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Acid Mucopolysaccharide Metabolism in Leprosy 1. Storage of Hyaluronic Acid and its Possible Significance in the Pathogenesis of Leprosy[1, 2]

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Lipid storage in leprosy has been well recognized ([7, 9, 28, 29, 30]) but information regarding the storage or metabolism of other substances, especially saccharides, is limited despite their possible significance in mycobacterial metabolism. The present study is directed at examining the role of hyaluronic acid in the pathogenesis of leprosy. In an analysis of 102 routine biopsies from Zaire, Africa, abundant hyaluronic acid was found diffusely spread in the lepromas of early and relapsed lepromatous leprosy cases. It appears to decrease with increasing chronicity but it does not completely disappear in the majority of instances, even in very chronic cases. On the other hand, in tuberculoid leprosy, hyaluronic acid was present in early stages in the centers of the granulomas and seems to disappear within a couple of years, or in most cases less than that. In borderline cases, cells that are hyaluronic acid positive or that are negative or have moderate quantities of the substance are mixed and the hyaluronic acid positive cells are usually located in the periphery of the infiltrated foci. In other studies, to be reported, we have noted an enhancing effect of hyaluronic acid on the development of experimental lepromas. Those experiments also suggest that the ability of the host to dispose of hyaluronic acid in the vicinity of infecting *Mycobacterium leprae* plays more than a secondary role in the expression of the host's defense mechanism against leprosy. The results of the present study are suggestive of this possibility.

MATERIALS AND METHODS

Sources of materials. One hundred and two leprosy skin biopsies, fixed in formalin, were sent to our laboratory from the Institut Médical Evangélique, Kimpese, Republic of Zaire. Routine paraffin section processing was followed by Triff, acid-fast, and Mowry's colloid iron stain for the detection of acid mucopolysaccharide (AMPS), counter stained with periodic acid Schiff (PAS) in all instances ([21]). Toluidine blue and alcian blue staining at pH 0.4, 1.0 and 2.5 ([21]) and hyaluronidase ([21]) extraction were applied to selected cases.

Classification of cases. The cases were classified according to the five group system proposed by Ridley and Jopling ([26, 27]) and arranged according to the duration of their leprosy, as summarized in Table 1. Unfortunately, lepromin testing for these patients was not available.

Evaluation of the amount of acid mucopolysaccharide (AMPS). The amount of AMPS was evaluated in histopathologic sections, double stained by the Mowry PAS methods. The grading of AMPS concentration was as follows: –, negative; ±, trace; +, slight; ++, moderate; and +++, abundant. This evaluation was studied for relationships or differences related to leprosy type and disease chronicity. Of necessity, the duration of the disease largely depended on the patient's own witness. Attention was also paid to the differences in AMPS distribution as related to the central or peripheral portions of the inflammatory infiltrations.

RESULTS

As shown in Table 1 and illustrated in Figure 1, a fairly large proportion of early lepromatous cases showed significant uniform accumulations of acid mucopolysaccha-

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TABLE 1. *Grading of hyaluronic acid concentration in the infiltration foci of the skin of various types and durations of leprosy.*

Types	Disease duration (years)	No. of cases	Age distribution	Sex		Hyaluronic acid in the foci of cellular infiltration					
				male	female	–	±	+	++	+++	
LL	less than 2	6	28-46	6	0	0	1	1	0	4	[a]
	2-4	9	23-55	6	3	2	1	1	2	3	
	5-7	6	33-57	4	2	3	1	1	1	0	
	11-13	3	23-40	1	2	0	1	1	0	1	
	more than 14	6	27-48	5	1	1	1	4	0	0	
BL	less than 2	2	25-35	1	1	0	0	0	1	1	[a]
	2-4	2	39-50	0	2	0	0	0	1	1	
	5-7	1	25	1	0	0	1	0	0	0	
	11-13	1	60	0	1	0	0	1	0	0	
	more than 14	1	35	0	1	0	0	1	0	0	
BB	less than 2	7	17-72	4	3	1	3	1	2	0	[a]
	2-4	4	22-62	2	2	0	1	3	1	0	
	5-7	3	12-50	2	1	0	0	3	0	0	
BT	less than 2	24	17-20	9	15	3	6	7	5	2	
	2-4	8	19-55	4	4	1	4	3	0	0	[a]
	5-7	1	40	0	1	1	0	0	0	0	
	8-10	1	60	1	0	0	0	1	0	0	
	11-13	2	36-50	0	2	0	1	1	0	0	
TT	less than 2	7	13-60	2	5	3	4	0	0	0	[a]
	2-4	3	23-35	2	1	0	3	0	0	0	
	5-7	2	35-40	0	2	1	0	0	1	0	
	11-13	3	46-60	0	3	3	0	0	0	0	

[a] Distribution of H.A. positive (•) and negative (○) cells in granuloma.

ride throughout the lepromas. Saccharide staining seemed to decrease chronologically but not markedly, even after a few years, except for the effectively treated cases.

