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Histochemical Study of
Erythema Nodosum Leprosum (ENL) Lesions^{1, 2}R. M. Abalos, J. G. Tolentino and C. C. Bustillo³

Few investigators have employed histochemical examinations in the study of leprosy patients. Hollander and Sommers (14) in a histochemical study of mucopolysaccharides of the skin of leprosy patients demonstrated the presence of acid mucopolysaccharide in normal amounts and a decrease in neutral polysaccharide. Ghosh (12) showed the presence of neutral fat and phospholipid in bacilli-laden lepra cells. Sakurai and Skinsnes (29), in a study of lipids in leprosy, showed the presence of phospholipids, fatty acids and neutral fat in higher content in lepromatous than in tuberculoid leprosy. Pepler *et al* (27) reported the presence of higher content of acid phosphatase in lepromatous than in tuberculoid leprosy. They suggested that this enzyme may be of importance in the lipid metabolism of the lepra bacillus. Job (17), in the study of lysosomal activity of macrophages in leprosy, showed increased acid phosphatase activity both in lepromatous and tuberculoid leprosy. Aquino and Skins-

nes (2) demonstrated cytochemically the presence of acid phosphatase in the various subcellular components of Virchow cells and attempted to formulate a structural pathway for the degradation of phagocytosed leprosy bacilli.

The foregoing investigations were conducted on leprosy patients not undergoing reaction. Mabalay *et al* (20) first reported on the histochemical changes seen in leprosy patients with *erythema nodosum leprosum* (ENL) lesions. They demonstrated that the amount of dermal acid mucopolysaccharide did not differ very much from that of a normal skin except in areas of acute inflammation where the nonsulfated hyaluronidase labile acid mucopolysaccharide was slight or absent but was present in moderate amount in areas not undergoing reaction. Their study showed the presence of periodic acid Schiff (PAS) positive diastase resistant materials in histiocytes and mast cells and observed it to be increased in early lesions of ENL. They suggested that these cells may play a role in production of ENL.

This paper presents the results of a histochemical study of ENL lesions and discusses the possible role that lysosomal enzymes may play in the pathogenesis of ENL.

MATERIALS AND METHODS

Biopsy materials were taken from 14 carefully selected lepromatous patients with ENL lesions. Each biopsy specimen was divided

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into three portions: the first was fixed in buffered formalin, processed, sectioned and stained with hematoxylin and eosin to confirm the diagnosis while another section was stained by the Fite-Faraco method ⁽²¹⁾ for acid-fast bacilli; the second portion was fixed in formol-calcium, processed, sectioned for neutral fat and phospholipid; the third portion was immediately frozen for enzyme studies. Sections were cut at four microns in a cryostat (-30°C). The following histochemical procedures were performed.

A. Carbohydrates

1. Periodic acid Schiff (PAS) with and without diastase digestion and with pyridine extraction ⁽²¹⁾.
2. Alcian blue with and without hyaluronidase digestion (pH 2.5) ⁽²¹⁾.
3. Colloidal iron stain with and without hyaluronidase digestion ⁽¹⁹⁾.
4. Gomori's aldehyde-fuchsin stain ⁽²¹⁾.
5. Gomori's reticulum stain ⁽²¹⁾.

B. Lipids

1. Neutral fat ⁽²²⁾.
2. Phospholipid—Baker's acid hematein with and without pyridine extraction ⁽³⁾.
3. Cholesterol ⁽²⁴⁾.

C. Enzymes

1. Alkaline phosphatase ⁽⁴⁾.
2. Aryl sulfatase ⁽²⁵⁾.
3. Gomori's acid phosphatase ⁽⁵⁾.

Negative controls for enzyme studies consisted of heating sections at 60°C for 30 minutes and incubating them without substrate or capture reagent in the media.

RESULTS

Hematoxylin and eosin stain. Sections generally showed a mild to moderate degree of interstitial edema mostly in the deeper corium. The presence of infiltrate varied in degree, some being very rich in polymorphonuclear leukocytes and others mostly histiocytic. Microabscesses⁴ were frequently observed and vascular changes affecting mostly the smaller vascular channels, varying from edema of the wall to severe destruction producing necrotizing arteriolitis, were occasionally observed.

Fite-Faraco stain. Acid-fast bacilli were numerous in areas infiltrated mostly by his-

tiocytes and few to completely absent in the centers of microabscesses. Generally the bacilli appeared granular.

PAS with and without diastase digestion and with pyridine extraction. PAS positive materials were observed in the epidermis, reticular stroma, blood vessels, nerves, sweat glands, sebaceous glands, hair follicles, mast cells, macrophages, bacilli and in areas of microabscesses. The intensity of the PAS positive reaction diminished after pyridine extraction, especially in areas of lepromatous cells infiltrate. The results suggested the presence of lipid component as one of the PAS positive materials. Diastase digestion failed to remove or diminish the PAS positive reaction in all the areas mentioned except in the zones of acute inflammation. The results confirmed the presence of glycogen in neutrophilic granulocytes.

