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Demonstration of Mycobacterial Antigens in Leprosy Tissues[1]

Robert N. Mshana, Ayele Belehu, Gerald L. Stoner,
Morton Harboe, and Abebe Haregewoin[2]

Leprosy, a chronic disease caused by *Mycobacterium leprae*, is characterized by a clinico-pathological spectrum ranging from a high resistant form, tuberculoid leprosy (TT), through a range of borderline forms, to a very low resistant form, lepromatous leprosy (LL) ([26, 27]). In the borderline and lepromatous forms, demonstration of *M. leprae* in tissues using conventional mycobacterial stains is relatively easy. It is, however, difficult in the high resistant forms despite evidence of tissue hypersensitivity and damage ([28]).

The presence of *M. leprae* in the tissues is not necessarily accompanied by host tissue reaction and conversely host reaction is not always directed towards demonstrable bacilli. This discrepancy and the failure to directly correlate the presence of the bacilli and the sometimes very severe tissue damage has led to several theories, the most recent being autoimmune delayed type hypersensitivity to non-myelin components of sensory nerves ([10, 11, 18]). *M. leprae* is an acid-fast bacterium, that is, it can be stained with various triphenylmethane dyes and is not subsequently decolorized by acid. This, however, is not a universal property of the bacilli, and the biochemical mechanisms involved are not fully understood; and modifications of the staining technique can lead to different bacteriological indices. The persistence of a granuloma in the absence of demonstrable bacilli may indicate that the organism is no longer acid-fast or that free antigenic products are initiating and perpetuating the granuloma. In liver biopsies of patients with borderline tuberculoid (BT) and tuberculoid leprosy (TT), acid-fast bacilli could be demonstrated in only three out of 58 biopsies, whereas 68% of them had tuberculoid granulomas ([23]). This suggests that the mechanisms of perpetuation of a granuloma are not directly dependent on acid-fast bacilli or that the bacilli need not be intact to induce and/or perpetuate it. Purified chemicals from my-

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[2] R. N. Mshana, M.D., Research Scientist; A. Belehu, Ph.D., Director; G. L. Stoner, Ph.D., Research Scientist, Armauer Hansen Research Institute, P.O. Box 1005, Addis Ababa, Ethiopia; M. Harboe, M.D., Professor, Institute for Experimental Medical Research, University of Oslo, Ullevaal Hospital, Oslo-1, Oslo, Norway; A. Haregewoin, M.D., Research Scientist, Armauer Hansen Research Institute, P.O. Box 1005, Addis Ababa, Ethiopia.

cobacterial cell walls do induce the formation of a granuloma.

Histopathological examination of tissue sections may not reveal the presence of small numbers of microorganisms. On these occasions, immunologically based techniques ought to be of value since they combine both specificity and sensitivity and are not dependent on the presence of viable organisms. Recent advances in immunochemistry have shown that immunoperoxidase staining can be used for the demonstration of infectious organisms and their antigens in tissues ([4,5,7,12,29,31]). Counter staining of immunoperoxidase stained sections not only gives an insight into the functional aspect but also into morphology, making it a powerful histopathological tool.

Antigenic analysis using crossed immunoelectrophoresis indicates that all antigens detected in *M. leprae* sonicates are widely cross reactive among mycobacterial species, and especially so with *M. bovis* (BCG) ([9,15,16,25]), although radioimmunoassay indicates the presence of *M. leprae* specific determinants ([17]). It was on this basis that we chose to use rabbit anti-BCG as the primary antibody to demonstrate both the bacilli and its antigens in leprosy tissues.

MATERIALS AND METHODS

Skin biopsy blocks were retrieved from the AHRI histology archives. The biopsies had been fixed in formaldehyde-mercuric chloride-acetic acid (FMA) fixative for 2 hr, transferred to 70% alcohol for 24 hr and then paraffin embedded ([28]). Five μ thick sections were cut, left to dry in an oven at 56°C overnight, dewaxed in xylene, passed through graded alcohols and into water. Fite-Faraco, TRIFF ([28]) and hematoxylin and eosin staining were done for classification and demonstration of *M. leprae*. The classification of the patients was based on the Ridley-Jopling scale ([27]). In indeterminate leprosy, classification was based on both clinical and histological findings. Clinical diagnosis was based on presence of ill-defined, slightly hypopigmented skin lesions with localized sensory changes. The finding of a small epithelioid cell granuloma around skin structures, even in the absence of demonstrable acid-fast bacilli and/or the presence of Schwann cell proliferation of nerves in such lesions, was taken as confirming the diagnosis of indeterminate leprosy.