Figure 2 represents the findings in early tuberculoid cases. Within a few months after the stated onset of skin lesions, acid mucopolysaccharide was seen centrally in the granulomas, the peripheral portions of which appeared somewhat edematous. Later, within one year in most cases, the central portions of the granulomas, while still staining for AMPS, were surrounded by lymphocytes which did not stain for AMPS. This infiltration separated the acid mucopolysaccharide positive areas from the surrounding dermis (Fig. 3). In the more fully developed granulomas, in later stages, the acid mucopolysaccharide decreased significantly in their central portions (Fig. 4).

In borderline lepromatous (BL) cases, the distribution of acid mucopolysaccharide seems to be essentially the same as that found in the lepromatous type although the deposition is not as heavy as in the lepromatous type.

In borderline (BB) type, macrophages with quantitatively varying staining for AMPS were mixed.

In the borderline tuberculoid (BT) type (Fig. 5), the granuloma in the early stage was characterized by the presence of histiocytes with stronger AMPS staining in the peripheral portion. Later, the AMPS of the central portion of the granuloma decreased or disappeared, but that of the periphery did not (Fig. 6). This distribution of acid mucopolysaccharide is contrary to that found in tuberculoid lesions where AMPS stained more intensely at the centers.

The acid mucopolysaccharide studied has the characteristics of hyaluronic acid, giving a stronger alcian blue coloration at pH 2.5 than at a lower pH and being destroyed by hyaluronidase extraction.

DISCUSSION

Because of the marked lipid storage evident in leprosy, particularly in lepromatous leprosy ([7, 9, 28, 29, 30]), there is reason to think

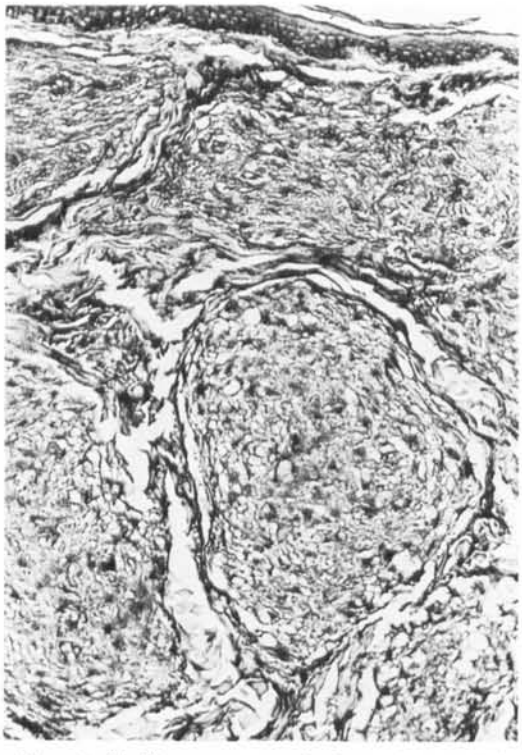

FIG. 1. Uniform accumulation of AMPS in lepromas (stained black). Mowry stain. Original mag. ×100.

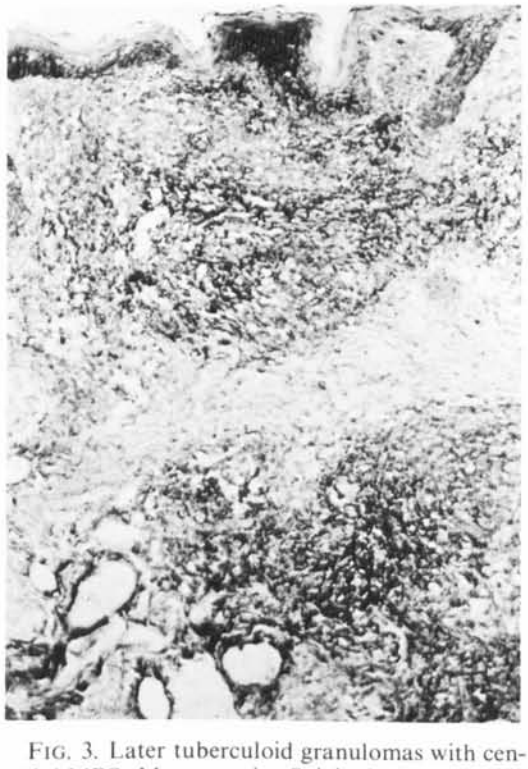

FIG. 3. Later tuberculoid granulomas with central AMPS. Mowry stain. Original mag. ×100.

