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# Erythema Nodosum Leprosum (ENL). Ultrastructure of the Connective Tissue Response[1]

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Recently we concluded that erythema nodosum leprosum (ENL) supervenes in lepromatous leprosy (LL) at a particular local antigen-antibody ratio when extravascular immune complexes are formed in slight antigen excess. The antigen is degraded cell walls, particulate acid-fast debris and a diffuse BCG positive component, which can be detected in macrophages and neutrophils at the reaction site. Immunoglobulin and complement are also present in these cells ([4]). We postulated that necrosis and edema associated with the reaction could cause dispersal of antigen which might account for the connective tissue response in ENL. Although we could demonstrate immunoglobulins, complement, lysozyme, the coagulation protein plasminogen, and the acute phase reactants C-reactive protein (CRP) and $\beta$-lipoprotein, bound to damaged connective tissue and elastic fibrils in the dermis, we failed to demonstrate any antigen by light microscopy. Thus it was not clear whether the collagen damage was directly related to the leprosy bacilli. We therefore undertook this electron microscopic study to search for *Mycobacterium leprae* antigen in ENL of the New Guinea type with particular attention to connective tissue. In these patients, the connective tissue response in the dermis is often very severe ([2]). Okada, *et al.* ([1]) have already demonstrated the presence of mycobacteria in association with ferritin-bound antibody in an ultrastructural study of ENL, but they were not concerned with the connective tissue far removed from the area of reaction.

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## MATERIAL AND METHODS

Two biopsies of ENL included previously in histopathological studies ([2, 4]) were available for electron microscopy. The samples were finely divided into 1 $mm^3$ pieces and fixed in cold 3% glutaraldehyde in cacodylate buffer, pH 7.4, for 3 hr before being transferred to a cacodylate-sucrose washing buffer. They were post-fixed in 1% osmium tetroxide, processed and embedded in epon. One micron sections were used for orientation of the block. Both biopsies came from New Guinea; biopsy A was from an acute dermal ENL and biopsy B was from an early resolving subacute lesion.

## RESULTS

The connective tissue of both the acute as well as the subacute lesions is equally involved in the reaction. In both cases, most striking is the abundance of foamy membrane-bound sacs containing bacterial debris, widely distributed over the connective tissue, intimately impacted between collagen (Figs. 1 and 2). They appear to be intact phagosomes containing a serous material in which the debris is embedded. Some of the debris has the electron-transparent halo characteristic of mycobacteria. The debris comprises bits of cell wall which is the main component, and some electron-dense bacterial cell bodies, presumably cytoplasm which is contained within degenerate cell walls. When the phagosome ruptures, the degenerate cell walls and debris are released into the extracellular space. The space is occupied by a homogeneous amorphous material and degenerate aggregated collagen fibrils on which bacterial debris is deposited. Elsewhere intact new collagen of banded appearance predominates, especially in the resolving lesion (Figs. 3 and 4).

In the acute lesion, rounded monocytes have ingested large quantities of dispersed phagosomes (Fig. 5). The nucleus of these cells is similar to that of tissue macrophages.

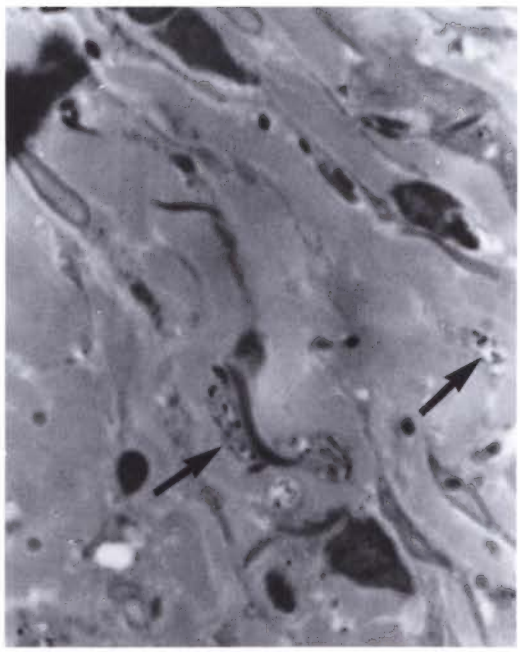

FIG. 1. Membrane-bound sacs containing unstainable bacterial debris are distributed over collagen, causing disruption of elastic (↕) ( ×1000).