All antisera used for peroxidase staining were purchased from DAKO-immunoglobulin A/S, Copenhagen, Denmark. These were rabbit anti-BCG, swine anti-rabbit IgG, horse-radish peroxidase labelled swine anti-rabbit IgG, and horse-radish peroxidase-rabbit anti-peroxidase complex (PAP). Coupling of peroxidase to antibodies was a modification of the two-step glutaraldehyde method of Avrameas and Ternynck ([3]), giving conjugated antibody molecules of molecular weight of about 200,000–240,000. Peroxidase-rabbit anti-peroxidase (PAP) complex was prepared according to Sternberger, *et al.* ([31]). Free base 3,3′-diaminobenzidine (DAB) was purchased from Sigma, St. Louis, Missouri, U.S.A. (Lot: 44C-0780). Normal swine serum was kindly provided by Addis Ababa Abattoirs, Addis Ababa, Ethiopia. Sections from each biopsy were treated with freshly prepared 2.28% periodic acid (w/v) in distilled water for 10 min, washed in tap water and exposed to freshly prepared 0.02% sodium borohydride in distilled water for exactly 2 min ([22]). They were then washed in phosphate buffered saline (PBS), pH 7.6, for 5 min. To reduce background staining, normal swine serum diluted 1:5 in PBS was applied to the slides and tipped off after 30 min; the slides were not washed. For the indirect peroxidase method, anti-BCG was used at a 1:100 dilution for 2 hr at 37°C in a moist chamber. In the PAP method it was used at a 1:10,000 dilution. Labelled swine anti-rabbit IgG was used at 1:50 while unlabelled swine anti-rabbit IgG and PAP complex were used at 1:20 and 1:100 dilutions, respectively. All dilutions were done using PBS, and the same buffer was used for washing after each step. Each washing consisted of three changes of fresh PBS, each for 10 min, with intermittent stirring. A freshly prepared solution of 0.05% DAB containing 0.01% hydrogen peroxide in 0.05 M Tris saline buffer, pH 7.6, for 5 min was used to localize the site of the peroxidase reaction. After washing in tap water for 5 min, the slides were counter stained in Harris hematoxylin for 5 min, dehydrated, cleared and mounted in DPX. In control slides, normal rabbit serum was applied as the primary antibody.

RESULTS

Histologically, 19 patients were classified as BT, 4 as BL, 18 as LL and 10 as indeterminate leprosy. Eleven had reversal reaction and seven had erythema nodosum leprosum (ENL). Slides stained for the demonstration of *M. leprae* were read for a minimum of 5 min per section and bacilli noted as being present or not, irrespective of their staining characteristics. The distribution of acid-fast bacilli and granulomata in the skin biopsies is shown in Table 1. In indeterminate leprosy sections, few bacilli were found in only two of ten biopsies. The criteria used for the diagnosis of this type of leprosy has been stated under Methods. Two bacilli were found around destroyed superficial nerves and within areas of intense inflammatory response in one biopsy while in the other biopsy only one bacillus was found in a deep dermal nerve which looked normal and was not involved in the cellular infiltrate. In BT leprosy, bacilli were found in or around nerves and were surrounded by histiocytic and lymphocytic infiltrates. No bacilli were found within blood vessels. In BL and LL leprosy, many bacilli could be seen, but there was no evidence of tissue hypersensitivity towards them. During reversal reaction, conspicuous giant cell formation could be seen, but these giant cells were not related to the presence or absence of the bacilli. Both the giant cells and epithelioid cells contained no bacilli. An intense inflammatory response, composed of lymphocytes and epithelioid cells around nerves, was seen in most of the biopsies from patients with reversal reaction, but there were no demonstrable bacilli in these areas. Most bacilli around the blood vessels in ENL were fragmented and within macrophages. The polymorphonuclear leukocytes around the blood vessels showing perivasculitis were not related to the presence of bacilli or macrophages containing them.

