid and efficient phagocytosis ensues. In the second complement is fixed and the organism is lysed. Neither of these mechanisms seems to be important in defence against leprosy.

3. Antibody passively coats host cells and is available to react with antigen. This antibody is one of two kinds. It may be cytophilic for macrophages, in which case that cell is better enabled to take up micro-organisms; this mechanism may be important in cell mediated immunity to certain facultative intracellular organisms, but it does not increase the digestive power of that macrophage, and may not be relevant in leprosy. Alternatively the antibody is reaginic of the class IgE and is fixed to mast cells. Circulating antigen reacts with the fixed antibody giving rise to the phenomena of local or systemic anaphylaxis. This does not seem to happen in leprosy.

4. Precipitating antibody combines with antigen, which is present in moderate excess, and forms complexes to which complement is fixed. This may take place either in the circulation or in the tissues. Complexes formed in the circulation are deposited, depending on their size, in the endothelial spaces of vessels, notably those in the glomerulus, the skin and synovial membranes. A depot of antigen, in the tissues, such as a lepromatous nodule, can be the origin of a gradient of antigen concentration. Antibody diffuses from the circulation and at the appropriate relative concentrations complexes are formed and deposited. Complement is fixed and the release of its third component attracts polymorphonuclear leucocytes which accumulate, phagocytose the complexes and release enzymes of which the most potent are proteases and which cause tissue damage. Activation of vascular endothelium further encourages adhesion of leucocytes and platelets, thrombosis and haemorrhage. This process is important in the genesis of type 2 reactions in leprosy (erythema nodosum leprosum).

Cell-mediated immunity (lymphocyte and macrophage mediated)

Antibodies do not normally penetrate host cells. Certain organisms have become adapted to intracellular life, and those which are adapted to intramacrophage life are especially well protected. These organisms include *Leishmania*, *M. leprae*, *M. tuberculosis*, certain fungi and, to a lesser extent, the more facultative intracellular organisms, *Salmonella* and *Brucella*. Cellular immunity is of especial importance in dealing with infections caused by these organisms. It is also of importance in some viral infections, in graft rejection and in tumour immunity.

There are two main ways in which cells may produce and maintain a state of immunity. They are macrophage activation and lymphocyte cytotoxicity. There are also several mechanisms involved in the production of a state of delayed hypersensitivity which contribute to pathology.

Specifically sensitized T-lymphocytes, produced in the paracortical areas of lymphoid tissues, enter the circulation and 'home' onto the site or sites containing antigen. In the case of leprosy this is predominantly the infected macrophages of the skin and nerves. The phenomena of cell-mediated immunity and delayed hypersensitivity follow. These are:

1. Focalization and division of macrophages.

2. Accumulation of lymphocytes around the macrophages.

3. Alteration of macrophages into epithelioid cells, often with the formation of giant cells. Steps 1, 2 and 3 produce a tubercle, the characteristic histological picture of cell-mediated immunity.

4. Increased enzymatic activity of macrophages and increased ability to digest organisms. This increased activity is not specific to the organism inducing the immune response.

5. Central necrosis, or ulceration of overlying skin.

These phenomena are probably mediated by lymphokines released by lymphocytes as they divide during contact with antigen, which is usually on the surface of macrophages. Lymphokines are not immunoglobulins and have a very short range of action. Their action is therefore only local, in contrast to that of circulating antibody. Lymphokines are assayed by their biological activities.

The most important of the lymphokine factors are:

skin reactive factor: this increases capillary permeability and may facilitate the ingress of cells into the lesion,

chemotactic factor: this attracts macrophages,

macrophage migration inhibition factor: this inhibits the natural migratory properties of macrophages and may play a part in the localization of infection. It also increases the digestive powers of macrophages, a property which is probably important in leprosy.

Cytotoxic factors (lymphotoxins): there are several of these, antigen specific and non-specific, acting in different ways. They may be responsible for caseation in tuberculosis, ulceration in cutaneous leishmaniasis and for rejection of homografts. They are probably not so important in defence against leprosy, though they might contribute to type I reactions.

Mitogenic (blastogenic) factor: this causes lymphocytes, both specifically sensitized and non-sensitized, to divide and in doing so to secrete more lymphokines and so increase the reaction and, so long as antigen remains, prolong the reaction.

When the end result of these processes is elimination of invading organisms and protection against reinfection a state of cell-mediated immunity is said to exist; when inflammation alone, cellular or delayed hypersensitivity exists.

Fig. 1. Cell-mediated immune response, as shown by lymphocytic infiltration of lesions and by lepromin positivity.

Immunological Findings in Leprosy

The pathological and clinical characteristics of leprosy form a spectrum from LL to TT. The factor which mainly determines the place of an individual patient's disease on this spectrum is the extent to which cell-mediated immunity is expressed. In tuberculoid leprosy cellular immunity and hypersensitivity are well developed. In lepromatous leprosy they are absent; in LL induction and/or expression may be impaired. In borderline disease expression rather than induction is impaired. In addition the antibody responses at the poles differ and although this difference does not directly contribute to the patient's position on the spectrum it does contribute to certain other aspects of the disease.

CHARACTERISTICS OF THE IMMUNE RESPONSE AT THE POLES

Tuberculoid leprosy

1. Cellular response

The presence of cellular immunity is shown by certain characteristics of disease at this pole and by some experimental data.