FIG. 2. Early tuberculoid granulomas with AMPS centrally distributed. Mowry stain. Original mag. ×100.

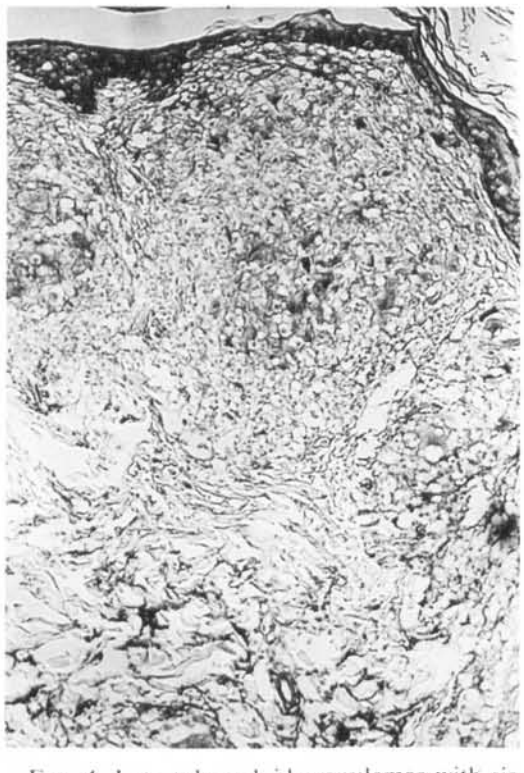

FIG. 4. Late tuberculoid granulomas with significantly decreased AMPS. Mowry stain. Original mag. ×100.

FIG. 5. Early borderline tuberculoid (BT) granulomas with peripheral AMPS staining. Mowry stain. Original mag. ×100.

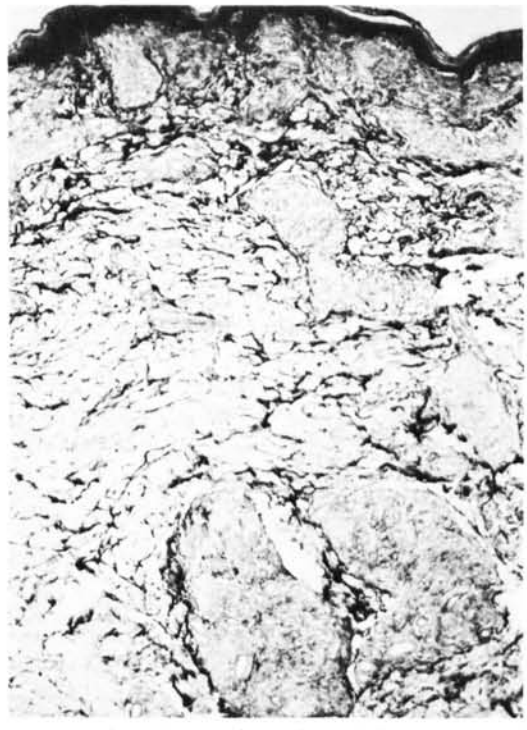

FIG. 6. Late borderline tuberculoid (BT) granulomas with peripheral AMPS staining only. Mowry stain. Original mag. ×100.

that leprosy, particularly of the lepromatous type, is related to some patient defect in ability to dispose of *M. leprae*, especially its lipid fraction. Barbieri and Correa [2], utilizing tissue cultures of human white blood cells, 35 of which were from tuberculoid leprosy, 40 from lepromatous patients and 50 from healthy persons, found that macrophages from tuberculoid and from lepromin positive healthy persons showed lytic activity against autoclaved *M. leprae* whereas those from lepromatous and Mitsuda negative healthy persons did not. Beiguelman [3, 4] showed similar results with macrophages from tuberculoid and lepromatous patients. Godal and Rees [8] in a study of five tuberculoid and five lepromatous patients were unable to confirm these findings. Nevertheless, Pisani and associates [23], from similar continuing studies involving 10 lepromatous, 10 tuberculoid, 10 dimorphous, 17 indeterminate and 7 cases of uncertain classification and utilizing a refined technic, report that tuberculoid macrophages showed lytic activity while lepromatous macrophages did not, and that dimorphous macrophages were predominantly weakly lytic while indeterminate type macrophages were represented by all three types of reaction.