Figure 1 shows the flow diagram and interpretation of the peroxidase staining. The brown precipitate locates the presence of the antigen.

Indirect immunoperoxidase staining was less sensitive than the PAP method and had a higher background staining. Thus PAP staining was used in most of the slides. The skin biopsies from patients with non-mycobacterial skin diseases (Figs. 2a, 2b), as well as control biopsies from leprosy patients where normal rabbit serum was used as the primary antibody, were all negative. The periodic acid and sodium borohydride treatment blocked the endogenous peroxidase and, in addition, resulted in better clarity and definition of tissue components than when using 0.5% hydrogen peroxide in methanol for 30 min ([7, 8]).

PAP staining, as compared to Fite-Faraco technique, demonstrated bacilli and antigens in 12 and 17 BT biopsies, respectively. It was not possible to define the morphological characteristics of these bacilli due to a diffuse light brown coloration around

TABLE 1. *Acid-fast bacilli and presence of granuloma in skin according to leprosy classification.*

Classification	No. studied	Histological type of granuloma		Presence of acid-fast bacilli using Fite-Faraco	
		Lepromatous	Tuberculoid	No.	%
Indeterminate	10	0	0[a]	2	20
Borderline tuberculoid	19	0	16	10	57
Borderline lepromatous	4	4	0	4	100
Lepromatous leprosy	18	18	0	18	100
Reversal reaction[b]	11	0	conspicuous giant cell formation	4	36
Erythema nodosum leprosum[c]	7	7	0	6	85

[a] Lymphocytic infiltration around neurovascular bundles and adnexa.
[b] Reversal reaction occurring in BT patients only.
[c] ENL occurring in LL patients only.

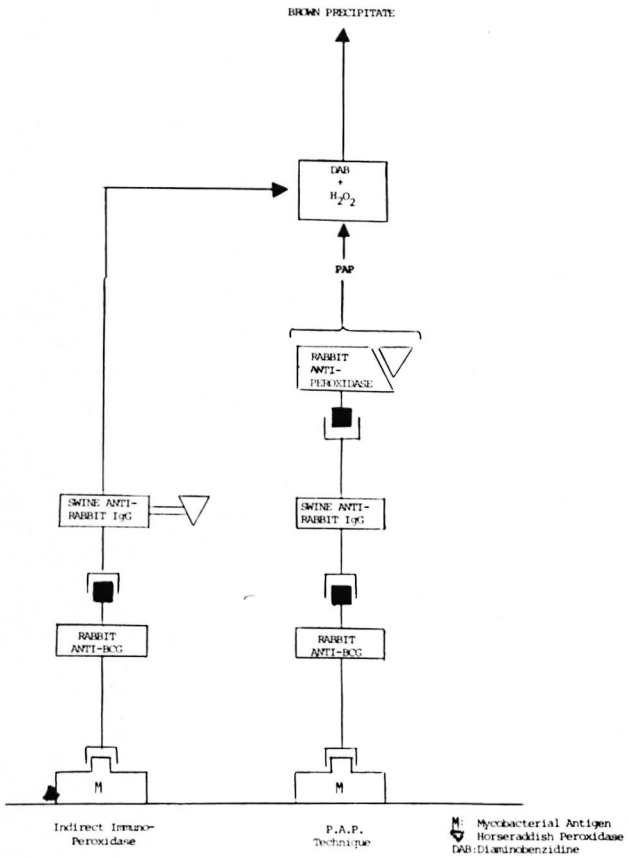

FIG. 1. Flow diagram for the immunoperoxidase staining.

them. Giant cells could be seen in a few biopsies, and these contained no antigen within them.