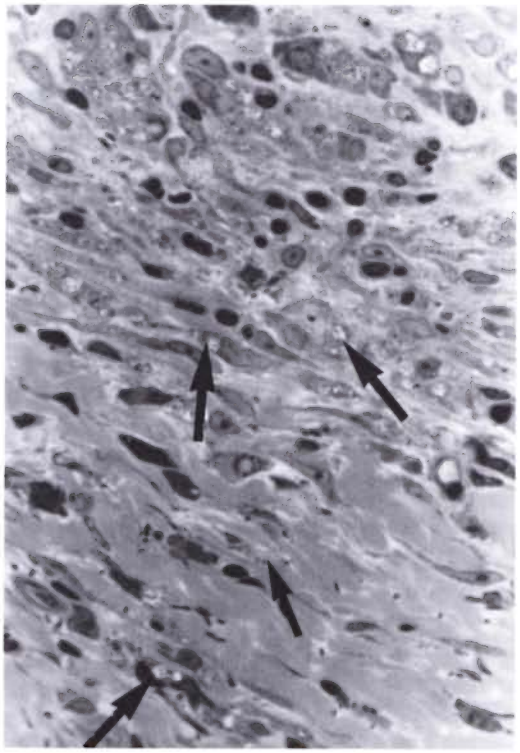

FIG. 3. Abundant degenerate bacterial debris deposits in connective tissue far removed from the reaction site (↕) ( ×500).

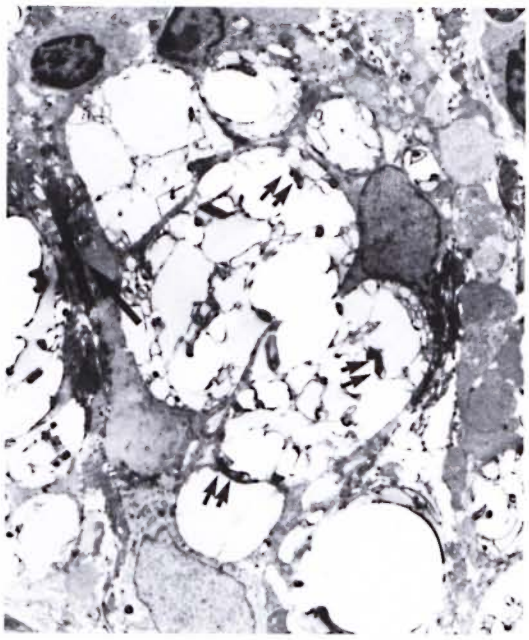

FIG. 2. Membrane-bound sacs appear to be intact phagosomes containing decayed bacterial debris (↕↕). Degenerate collagen fibrils are seen in a homogenous matrix (↕) ( ×10,000).

It is large with marginated chromatin and sometimes a conspicuous nucleolus is present. The scanty cytoplasm contains few organelles. Other cells present in the edematous connective tissue are fibrocytes which are very elongated cells with a slender pale nucleus and inactive cytoplasm. Mast cells are also seen, many of them showing degenerative changes.

Spindle-shaped tissue macrophages are recognized by a large pale oval nucleus with a rim of chromatin and prominent nucleolus. The cytoplasm is uniformly granular with few organelles, inconspicuous Golgi apparatus, mitochondria, secretory vesicles, and varying amounts of ingested phagosomes containing bacterial debris. They have a notably smooth plasmalemma (Fig. 6). Inflammatory macrophages, by contrast, have an irregular nucleus and active plasmalemma. Closely associated with the tissue macrophages are fibroblasts, distinguished by distended rough endoplasmic

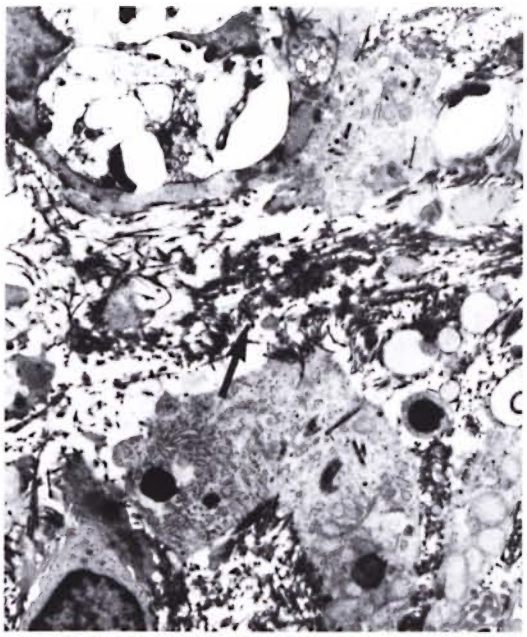

FIG. 4. Homogenous amorphous material is abundant. New collagen fibrils appear in resolving lesions (↕) ( ×10,000).

