COMMITTEE 4: ADVANCES IN IMMUNOPATHOLOGY

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Since the last congress panel report in 1963, great strides have been made in the understanding of the immunopathology of leprosy. These advances, however, have been possible because of the great amount of pathologic information available from the work of the past century which has given direction to and formed a basis for the application of newer technics and hypotheses in immunopathology generally.

Leprosy still remains clinically and histologically the best characterized single host *vs* single pathogen infectious disease which covers the range from a stage of effective immunity at one end to a state of profound immunologic deficiency towards the infectious agent at the other, but manifesting in its spectrum effects of both cell-mediated and humoral antibody-mediated immune phenomena.

Although advances in animal transmission of *M. leprae* have contributed significantly to the understanding of pathogenic mechanisms in leprosy, recent progress in basic medical research has provided methods by which it has become feasible to study in detail the host/parasite interaction in the leprosy patient himself. This fact makes it increasingly important to establish and support laboratories in leprosy-endemic areas where immunopathological studies can be undertaken on materials from leprosy patients. Such studies may not only contribute to our understanding of disease processes in leprosy and related diseases but also give a lead to better care and control of the disease.

CLASSIFICATION

The classification adopted by the VIth International Congress, Madrid, still seems basically operative, adequate, and in conformity with new developments in understanding. Essentially, this classification recognized the two polar immunopathologic expressions of leprosy as "tuberculoid" and "lepromatous" with an interlying spectrum of variable manifestations, termed "borderline" (dimorphous) in place of the previously adopted "indeterminate" and a category designated "indeterminate" of use particularly in early cases where the eventual classification characterization is not clear. This classification has been neatly summarized and correlated with its clinical, immunologic and morphologic expressions (Leprosy Rev. **33** [1962] 119-128; Internat. J. Leprosy **34** [1966] 255-273) with the convenient code designations of TT, BT, BB, BL and LL added.

It is recommended that this system of classification and notation be generally used; and for ease of communication that this system be utilized in publications. It is recognized that some workers and groups of workers feel that on occasion additional designative terms are necessary. It is suggested that when such are used, their relative position in the classification scale be stated and they be clearly defined either in terms of the full scale of immunopathologic and microbiologic characterizations now used in classification, or if they be used for clinical convenience, that this be stated. For reference purposes it is recognized that both the "Lucio" and the "histoid" expressions of leprosy are unexplained variants at the lepromatous end of the classification scale and it is desirable that these terms be retained and used appropriately.

The term "lazarine" leprosy has historical associations with both ulceration seen in "Lucio" leprosy and otherwise seen, often in association with debilitation. It is recommended that, since the cause of ulceration in "Lucio" leprosy is recognized to be vascular thrombosis with dermal infarction, that the designation "Lucio phenomenon" be retained for this manifesta-

tion and the term "lazarine" be reserved for complicating ulcerative manifestations, particularly as associated with debilitation.

HISTOPATHOLOGY IN LEPROSY

The use of the biopsy for the diagnosis and classification of leprosy is well established. The significance of neural involvement in leprosy has long been recognized and is increasingly coming to the fore. It is urged that no histopathologic report on skin biopsies relating to the possibility of leprosy be regarded as complete or acceptable unless mention is made of evaluation of nerve involvement. It is of importance that all biopsies include full thickness of the dermis to provide probable inclusion of adequate cutaneous nerve samples.

MEASUREMENT INDICES IN LEPROSY

Bacteriologic Index (BI). The BI in use is of proven value. Variations in the standards employed in different laboratories impair its usefulness as a comparative measurement. Nevertheless, its standardized use in the form of log scale increments is recommended.

Morphologic Index (MI). This index, expressed as the percentage of solid form bacilli among the total counted, has come to be generally used as a measure of the percentage of viable organisms present, and therefore as a measure of therapeutic response. Variations in its performance and in staining related thereto in various laboratories precludes its usefulness as a precisely comparative measure. The statistical confidence interval [Internat.J. Leprosy **39** (1971) 857-862] is considerably greater when 100 bacilli are counted than when 200 are the basis of enumeration. The difference appearing when 400 are counted is less significant and it seems reasonable to suggest, in balance, that the counting of 200 bacilli gives a more valid percentage whereas the counting of 400 is probably not worth the extra time and effort required. In patients in relapse it is important to utilize one or more of the most active lesions.