In the bacilliferous forms of leprosy, bacilli could be demonstrated in all biopsies. Due to the large numbers of these bacilli, in most cases a cloud of brown precipitate (antigenic cloud) was seen frequently, and this suggested leakage of antigen from dying or multiplying bacilli (Figs. 3a, 3b). However, this made it impossible to study the morphology of the organisms. It can also be seen that macrophages containing large numbers of bacilli or their products were also stained on the surfaces. Foamy cells which contained no demonstrable bacilli were shown to be full of antigens. (It should be noted in passing that the Gomori-methamine-silver staining method has been used to demonstrate non-acid-fast material in borderline and lepromatous patients. Since this method does not specifically stain for mycobacteria or their antigens, our findings offer evidence that the non-acid-fast materials seen by using such stains are actually of mycobacterial origin.) The amount of antigen demonstrated confirmed the view that in these types of patients there is both a heavy bacillary and antigenic load.

In indeterminate leprosy, areas showing lymphocytic infiltration were closely searched for the presence of the bacilli. These were found in two biopsies, whereas mycobacterial antigens could be seen in eight biopsies.

Conspicuous giant cell formation was seen during reversal reaction, but no antigens or bacilli could be seen within them. Antigens could, however, be seen around the diffuse lymphohistiocytic infiltrate. Only one biopsy was taken after the reversal reaction had clinically subsided, and in this specimen no antigen could be demonstrated.

Antigens and bacilli could easily be dem-

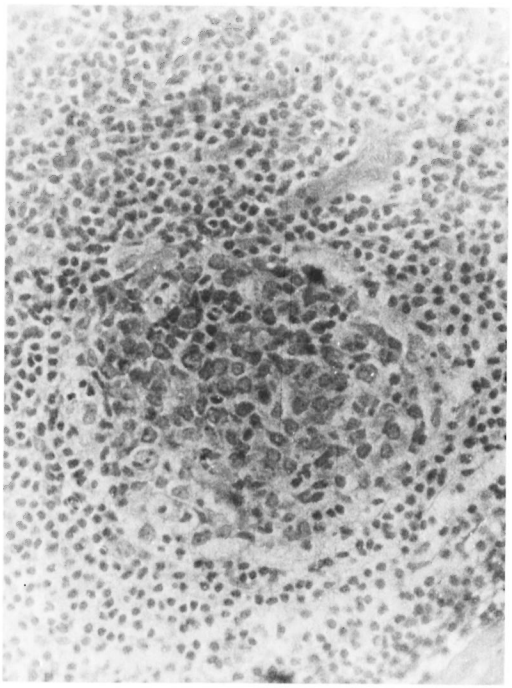

FIG. 2a. Sarcoid granuloma in a lymph node (77/108). Hematoxylin-eosin (×250).

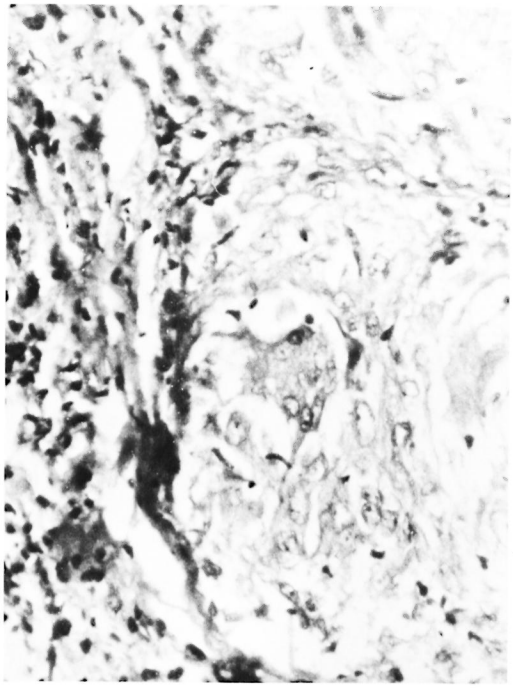

FIG. 2b. Sarcoid granuloma in a lymph node (77/108). PAP stain using anti-BCG (×480). No acid-fast bacilli were seen using the Fite-Faraco staining.