Recently, Drutz *et al* [6] found that blood-derived macrophages had the same ability to digest heat-killed *Mycobacteria leprae* regardless of the types of leprosy from which they were derived. This seems to be a completely different result from the report by Saul [31] who showed differences in the ability of macrophages to handle *M. leprae*.

Comparative review of the lipid reticulocytoses for resemblance to the leprosy lipid storage phenomenon calls attention to Fabry's disease in which there is combined storage of fat and acid mucopolysaccharide [18]. Even the more common atherosclerosis of the aorta has been reported by Hartroft [10] as presenting combined storage of hyaluronic acid and ceroid, which is a variety of peroxidized fat. Sakurai and Skinsnes [29] noted dermal ceroid accumulation in B663 treated lepromatous leprosy. This combined storage might be explained by the fact that some lipids, such as β-lipoprotein of serum and acid mucopolysaccharide, generally form

insoluble complexes [14] and if such complexes are ingested by histiocytes, storage of the involved substances might develop. In the light of these considerations, it seems reasonable to think that understanding of the phenomenon of lipid storage in leprosy may be closely related also to understanding of the storage of acid mucopolysaccharides.

Mabalay *et al* [16], Hollander and Sommers [13] and Reyes [24] reported the presence of AMPS in lepromatous leprosy and its disappearance in *erythema nodosum leprosum*. However, Ribicini and Giménez [25] and Ghosh *et al* [7] denied its presence in lepromatous leprosy. In the present study, acid mucopolysaccharide having the characteristics of hyaluronic acid is not effectively stained in some cases by alcian blue. However, Mowry's colloid iron stain, counterstained with the PAS-reaction [21] showed quite striking accumulation of this AMPS, especially in some of the lepromatous cases. This acid mucopolysaccharide in histiocytes is especially strongly positive in early or recurrent lepromatous leprosy cases and less markedly positive in chronic and treated cases, but is still present to some degree in the majority of the latter. Its distribution in the leproma is homogenous in both lepromatous and borderline lepromatous types. In the course of tuberculoid cases, however, the acid mucopolysaccharide is seen in epithelioid cells and Langhans type giant cells in the centers of granulomas and surrounded by lymphocytes not staining for acid mucopolysaccharide. This infiltration of lymphocytes separates the acid mucopolysaccharide positive area from the surrounding dermis before the central portion of the granuloma loses this saccharide. This arrangement of the saccharide positive and negative areas in infiltrates is quite contrary to that found in borderline tuberculoid leprosy in which the positive staining area in the periphery of the granuloma is not separated from the surrounding tissue, as shown in Figures 5 and 6. The difference is striking, although the ordinarily stained tissues do not reveal the difference. That the strongest concentration of acid mucopolysaccharide occurs in the leproma of lepromatous leprosy and not in the tuberculoid type and shows the same pattern of deposition as that of lipid, might suggest the combined storage of lipid and hyaluronic acid in leprosy.

The hyaluronic acid in the foci of cellular infiltration must be regarded as being derived from either tissue fluid and serum, which formed complexes with *M. leprae* before ingestion by macrophages, or from synthesis by macrophages. There is no available evidence that any microorganisms, except group-A streptococci [5] and *Treponema pallidum* [34], can synthesize hyaluronic acid.

We have recently found that hyaluronic acid has an enhancing effect on the growth of *M. leprae* and *M. lepraemurium* subcutaneously inoculated into mice. Therefore, its activity may also be similar in human beings. The presented findings can be considered to be compatible with these experimental results. First, in active lepromatous leprosy the accumulation of acid mucopolysaccharide is marked. Secondly, in tuberculoid type the disappearance of the saccharide coincides with the development of granuloma. Thirdly, in borderline tuberculoid type, the acid mucopolysaccharide is not separated from the surrounding tissue and does not disappear quickly as in the tuberculoid type. This sequence may reflect the grades of the hosts' defense mechanism expressed by the grades of effective handling of hyaluronic acid, which may be an enhancing factor for the growth of *M. leprae in vivo*. Therefore, since Nakamura and Shingu [19] reported the spreading effect of hyaluronidase upon *M. lepraemurium* inoculated into mice, the activity of the hyaluronic acid in leprosy is not likely to be something to keep *M. leprae* from spreading.