Histopathologic Index (HI). Also known as the Biopsy or Numerical Index. Based on the examination of an acid-fast stained paraffin section cut at 5 microns thickness, this assay utilizes the Bacterial Index system of bacterial concentration combined with an assessment of the proportion of the specimen occupied by bacilliferous lesion. Under controlled conditions it is more accurate than the slit-smear method and has proved its value in research. It has not generally found a place in the routine management of patients.

SIGNIFICANCE OF VISCERAL LESIONS

The presence of bacillemia in leprosy postulated by the First International Leprosy Congress and subsequently demonstrated by a number of workers has recently been shown to be virtually continuous in untreated lepromatous leprosy. The circulating bacilli have been shown to be, in part at least, viable.

Significant morphologic evidence of leprous lesions in visceral organs, most particularly the liver, spleen, adrenal glands, bone marrow and lymph nodes has also been long available. More recently, however, biopsy studies have reiterated and extended the recognition that the visceral lesions essentially reflect the morphologic characteristics of the immunopathologic skin lesion type including episodes of lepra reaction. Such studies have also demonstrated that viable *M. leprae* are present in lepromatous leprosy in the liver and bone marrow, and it is therefore to be expected that they are present also in other areas of deposition. Though morphologic evidence suggests that bacilli in these lesions may be viable, the evidence is not conclusive that they are proliferating. The presence of viable bacilli in these organs can also be interpreted as merely the presence of recent hematogenous bacillary deposition.

Laryngeal lesions, at one time not infrequent and troublesome enough to require tracheostomy, have virtually disappeared under sulfone therapy. In contrast, nasal lesions, though also responsive to sulfone therapy, have increasingly been noted as a probable source of discharged bacilli for the spread of leprosy.

The nasal lesions in untreated lepromatous patients infiltrate cartilage and bone which form the framework of the nose, resulting in its collapse and deformity in some of them. Infection by secondary organisms may hasten this process of destruction. Similarly in the small bones of the hands and feet specific leprous osteomyelitis may

be responsible for the bone erosion. But in this instance, more often invasion by secondary organisms is responsible for loss of tissue, including bones. There are also the underlying vascular alterations following nerve paralysis which create a complex pathogenic process. In addition, factors such as disuse atrophy, pressure atrophy etc., combine to produce the severe deformity that is often seen.

The involvement of the eye in lepromatous patients is a serious complication. There is infiltration of the iris, ciliary body, sclera and episclera by macrophages to form lepromas. During the reactive phase there may be infiltration of the iris and ciliary body by neutrophils with fibrinous exudate into the anterior chamber resulting in loss of vision. Anesthesia of the cornea and lagophthalmos due to paralysis of the 5th and 7th cranial nerves respectively, found in all forms of leprosy, may lead to corneal ulceration and ultimate loss of the affected eye.

PATHOLOGY OF NERVE INVOLVEMENT IN LEPROSY

Peripheral nerve involvement is a characteristic finding in all forms of leprosy, although the nature of the lesion varies depending on the type of this disease. In lepromatous leprosy the predominant picture is the invasion of the nerve by a large number of organisms which are present in perineural cells, Schwann cells and intraneural macrophages. There is also intraneural proliferation of collagen and edema. In tuberculoid leprosy there is inflammatory response to bacillary antigen both intra- and perineurally and the nerve parenchyma is replaced by tuberculoid granulomas which in some instances may even caseate and form an abscess. *M. leprae* are demonstrable with difficulty. In borderline leprosy the appearances are very variable, depending on the type of the disease.

Further, the nerve involvement is generalized in lepromatous and borderline leprosy and localized to one or few nerves in tuberculoid cases. However, the route of entry of organisms into the nerves is still a matter to be elucidated.

The onset of paralysis is slow and insidious in lepromatous leprosy, taking several years, and may improve under effective chemotherapy. In tuberculoid leprosy, however, the onset is comparatively rapid and usually irreversible. In borderline cases also the nerves are rapidly damaged and very severe, and rapidly developing paralysis can occur in the presence of reactions.