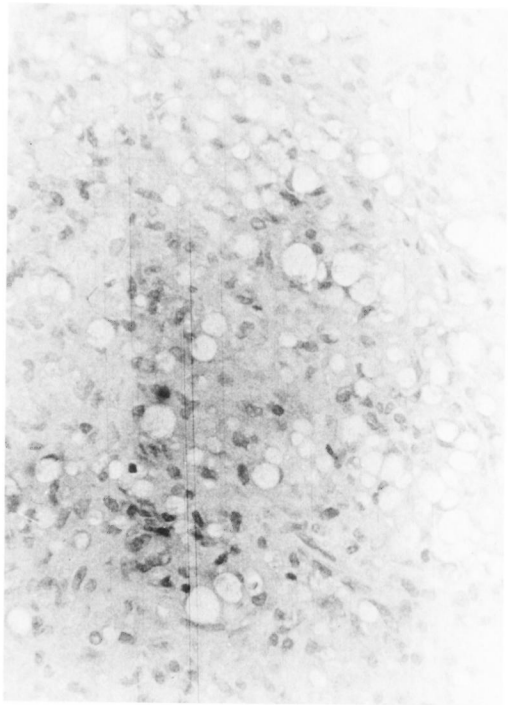

FIG. 3a. Lepromatous leprosy skin (903/80). Control section using normal rabbit serum as the primary antibody (×250).

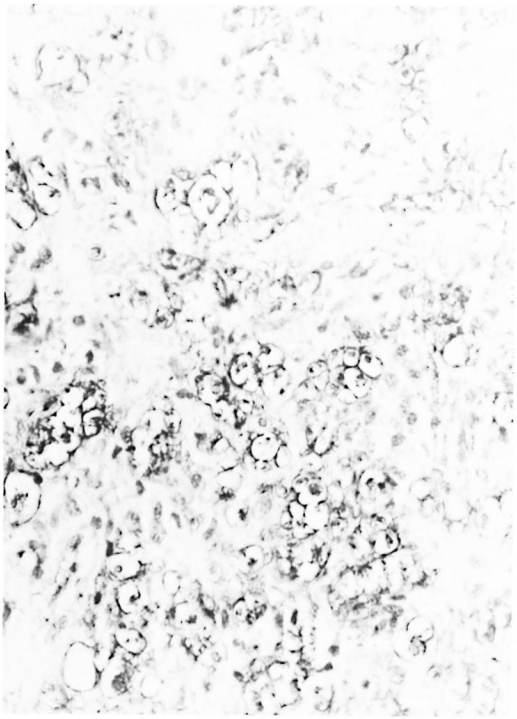

FIG. 3b. Lepromatous leprosy skin (903/80). PAP stain using anti-BCG (×250).

TABLE 2. *Presence of mycobacterial antigens and host response in leprosy.*

Classification	No. studied	Presence of mycobacterial antigens using anti-BCG in the PAP staining		Presence of host tissue reaction towards antigens[a]	
		No.	%	No.	%
Indeterminate	10	8	80	4	40
Borderline tuberculoid	19	17	89	15	88
Borderline lepromatous	4	4	100	0	0
Lepromatous leprosy	18	18	100	0	0

[a] Host responses in indeterminate leprosy were taken to be a round cell infiltrate around skin adnexa and nerves; in borderline tuberculoid, borderline lepromatous, and lepromatous leprosy, an epithelioid granuloma in relation to antigen.

onstrated around blood vessels showing signs of perivasculitis and neutrophil infiltration during ENL. Antigens were also seen around some blood vessels where there was no accompanying inflammatory response.

By using rabbit anti-human IgM as the primary antibody, linear immunoglobulin deposits could be seen in the dermo-epidermal junctions in some biopsies from the lepromatous end of the spectrum. These deposits were not related to the presence of ENL. IgM deposits could also be seen around some blood vessels, but these were not related to signs of perivasculitis even in patients with ENL. In only three biopsies could these immunoglobulin deposits be found in the same sites as mycobacterial antigens.