Alcian blue with and without hyaluronidase digestion (pH 2.5). The supporting stroma, especially the papillary portion of the upper dermis, showed a moderate to marked degree of reaction. Reaction was observed in blood vessels, nerves, sweat glands, hair follicles and mast cells. Slight to complete absence of reaction was noted in zones of microabscess formation. After hyaluronidase digestion, the reaction in the reticular stroma was markedly diminished, whereas in other areas the reaction remained unchanged. The results suggested the presence of nonsulfated hyaluronidase labile acid mucopolysaccharide in the reticular stroma and of a more sulfated type of acid mucopolysaccharide in the different specialized dermal structures.

Colloidal iron with and without hyaluronidase digestion. Positive reaction was mainly observed in areas of histiocytic cell infiltrate, sweat glands and blood vessels. The intensity of staining reaction in areas of histiocytic cell infiltration appeared to be dependent on the bacillary content of the macrophages. Reaction was markedly diminished or absent in areas of microabscesses. Slight or no change in the intensity of reaction was observed after hyaluronidase digestion. The reaction was mostly due to the presence of a sulfated type of acid mucopolysaccharide.

Gomori's aldehyde-fuchsin stain. Delicate elastic fibers were seen especially in the upper dermis between collagen bundles and around areas of histiocytic cell infiltration. Little or no elastic fibers were observed in

⁴Small pocket collections of neutrophilic granulocytes.

central portions of microabscesses. Mast cells stained predominantly and were seen mostly around blood vessels and in reactive zones. They were more evident in early lesions.

Gomori's reticulum stain. Fine reticulum fibers were observed around and within areas of histiocytic infiltration. They were also seen in areas of abscesses but were fragmented, few or absent in the central portion of microabscesses.

Oil red O stain for neutral fat. Reaction was prominently demonstrated in areas of histiocytic cell infiltration. The reaction varied markedly in different areas and appeared related to the bacillary content of the macrophages. Little or no reaction was demonstrated in zones of acute inflammation.

Baker's acid hematein with and without pyridine extraction for phospholipid. Reaction was mainly demonstrated in histiocytes and bacilli. Little or no reaction was seen in areas of acute inflammation. Reaction disappeared completely after pyridine extraction.

Schultz method for cholesterol. Reaction was absent in all areas of the section.

Alkaline phosphatase. Reaction was observed in capillaries and in areas of microabscesses where the reaction was diffused and particulate. This finding may be due to the presence of alkaline phosphatase in neutrophilic granulocytes.

Aryl sulfatase. Reaction was particulate and localized within macrophages. Little or no reaction was seen in areas of acute inflammation.

Acid phosphatase. Mild reaction was observed in the keratin layer of epidermis. Marked reaction was demonstrated within lepromatous cell infiltrates and appeared well-localized within macrophages (Fig. 1). The reaction was less intense, diffuse and particulate in zones of acute inflammation. Reaction was almost absent in the central portions of microabscesses (Fig. 2).

Table 1 summarizes the results of the histochemical findings.

DISCUSSION

This study showed 1) the presence of moderate amounts of PAS positive diastase resistant material, acid mucopolysaccharide, neutral fat, phospholipid, aryl sulfatase, and acid phosphatase in areas around and away from reactional sites; and 2) the presence of

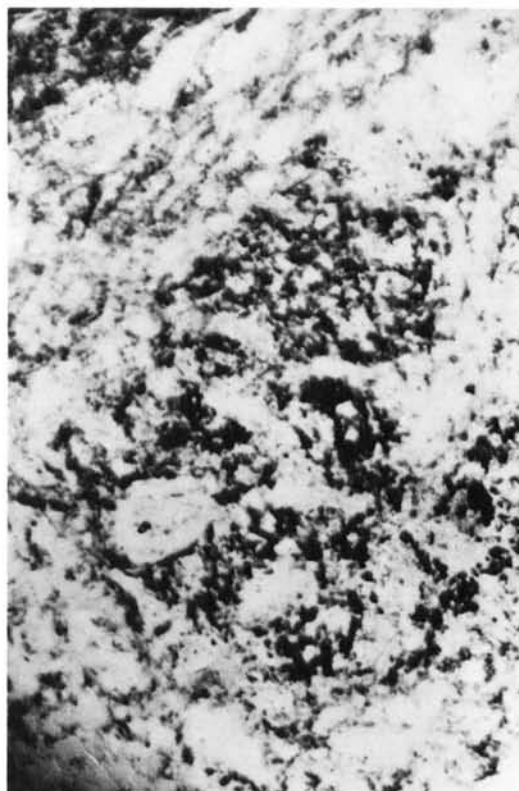


FIG. 1. A low power photomicrograph showing a marked increase in acid phosphatase activity within a lepromatous cell infiltrate and which appeared to be localized within macrophages. Acid phosphatase, Gomori. $\times 100$.