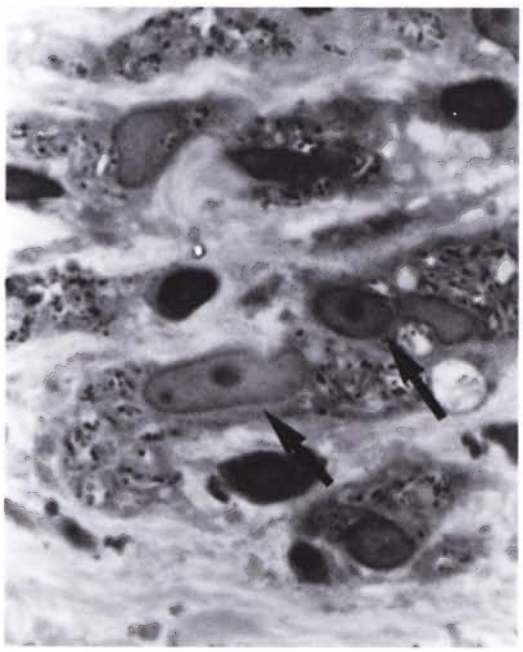

FIG. 6. Tissue macrophage with ingested bacterial debris has a large oval nucleus, prominent nucleolus and smooth plasmalemma (↕) ( ×1000).

FIG. 5. Monocyte with ingested phagosomes containing degenerate bacilli. Some organisms lie free in the extracellular matrix (↕) ( ×10,000).

reticulum and newly formed banded collagen in and around them. Lymphocytes and plasma cells are commonly seen among the fibroblasts, and plasma cells are sometimes closely aligned to degenerate phagosomes and bacterial debris. Giant cells with the nuclear and cytoplasmic characteristics of tissue macrophages are sometimes seen in intercollagenous bundles (Fig. 7). They contain 2–3 nuclei. The cytoplasm is conspicuously granular with few organelles, and in this they are different from giant cells of inflammatory origin ([4]). Ingested phagosomal debris is found in the giant cells.

The small blood vessels in the acute lesion have marked hyperplasia of endothelial cells, closely apposed in the luminal space. The basal lamina in some vessels is reduplicated with varying amounts of fine amorphous material deposited between layers of increased coarse basement membrane. Capillaries in the subacute lesion are plentiful and patent. The myoblasts of medium-size vessels are intact, with typical myofibrils in the cytoplasm. Phagosomal-

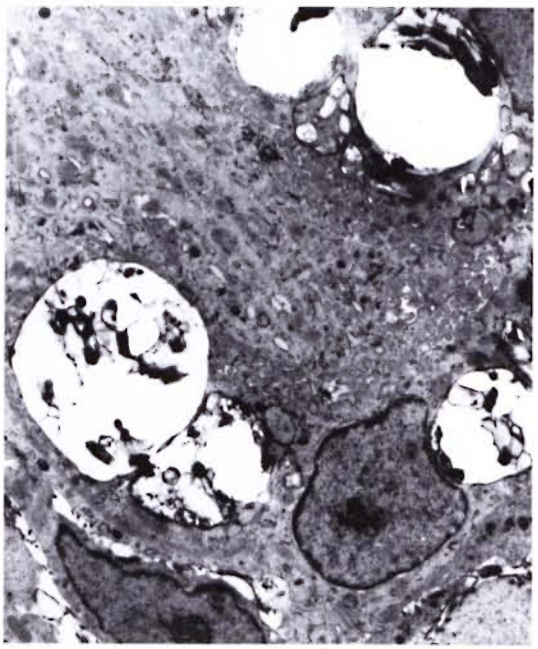

FIG. 7. Giant cell has oval nuclei with prominent nucleolus and cytoplasm with few organelles. Ingested phagosomes are seen containing decaying bacterial debris ( ×10,000).

bacterial debris is found between adjacent cells. Some perithelial cells have small amounts of ingested bacterial debris and perithelial edema is present. The dermal nerves are unaffected by the reaction although they contain leprosy bacilli. Neutrophils are not seen around degenerating collagen.

## DISCUSSION

Bacterial antigen is detected with difficulty in ENL using immunofluorescence, possibly because the antigen is degraded to an undetectable form ([6, 7, 8]). By the immunoperoxidase technique and silver impregnation, bacterial components could be detected more readily in and around the degenerate macrophages on which the reaction was centered ([4]). But bacterial antigen was often sparse or absent in the areas of severe acute and subacute connective tissue damage in the dermis, which typifies the New Guinean form of ENL ([2]) and is present to some extent in other forms of ENL. In the present study, electron microscopy demonstrated a much greater amount of extracellular bacterial antigen in the form of degenerate organisms and debris, much of it in the phagosomes set free by the disintegration of the macrophages. Although the study was confined to the New Guinea type of ENL, it would be surprising if this finding did not apply also to the other types in which the granulomatous involvement is more prominent.