The relationship between the presence of mycobacterial antigens and host tissue reaction is shown in Table 2. In BT leprosy, host response was judged by the presence of an epithelioid cell granuloma in relation to the antigen. In indeterminate leprosy, the host response was taken to be a round cell infiltration around skin adnexa and nerves. The degree of this infiltration and the formation of an epithelioid granuloma were taken as a measure of the intensity of the response. The two biopsies which had revealed AFB by the Fite-Faraco stain also showed the bacilli and antigens by using PAP. The numbers of bacilli did not increase by using the peroxidase stain. In the biopsy where the bacilli were found in destroyed nerves, bacillary antigens could also be seen to be surrounded by an intense lymphocytic infiltration with aggregates of poorly differentiated epithelioid cells. This was the case in three other biopsies where no AFB could be seen by either Fite-Faraco or PAP. In the biopsy where an AFB was in a normal looking nerve, there was no intense host response to the AFB or its antigens. Three other biopsies showed bacillary antigens but no identifiable bacilli when PAP was used. These antigens were found inside deep dermal nerves and around sweat glands and their ducts as well as around hair follicles and blood vessels. They were surrounded by scarce lymphocytic infiltrations without a tendency to epithelioid cell granuloma formation, and thus the host response was judged as being insignificant. These biopsies were small and serial sections could not be cut. The remaining two biopsies did not reveal bacillary antigens at all. In total therefore, out of eight biopsies where bacillary antigens were seen, in only four was the host response judged to be intense and significant. The response was seen to surround bacillary antigens. During ENL the response was predominantly perivasculitis with intense polymorphonuclear leukocyte infiltration, while during reversal reaction it was a diffuse lymphohistiocytic infiltration, fibroblast proliferation, and conspicuous giant cell formation. Bacilli or antigens within macrophages and in nerves did not seem to elicit any host reactions, whereas extracellular antigens were surrounded by intense inflammatory responses in the BT and indeterminate leprosy biopsies. These bacilli and antigens, although found in vast amounts, did not elicit any host response in BL and LL patients. The bulk of these antigens were intracellular, although heavily stained cells showed surface bacillary

antigens (Fig. 3b). During ENL, the neutrophillic infiltration was seen around extracellular perivascular antigens.

DISCUSSION

There is an obvious discrepancy between the numbers of bacilli demonstrated in tissues and the host reaction that accrues in the high resistant forms of leprosy. This discrepancy could be due to host factors or to inherent properties of *M. leprae*. Granuloma formation is accelerated when hypersensitivity to the inducing antigen exists *pari pasu* ([1]), so that in BT and TT leprosy epithelioid cell granulomas can be readily elicited with *M. leprae* antigens. The natural history of a granuloma is to disappear or regress once the eliciting agent is removed, so that the continued presence of a granuloma suggests the persistence of antigen. The presence of intact bacilli is not essential for the induction of a granuloma; and skin biopsies from patients with papulonecrotic tuberculids secondary to *M. bovis* ([23]) and disseminated cutaneous granulomas from BCG therapy ([25]), all showing typical lymphohistiocytic infiltrates with granuloma formation histologically, did not contain demonstrable bacilli and had negative culture results ([25]). Synthetic products, like muramyl dipeptide, which is a component of mycobacterial cell walls, can induce epithelioid cell granuloma formation.

There is no reason to believe that *M. leprae* is different from other mycobacteria. It has a complex number of antigens, at least 20 having been demonstrated ([9]). The reasons why LL and BL leprosy patients do not show any significant tissue response to antigens to which TT and BT patients do respond are unknown. Since this lack of response seems to be restricted only to *M. leprae* antigens, lack of specific T-lymphocytes, suppressor cells, and genetic factors have all been cited as probable causes. Recently, *in vitro* experiments have suggested that antigenic overload in these patients may also be involved ([35]).

Intradermal injections of *M. leprae* antigens into individuals able to mount a proper immune response result in an epithelioid cell granuloma, thus indicating that *M. leprae* antigens are able to induce such a reaction and that the host response is directed towards *M. leprae* antigens. Further evidence that the epithelioid cell granuloma is a result of cell mediated immune mechanisms, specific or otherwise, to *M. leprae* antigens is provided. In these patients, epithelioid cell granulomas, sometimes with giant cell formation, could be seen in the "reactive" lesions, and in these areas there were no bacilli demonstrable. Failure to detect mycobacterial antigens in some biopsies need not mean their absence since the method used, by definition, detects only cross-reacting antigens. It has been shown previously that these antigens are potent inducers of antibody formation ([26]) and therefore the antigenic determinants may have been blocked by antibodies. The possibility that the *M. leprae* antigens might have already been removed by the host response can not be eliminated.