From these findings, two variant possibilities for the activity of hyaluronic acid in leprosy appear: 1) that it provides a nutrient energy source for *M. leprae;* or 2) that it serves as a factor in suppressing the defense mechanism of the host. There are two supportive reports for the first possibility derived from studies with *M. lepraemurium*. The first is that by Sula and Dubina [33] who reported that placental extract has an enhancing effect in *M. lepraemurium* cultivation. It is well known that the placenta contains large amounts of hyaluronic acid. The second one is that by Kato [15] who described heparin as a nutrient for *M. lepraemurium*. Although Kato did not use hyaluronic acid, heparin is also one of the acid mucopolysaccharides having beta-glucuronic acid as a common component with hyaluronic acid [20, 22].

In considering hyaluronic acid as a possible factor in the suppression of the immune mechanism of the host, there are some reports which should be taken into consideration although these have not as yet been related directly to leprosy. One of these concerns the "thymus factor" which plays some role in the defense mechanism against mycobacteria, according to Hirsch and Dubos ([11, 12]) and the suppression of its activity by heparin ([12]) or hyaluronic acid ([1, 32]). Martz and Benaceraf ([17]) showed heparin to retard the cell-mediated lysis of mouse ascitic tumor cells. Although they used heparin, it is possible that hyaluronic acid might show the same activity in leprosy because of the similarity of the two saccharides.

Acid mucopolysaccharide is generally regarded as playing some role in the early stages of granuloma formation, disappearing or decreasing in amount after cicatrization ([20]). As shown in this study, this general tendency is applicable only in tuberculoid type leprosy. This also suggests that progressive leprosy is correlated with an inability of the host to dispose of hyaluronic acid from the vicinity of *M. leprae*. This problem will be discussed in a subsequent report.

SUMMARY

A histochemical analysis of 102 skin biopsies from a variety of leprosy types revealed the persistent presence of hyaluronic acid in lepra cells of lepromas. In contrast, the hyaluronic acid content of tuberculoid epithelioid cells showed a minimum amount of hyaluronic acid and hyaluronic acid tended to disappear from these granulomas as they aged. The macrophages of dimorphous leprosy occupied an intermediate position with respect to hyaluronic acid content and distribution, resembling the tuberculoid in BT cases and the lepromatous expression in BL cases.

It is suggested that hyaluronic acid, in a manner similar to *M. leprae* and lipid, has a quantitatively varied distribution reflecting the immunopathologic spectrum of leprosy. This finding suggests that acid mucopolysaccharide may be significantly involved in the host/parasite interaction in leprosy.

RESUMEN

El análisis histoquímico de 102 biopsias cutaneas con una variedad de lesiones leprosas reveló la presencia persistente de ácido hyalurónico en celulas leprosas de lepromas. En contraste, el contenido de ácido hyalurónico de células epiteloides tuberculoides mostró que este ácido se hallaba presente en mínimas cantidades y que tendía a desaparecer de los granulomas con el tiempo. Los macrófagos de la lepra dimorfa ocupaban una posición intermedia con respecto al contenido y distribución del ácido hyalurónico, asemejando a la expresión tuberculoide en casos de BT y a la expresión lepromatosa en casos de BL.

Se sugiere que el ácido hyalurónico, en una forma similar al *M. leprae* y a los lípidos, tiene una distribución cuantitativa variable reflejando el espectro inmunopatológico de la lepra. Estos hallazgos sugieren que el ácido mucopolisacárido puede jugar un rol significante en la interacción "huesped-parásito" de la lepra.

RÉSUMÉ

On a analysé histochimiquement pour l'acide hyaluronique 102 biopsies cutanées prélevées des malades de lèpre des formes différentes. Ces études ont révélé aux lépromes une présence persistante de l'acide hyaluronique dans les cellules de Virchow. Au contraire, la quantité d'acide hyaluronique dans les cellules épithélioïdes tuberculoïdes a été réduite au minimum et avec la viellesse des granulomes l'acide hyaluronique avait une disposition à disparaître. Les macrophages de l'origine des malades de forme dimorphe ont occupé une position intermédiaire à l'égard de la distribution et la quantité d'acide hyaluronique—les malades BT resemblants à la forme tuberculoïde et les malades BL à la forme lépromateuse.

On suggère que la distribution quantitative de l'acide hyaluronique est une expression de la classe immunologique de la lèpre, de même que la distribution de *M. lepre* et lipide. Cette découverte suggère que le mucopolysaccharide acidique joue un rôle significatif dans le rapport entre l'hôte et parasite.

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