slight amounts or absence of these materials within zones of acute inflammation. The loss of staining reaction for hydrolytic enzymes in areas of acute inflammation leads one to suspect solubilization and leakage of these enzymes into the surrounding and supporting tissue, thus producing cellular destruction with resultant inflammatory processes.

De Duve, in his tissue fractionization studies, postulated the presence of a group of cytoplasmic organelles enclosed within a lipoprotein membrane containing various hydrolytic enzymes called lysosomes⁽⁹⁾. They were believed to perform vital intracellular digestive functions and it has been thought that the release of these hydrolases into the cell sap or surrounding tissue may produce localized tissue destruction and catabolism of cell constituents.

Several factors and substances such as Vitamin A and streptolysin toxin are known to promote release of enzymes, while corti-

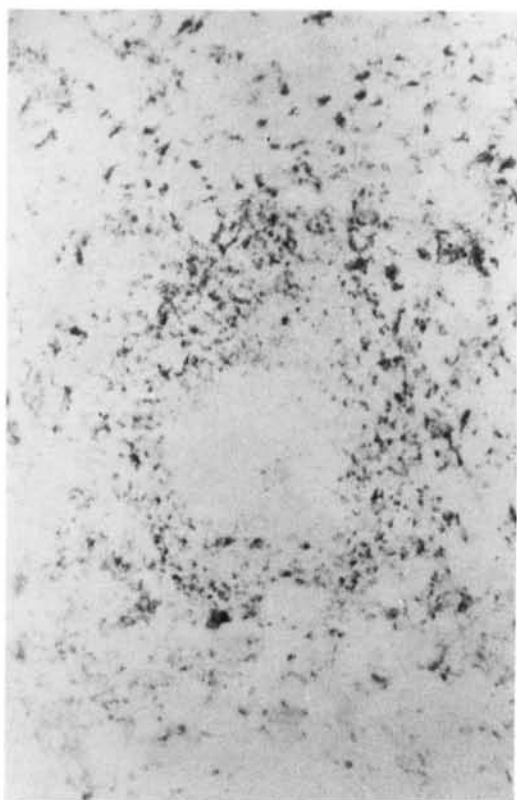


FIG. 2. An area of an acute inflammation. The reaction was less intense, diffuse and particulate in zones of acute inflammation. Acid phosphatase, Gomori. $\times 100$.

sone, chloroquin and phenergan inhibit release of enzymes by stabilization of the lysosomal membrane (¹⁰). It is interesting to note that both cortisone and chloroquin are being used in the treatment of ENL.

Yamamoto *et al* (³⁴) described the opaque droplets in leprosy and Brieger and Allen (⁶) identified a cytosome-like substance by electron microscopy. Imaeda in his histochemical study observed these to be lysosomal. Imaeda also observed that the amount of lysosomal substance appeared more increased in patients undergoing DDS therapy than in untreated patients (¹⁶). Palekar and Magar (²³) showed that the lysosomal enzymes of leprosy patients decreased significantly after DDS treatment and suggested that DDS may act on the lysosomes in such a way as to render the cell cytoplasm unsuitable for multiplication and survival of the bacilli. Prabhakaran and Bapat (²⁸) suggested that DDS induces bacterial lysis indirectly by effecting the release of lysosomal enzymes

in Virchow cells.

It is known that ENL is commonly precipitated by chemotherapy. Other factors such as emotional upsets, stress and infections have been implicated as precipitating attacks of ENL. Abe *et al* (¹) observed a significant rise in ASO titer in lepromatous patients with ENL and considered this an indication of a hidden infection by hemolytic streptococci. They pointed out that such an infection may play a role in the pathogenesis of ENL. Streptococcal toxin was shown to promote release of lysosomal enzymes (³¹).