The discrepancy between the paucity of the infecting organism and the amount of tissue damage may indicate the presence of autoimmune mechanisms. The myocarditis found in Chagas' disease is characterized by lymphocytic infiltration and destruction of normal heart cells in the absence of the parasite *in situ*. *In vitro* heart cell lysis by *T. cruzi*-sensitized T-lymphocytes lends support to autoimmunity in Chagas' disease ([33]). Recently, the induction of granulomatous hypersensitivity, hypopigmentation, and nerve damage has been achieved in rabbits by immunizing with non-myelin components of human sensory nerves ([10, 11]). This triad of symptoms is found in non-lepromatous leprosy patients. Although the pathogenesis of non-lepromatous leprosy is unclear, our findings seem to indicate a primary role for the mycobacterial antigens *in situ*. Furthermore, *in vitro* lymphoproliferative responses to peripheral nerve antigens do not indicate the presence of systemic sensitization to these antigens in leprosy patients (unpublished observation). Extensive studies will be needed to settle the question of autoimmunity.

Mycobacterial antigens could be detected in eight indeterminate patients, although in only half of them were the antigens related to the host tissue response. Studies on the immune function of indeterminate leprosy patients are at variance, but it is known that a proportion of them progress

to lepromatous leprosy ([2]). Also a number of these patients do not respond to *M. leprae* antigens *in vivo* and *in vitro*. It is those who show anergy to these antigens that progress to LL. Our results do not support the contention that indeterminate patients are immunologically "virgins" but rather that these patients can be divided into two broad groups, those who respond and those who do not respond to *M. leprae* antigens and are thus able or not able to localize the disease.

The high percentage of patients with BT leprosy who showed histological evidence of recognizing *M. leprae* antigens supports the immunological basis of the leprosy spectrum. Although we do not have a clear explanation why 12% of our BT patients did not respond to bacillary antigens, it must be noted that BT leprosy is not static and patients in this category can progress to lower (less immune) forms of the disease. The lymphocyte transformation test, which is probably a correlate of delayed hypersensitivity, also varies greatly in this group. Epithelioid cell granuloma formation, which has been shown to be influenced by delayed hypersensitivity, may actually be minimal in some of these patients. It has now been shown that during reversal reaction, cell mediated immune responses to *M. leprae* antigens, measured *in vitro*, are increased ([6, 14]). These *in vitro* responses have been found to be related more to hypersensitivity and inflammation than to protective function. That we could not detect antigens after reversal reaction indicates that the increased lymphoproliferative reactions are associated with clearing of the antigen also. The elimination of the antigens during this reaction perhaps can also explain why reversal reaction usually occurs only once or twice in borderline tuberculoid patients.

The pathogenesis of ENL is poorly understood and many theories have been put forward ([15, 40]). Our findings show that ENL is related to extracellular perivascular *M. leprae* antigens. That IgM and complement deposits could be found in LL patients without ENL indicates that the initiation of this complication may depend on other host factors apart from immune complexes. Work to clarify this issue is in progress, but preliminary findings suggest a disturbance of suppressor T cells during ENL.

Despite the disadvantages of expensive antisera and the possible carcinogenicity of diaminobenzidine, immunoperoxidase staining can be a powerful research tool. The problem of false positives, which can be obtained in biopsies where there is a heavy inflammatory response, can be solved by using enzyme systems that are not found or induced in mammalian tissues. Glucose-oxidase is one such enzyme. Systematic studies of serial sections from early disease and during reactional episodes, using different types of antisera, e.g., anti-myelin basic proteins and monoclonal antisera specific to *M. leprae*, should be undertaken to elucidate the pathogenesis of leprosy as well as the fate and types of *M. leprae* antigens involved in tissue damage. Further, characterization of the cellular infiltrate in leprosy tissues can now be undertaken using specific monoclonal antibodies. Work to this end has been started.