The mechanism of destruction in the tuberculoid part of the spectrum is a delayed type hypersensitivity reaction to bacterial antigen in the nerve parenchyma. In lepromatous leprosy, although the presence of a large number of organisms ultimately causes much destruction, the exact pathogenic sequence of events is as yet unclear. The reactions which may complicate all forms of leprosy commonly contribute to further damage.

Nerve damage is often permanent and disabling, and therefore of paramount practical importance in leprosy. Advances in the prevention and management of nerve damage are only possible as a result of increased understanding of the various mechanisms involved. Further research in this field deserves high priority.

REACTIONS IN LEPROSY

A "reaction" in leprosy is regarded as a hypersensitivity phenomenon and does not include phenomena associated with the simple extension or regression of the infection. Biopsy studies are of value in elucidating whether or not such a reaction is taking place and for determining its nature. On occasion biopsies are essential.

Two types of reaction are well recognized, namely *erythema nodosum leprosum* (ENL) in lepromatous leprosy, and reversal reactions in borderline leprosy. ENL is associated with the infiltration of neutrophils as in the Arthus reaction. Necrosis and ulceration may follow. The presence of immune complex deposits has been reported in ENL lesions, together with alterations of complement levels in serum. Confirmation is awaited, but the precise nature of ENL remains to be fully elucidated. Reversal reactions are associated clinically and histologically with an increase of cell-mediated immunity which can be demonstrated by lymphocyte function tests. In experimental animals reversal reactions

can be precipitated by the injection of syngeneic lymphoid cells.

A number of other reactions are liable to occur and lack of fundamental knowledge precludes their classification. These include:

1. Reactions in tuberculoid leprosy which are presumably an expression of delayed hypersensitivity.
2. Reactions in borderline leprosy associated clinically and histologically with a downgrading within the immunological spectrum or with no change in immunity, though the results of lymphocyte function tests are variable.
3. Reactions in lepromatous leprosy which vary in form from a simple localized neutrophil infiltration or necrosis in a hyperactive nodule to severe ulcerating lesions associated with thrombosis and dermal infarction in the "Lucio phenomenon." The relation of these lepromatous reactions to ENL remains to be determined.

Further studies of lepra reactions are regarded as important. It is recommended that the most promising approach is a controlled longitudinal study with combined clinical, histopathologic and immunologic observations before, during and after the period of reaction. A further possible means of advance lies in the study of the antigenic structure of *M. leprae* since the antigenic components involved in the reactions are not yet determined. Two polysaccharides designated *beta* and *delta* have been demonstrated and a protein antigen able to elicit delayed type hypersensitivity has been isolated.

A most serious and possibly fatal sequel of severe lepromatous leprosy is the development of renal lesions, including amyloidosis. It is not yet clear to what extent this is due to ENL or to other forms of reaction. It is urged that renal biopsies on selected patients should be included in studies of reaction where facilities permit; and that the kidneys of patients who come to necropsy following reaction should be made available for study.

LEPROMIN

The following antigenic preparations of *M. leprae* appear at present to be used in the study of delayed (skin) hypersensitivity to *M. leprae* (Bull.WHO 1973, in press):

a) Suspension of whole autoclaved homogenized leproma ("integral" lepromin).
b) More purified bacillary suspensions ("bacillary" lepromin).
c) Noncoagulated soluble bacillary proteins ("leprolins").
d) Defatted and disrupted bacillary suspension ("Dharmendra antigen").

While lepromin containing whole bacilli elicits both an early (Fernandez) reaction (48-72 hours) and a late (Mitsuda) reaction (3-4 weeks) "leprolins" and Dharmendra antigen elicit mainly an early reaction and none or a weak late reaction.

The early reaction indicates existing hypersensitivity to the injected antigens. The late reaction, on the other hand, allows sufficient time for the test subject to become sensitized by the injected antigens. Thus, the late reaction may not only measure existing delayed type hypersensitivity, but perhaps also provide information about the test subject's capacity to initiate and/or amplify the response to the injected antigens. The high incidence of late lepromin positive individuals in leprosy nonendemic areas are in agreement with this view. The late reaction, therefore, does not indicate whether a subject has previously been exposed to *M. leprae* or not. Its primary importance appears to be limited to the determination of prognosis in leprosy patients. Further information about the lepromin reaction may be found in a recent WHO Report (Bull. WHO 1973, in press).