Job *et al* (¹⁸), among others (^{11, 26, 33}), described the pathologic changes in ENL as resembling those seen in the Arthus phenomenon. Cochrane *et al* (⁷) and Humphrey (¹⁵) demonstrated that the presence of polymorphonuclear leukocytes is essential in the production of the Arthus reaction. Animals rendered neutropenic with nitrogen mustard failed to exhibit the Arthus reaction (¹⁵). Cohn and Hirsch (⁸) demonstrated that the granules of leukocytes possess the properties and enzymatic composition of lysosomes. Thomas (³⁰) showed that injection of polymorphonuclear granules intensifies the Arthus reaction and also prepares the site for the Schwartzman reaction. Golub and Spitznagel (¹³) showed that the injection of polymorphonuclear granules into the skin produces lesions whose histologic picture is similar to that seen in the Arthus reaction. Wemambu *et al* (³²) have shown by fluorescent microscopy that deposits of immunoglobulin and complement were seen in areas of polymorphonuclear leukocyte infiltration of ENL lesions.

These observations suggest that the occurrence of ENL is probably precipitated by factors that promote leakage of lysosome. It is probable that DDS acts directly on the lysosomal membrane, or by its capacity to activate or stimulate production of lysosomes may also increase bacterial metabolites which accumulate and distend old decrepit lepra cells with resultant rupture of their membrane. The release of lysosomes within the cell sap will produce cellular death and, as a result, it is not unreasonable to speculate that the following immunologic mechanisms may take place: 1) release of lysosomal enzymes may produce denaturation of cell constituents and may induce the production of autoantibodies, and 2) the release of bac-

TABLE 1. Summary of histochemical findings in ENL lesions.

Stain	Microscopic results	
	Nonreactive areas	Reactive areas
Periodic acid-Schiff		
without diastase digestion	++++	++++
with diastase digestion	++++	++
with pyridine digestion	++	++
Alcian blue		
without hyaluronidase digestion	++++	±
with hyaluronidase digestion	+++	±
Colloidal iron		
without hyaluronidase digestion	++++	±
with hyaluronidase digestion	+++	±
Aldehyde-fuchsin	+++	±
Reticulum	+++	±
Neutral fat	++++	±
Phospholipid, Baker's acid hematein		
without pyridine extraction	++++	±
with pyridine extraction	0	0
Cholesterol	0	0
Alkaline phosphatase	+	++
Aryl sulfatase	++++	±
Acid phosphatase	++++	±

^aEstimated degree of stain intensity, 0 to ++++.

terial antigens into the extracellular fluid may incite antigen-antibody reactions.

Either one or both of these mechanisms may be operative and, as a result of antigen-antibody reaction, complexes may be formed and probably be deposited in the wall of blood vessels. These have chemotactic properties and attract polymorphonuclear leukocytes which most likely ingest the complexes. Ingestion of the complexes degranulates the polymorphonuclears releasing the lysosomal contents and these produce the various vascular changes seen in ENL and which resemble those seen in the Arthus reaction.

SUMMARY

A histochemical study of ENL lesions was performed on skin biopsies from 14 lepromatous patients. There was: 1) the presence of moderate amounts of PAS positive diastase resistant materials, acid mucopolysaccharide, neutral fat, phospholipid, aryl sulfatase and acid phosphatase in areas around and away from reactional sites; 2) the presence of slight amounts or complete absence of these materials within areas of acute inflammation. These changes were interpreted as the result of solubilization and leakage of hydrolytic enzymes into the surrounding tissue. A pos-

sible role of lysosomal enzymes in the pathogenesis of ENL lesions is discussed.

RESUMEN

Se hizo un estudio histoquímico de lesiones de ENL en biopsias de piel de 14 pacientes lepromatosos. Se encontró: 1) presencia de cantidades moderadas de materiales PAS positivo diastasa resistentes, mucopolisacáridos ácidos, grasa neutra, fosfolípidos, aryl sulfatasa y fosfatasa-ácido en las áreas alrededor y lejos de los sitios reaccionales; 2) presencia de pequeñas cantidades o completa ausencia de estos materiales en las zonas de inflamación aguda. Estos cambios se interpretan como el resultado de la solubilización y drenaje de enzimas hidrolíticas hacia el tejido circundante. Se discute el posible papel de las enzimas lisosomiales en la patogénesis de las lesiones de ENL.

RÉSUMÉ

On a procédé à des études histochimiques des lésions d'érythème noueux lépreux, sur des biopsies cutanées obtenues de 14 malades lépromateux. Les résultats ont été les suivants: 1) la présence de quantités modérées de tissus positifs au PAS et résistant à la diastase, d'acide mucopolysaccharidique, de graisse neutre, de phospholipides, d'aryl sulfatase et d'acide phosphatase, autour des endroits réactionnels, et dans des sites plus éloignés; 2) la présence de faibles

quantités, ou même l'absence complète, de ces composés dans des régions d'inflammation aiguë. Ces modifications ont été interprétées comme témoignant de la solubilisation et de la pénétration des enzymes hydrolytiques dans les tissus avoisinants. Le rôle possible d'enzymes des lysosomes dans la pathogénèse des lésions d'érythème noueux lépreux, est discuté.

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