SUMMARY

Biopsies from 69 patients with leprosy were stained to demonstrate mycobacterial antigens using immunoperoxidase methods. The same biopsies were cut and stained using Fite-Faraco, TRIFF and hematoxylin-eosin for classifying the patients and to demonstrate mycobacteria. Since *M. leprae* and BCG show extensive antigenic cross reactions, anti-BCG antibodies were used as primary antisera to demonstrate cross-reacting antigens of *M. leprae*. Cross-reacting mycobacterial antigens were, thus, found in all LL and BL leprosy patients. Eight out of 10 patients with indeterminate leprosy had mycobacterial antigens and 17 out of 19 BT leprosy patients were positive for antigens. In general, in the BT patients the presence of the antigen was related to the host tissue reaction; this relationship was found in only half of the patients with indeterminate leprosy studied. During ENL mycobacterial antigens were found both intra- and extra-cellularly in the inflammatory infiltrate, but the polymorphonuclear leukocyte infiltration was seen only around the extracellular perivascular antigen. In reversal reaction, the inflammatory response was towards extracellular mycobacterial antigens. After this reaction there were no antigens demonstrable.

RESUMEN

Usando el método de la inmunoperoxidasa se tiñeron biopsias de 69 pacientes con lepra para demostrar la presencia de antígenos micobacterianos en los tejidos. Las mismas biopsias fueron seccionadas y teñidas por las técnicas de Fite-Faraco, TRIFF, y hematoxilina-eosina, para clasificar a los pacientes y para demostrar micobacterias. Puesto que el *M. leprae* y el BCG muestran una extensa reactividad cruzada, se utilizaron los anticuerpos anti-BCG como antisueros primarios para demostrar antígenos de reacción cruzada en el *M. leprae*. Así se encontraron antígenos micobacterianos de reacción cruzada en todos los pacientes LL y BL. Ocho de 10 pacientes con lepra indeterminada tuvieron antígenos micobacterianos, y 17 de 19 pacientes con lepra BT tuvieron tales antígenos. En general, en los pacientes BT la presencia de antígenos micobacterianos estuvo relacionada con la reacción tisular del huésped; esta relación sólo se encontró en la mitad de los pacientes con lepra indeterminada estudiados. Durante los cuadros de ENL, los antígenos micobacterianos se encontraron tanto intra- como extra-celularmente en el infiltrado inflamatorio, pero la infiltración por leucocitos polimorfonucleares sólo se observó alrededor del antígeno extracelular perivascular. En la reacción reversa, la respuesta inflamatoria fue hacia los antígenos micobacterianos extracelulares. Después de esta reacción no quedaron antígenos demostrables.

RÉSUMÉ

On a coloré des biopsies provenant de 69 malades de la lèpre, en vue de démontrer les antigènes mycobactériens, par l'emploi de méthodes à l'immunoperoxydase. Les mêmes biopsies ont été débitées et colorées par les méthodes de Fite-Faraco, TRIFF, et l'hématoxyline eosine, afin de classer les malades et de mettre en évidence les mycobactéries. Du fait que *M. leprae* et le BCG présentent des réactions croisées antigéniques sur une gamme étendue, des anticorps anti-BCG ont été utilisés comme antisera primaires, dans le but de démontrer les antigènes réagissant de façon croisée avec *M. leprae*. Les antigènes mycobactériens, avec réactivité croisée ont été dès lors trouvés chez tous les malades LL et BL. Parmi 10 malades atteints de lèpre indéterminée, huit présentaient des antigènes mycobactériens, et 17 sur 19 malades atteints de lèpre BT étaient positifs pour les antigènes. En général, chez les malades BT, la présence de l'antigène était en relation avec la réaction tissulaire de l'hôte; cette relation n'a été trouvée que chez la moitié des malades présentant une lèpre indéterminée. Au cours de l'érythème noueux lépreux, on a observé des antigènes mycobactériens à la fois dans le milieu intra- et extra-cellulaire de l'infiltrat inflammatoire; l'infiltration à leucocytes morphonucléaires n'a toutefois été relevée qu'autour de l'antigène périvasculaire extra-cellulaire. Dans les réactions inverses (reversal reactions), la réponse inflammatoire était dirigée contre les antigènes mycobactériens extra-cellulaires. Lorsque cette réaction était terminée on ne pouvait plus mettre en évidence d'antigènes.

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