It is recommended that in publications relating to their use, the type of antigen used and the nature of the reaction measured should be clearly specified.

It is urged that, if current reports of massive *M. leprae* proliferation in the armadillo continue to show validity, efforts be made to develop and utilize this source of bacilli for the preparation of standardized lepromin and leprolin that could be made universally available.

THE USE OF BCG IN THE IMMUNOPROPHYLAXIS OF LEPROSY

In the absence of additional information, this panel echoes the recommendation of

the WHO Expert Committee on Leprosy (Fourth Report, WHO Tech. Rep. Series No. 549, 1970) to the effect that it is premature to recommend the general use of BCG vaccination and that final recommendation be postponed until the results of the controlled studies in progress achieve definitive evaluation.

IMMUNE RESPONSIVENESS IN LEPROSY

The concept of a host-determined immunologic spectrum in leprosy has received steadily increasing clinical and pathologic support during the last decades, to the point where leprosy today stands forth as a unique immunopathologic disease model. The immunologic support for this concept was initially based on delayed hypersensitivity skin testings. More recently a considerable number of other immunological methods recently applied in studies on humoral and cellular immune responsiveness in leprosy include:

Humoral immune responsiveness.

1. Quantitative examination of serum immunoglobulin levels.
2. Semiquantitative determination of antibody production to TAB (typhoid-paratyphoid A and B) after active vaccination.
3. Presence of antimycobacterial antibodies in serum by fluorescent antibody and gel precipitation technics.
4. Detection of antibody-coated bone marrow derived lymphocytes (B-cells) by immune fluorescence.

Cell-mediated immunity.

1. Delayed skin sensitivity to microbial antigens such as PPD and "artificial" antigens such as dinitrochlorobenzene and picryl chloride.
2. Detection of sheep red cell rosette forming lymphocytes (T-cells).
3. Blastoid transformation of peripheral blood lymphocytes. This may be measured by morphologic examination of stained lymphocytes or more quantitatively by uptake of radioactive thymidine in such cells.

   Substances used to stimulate lymphocytes may be divided into three categories: a) nonspecific mitogens such as phytohemagglutinin, b) antigens not related to *M. leprae*, c) antigenic preparations derived from *M. leprae*.
4. Production and release of molecular mediators (lymphokines) from sensitized lymphocytes. These may be monitored by various technics, including migration inhibition of autologous, homologous or heterologous phagocytes.

It should be noted that the last two groups of tests cannot *a priori* be judged as measurement of cell-mediated immune responsiveness as humoral immune responses can influence the results of these tests.

While the findings suggest that humoral immune responses are unimpaired in leprosy patients, there is increasing evidence of a depression of cell-mediated immunity in certain categories of such patients. The degree of depression appears to increase continuously from the TT to the LL end of the spectrum. Circulating antibody to mycobacterial antigens, on the other hand, increases towards the lepromatous end of the spectrum. In addition to this immunological imbalance in lepromatous leprosy which is specific to *M. leprae*, a nonspecific depression of cell-mediated immunity has been reported in various studies. In contrast to the specific depression which does not recover as a result of antileprosy chemotherapy, the nonspecific depression may become reduced by treatment.

MACROPHAGE FUNCTION IN LEPROSY

Most tissue macrophages are derived from blood monocytes. In the tissues, macrophages can adopt a wide variety of morphologic forms. The resulting pleomorphism is well exemplified in leprosy where macrophages may appear as epithelioid, multinucleate, histiocytic or foamy cells. This differentiation and this influx into tissues appears to take place as a result of various stimuli, including products of activated lymphocytes.

Some laboratories have reported a lack in the capacity of lepromatous macrophages to lyse autoclaved *M. leprae* while other

groups of workers have been unable to substantiate this finding.

Whether the deficiency of macrophages in lepromatous leprosy to dispose of the leprosy bacillus and its lipid degenerative products is a defect in the macrophage population *per se* or due to lack of stimuli from other cells, is not yet clear.

It would appear that the eventual solution to the problem will involve the concepts and technics of both immunopathology and cell enzymology.