- (a) The essential histological characteristic is the tubercle: specialized macrophages (epithelioid cells) focalized by lymphocytes.
- (b) Delayed cutaneous hypersensitivity is present and is shown by a positive lepromin test.
- (c) Lymph nodes show well developed paracortical areas containing pyroninophilic blast cells. Intermediary sinuses contain numerous non-pyroninophilic lymphocytes. Germinal centres are poorly developed and medullary cords contain few plasma cells.
- (d) Lymphocytes from patients with tuberculoid leprosy, cultured *in vitro* in the presence of leprosy bacilli, transform into blast cells, activate macrophages and inhibit their migration.
- (e) Macrophages from patients with tuberculoid leprosy can be stimulated, *in vitro*, to digest *M. leprae* if lymphocytes from a tuberculoid patient (but not from a lepromatous patient) are added to the culture.
- (f) The disease tends to heal spontaneously.

2. Humoral response

- (a) Antibodies to antigens of *M. leprae* and other mycobacteria can be detected in sera of under 10% of patients with TT leprosy by precipitation techniques.
- (b) Auto-antibodies are not produced.

Lepromatous leprosy

1. Cellular response

The absence of cellular immunity is shown by:—

- (a) There is no tubercle formation. The histological picture is that of the leproma: undifferentiated macrophages, often damaged by oedema and full of bacilli, with no surrounding lymphocytes.

- (b) The lepromin test is negative.
 - (c) Lymph nodes show well developed germinal centres and medullary cords full of plasma cells. Paracortical areas are poorly developed and are replaced by undifferentiated and often bacilliferous macrophages. Intermediary sinuses do not contain lymphocytes.
 - (d) Lymphocytes from patients with lepromatous leprosy do not transform *in vitro* into blast cells in the presence of *M. leprae*, nor do they activate macrophages or inhibit their migration. The proportion of circulating lymphocytes, capable of binding *M. leprae* to their surface, is much lower in lepromatous than in tuberculoid patients.
 - (e) Macrophages from lepromatous patients can be stimulated *in vitro* to digest *M. leprae* if lymphocytes from a tuberculoid patient (but not from a lepromatous) are added to the culture.
 - (f) The disease does not heal spontaneously.
2. Humoral response (Fig. 2)

Fig. 2. Humoral response, as shown by presence of circulating antibodies and liability to develop erythema nodosum leprosum.

- (a) Antibodies to antigens of *M. leprae* and other mycobacteria can be detected in high titre by complement fixation, precipitation and indirect haemagglutination. Precipitins to up to 5 antigens are present in 80% of lepromatous sera. Serum levels of IgG are raised.
- (b) Sera from lepromatous patients do not inhibit, *in vitro*, the blastogenic response to *M. leprae* of lymphocytes from tuberculoid patients. This observation implies the absence of enhancing antibodies in those sera.
- (c) Many auto-bodies are produced. They include rheumatoid factor, anti-thyroglobulin antibody, cryoglobulins, C-reactive protein and false positive biological tests for syphilis such as the Wasserman reaction.

Antibodies in leprosy do not seem to have any protective or useful role. They are unable to get at the intracellular organism. They play a role in type 2 reactions, but in doing so are of no benefit to the patient. It is not known whether autoimmune antibodies cause tissue damage, result from tissue damage or are a manifestation of the adjuvant activity of *M. leprae* in lymph nodes and not directly related to the clinical or pathological pattern of leprosy.

NATURE OF IMMUNE DEFICIENCY IN LEPROMATOUS LEPROSY

The immunological findings in leprosy indicate that there is a deficiency of cellular immunity to *M. leprae* in patients with lepromatous leprosy. The clone of lymphocytes which should respond is absent or unresponsive. As a result of this macrophages are not activated and are unable to digest *M. leprae*. Other features of cellular hypersensitivity are also absent. In LL this deficiency would appear to be absolute, lifelong and not reversible after treatment, which has led people to suggest that a genetic defect may be involved and there is some epidemiological evidence to support this view. On the other hand there is only a relative defect in BL and BB leprosy, and patients may move their position on the spectrum, towards the lepromatous pole if untreated or toward the tuberculoid pole if treated. These facts indicate that the presence of the bacillus itself specifically depresses cellular immunity.

Antibody response is unimpaired in LL. High titres are present to mycobacterial antigens and the response to immunization with typhoid vaccine (TAB) is normal. Enhancing antibody has not been demonstrated.

Thus, in lepromatous leprosy, there exists a state that resembles selective T-lymphocyte tolerance (mediated by antigen or suppressor T-cells) or immune deviation (mediated by antibody). In borderline disease these defects are not absolute and some additional modulating process, such as cellular desensitization, may be operating.

The severe deficiency of cellular immunity in LL is specific for *M. leprae*. There is, however, a partial generalized depression of cellular immunity in some patients with severe LL. They have a reduced number of circulating T-lymphocytes, and *in vitro* tests of lymphocyte function to other antigens may be slightly impaired. Their response to sensitization with some but not all chemical agents (e.g. dinitrochlorobenzene) is impaired, their response to several skin test antigens is depressed, they reject grafts of homologous skin more slowly and they may be more susceptible to incurrent disease. This depression, which tends to recover after treatment, is possibly due to disorganization of lymph node architecture and to antigenic competition.

Several attempts have been made to correct the specific defect of cell mediated immunity in lepromatous leprosy by giving patients lymphocytes or an extract of lymphocytes—transfer factor—from normal or immune individuals. So far there have been no convincing reports that these attempts have altered the course of lepromatous disease. There have as yet been no reports of the use of transfer factor to stop troublesome chronic erythema nodosum leprosum in well treated lepromatous patients, a situation which might be amenable to this sort of immunostimulating therapy.

Further Reading

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