In the areas of connective tissue damage away from the granuloma, degenerate collagen was seen to be embedded in a homogeneous and edematous mass of tissue debris, in which we had previously demonstrated immunoglobulins, complement components and CRP, but not bacterial antigen. By electron microscopy, however, bacterial debris was easily demonstrable and, where present, it was associated with degeneration of collagen or elastic fibers. The bacterial debris was in an advanced stage of decay, which suggests that the failure to detect it by light microscopy was because much of it had diffused away. The debris in the connective tissue areas persisted into the subacute phase of the reaction, in which collagen damage is still very evident. Combining the evidence of the present study and earlier immunoperoxidase results, it is demonstrated that all of the components of mycobacterial immune complexes are present in the areas of connective tissue damage, and there is no necessity to postulate non-mycobacterial antigens or auto-immune mechanisms to explain the connective tissue damage. However, it is not clear why the damage should be so much greater in some forms of ENL than in others, or why there should apparently be an ethnic predisposition to it.

It is highly unlikely that an organism with such a low toxicity for human tissue as *M. leprae* should be responsible, per se, for acute destruction of collagen and elastic tissue. Nor is there evidence that the degraded bacterial components or the free antigen is much more toxic than the intact bacilli in the absence of delayed hypersensitivity, which was, of course, lacking in our patients. This supports our contention that immune complexes at an appropriate antigen-antibody ratio (probably near equivalence) may be more damaging to tissue than uncomplexed antigen of a non-toxic sort ([4, 5]). It may be either that the complex itself is destructive

or, perhaps more likely, that the complexing of antigen bound to cells or fibers damages the membranes to which it is bound.

**Note Added in Proof.** Two additional biopsies received from India have now been studied. There was no substantial difference noted from those already reported, and dispersal of *M. leprae* antigen with subsequent binding to connective tissue elements in skin appears to be a feature of all ENL lesions. The persistence of soluble components, not visible by electron microscopy, may conceivably be related to the severity of collagen damage which follows, as observed in some cases.


## SUMMARY

In ENL lesions of the type associated with severe damage to the connective tissue of the dermis, large quantities of bacterial debris were demonstrated by electron microscopy, although not by light microscopy. The debris, in an advanced stage of degeneration, was present in the phagosomes of decrepit macrophages, in the extracellular compartment and, in particular, bound to degenerate collagen and elastic where immunoglobulin, complement, and inflammatory mediators had been demonstrated previously. It is suggested that the complexing of mycobacterial antigen is a major factor in the causation of connective tissue damage, as well as other aspects of ENL. The reason why connective tissue involvement is so variable has not been explained.

## RESUMEN

En lesiones ENL del tipo asociado can daño severo del tejido conectivo de la dermis, se demostró la presencia de grandes cantidades de detritus bacterianos sólo cuando se usó la microscopía electrónica. Los detritus, en un avanzado estado de degeneración, estuvieron presentes en los fagosomas de los macrófagos decrépitos, en el compartimento extracelular y, en particular, asociados al tejido colágeno y elástico donde previamente se han demostrado depósitos de inmunoglobulinas, complemento y mediadores inflamatorios. Se sugiere la importancia de los antígenos micobacterianos como participantes en el daño del tejido conectivo que acompaña al ENL. No se ha explicado el porqué la afección del tejido conectivo es tan variable.

## RÉSUMÉ

Au moyen de la microscopie électronique, on a pu mettre en évidence de grandes quantités de débris bactériens dans les lésions d'érythème noueux lépreux (ENL) du type associé avec un dommage grave des tissus conjonctifs du derme, alors qu'il n'était pas possible de démontrer un tel phénomène au moyen de la microscopie optique. Les débris étaient dans un état avancé de dégénération; ils étaient présents dans les phagosomes des macrophages décrépits, dans le compartiment extra-cellulaire, et en particulier on les a trouvés liés à du tissu collagène ou élastique, là où on avait pu mettre en évidence auparavant les immunoglobulines, le complément, et des médiateurs inflammatoires. On suggère que la transformation de l'antigène mycobactérien en complexe constitue un facteur majeur dans la cause du dommage tissulaire conjonctif, de même que dans l'apparition des autres aspects de l'ENL. La raison pour laquelle la teinte du tissu conjonctif est si variable n'a pas pu être expliquée.


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