# Lipid-laden macrophages in bone marrow of leprosy patients

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Summary While conducting a study to observe bone marrow cytomorphological changes in multibacillary leprosy, lipid laden macrophages as seen in sphingolipidoses were noted. The present study was planned to observe the occurrence and morphological characterization of these macrophages in various types of leprosy. Bone marrow records from 48 cases of paucibacillary and 72 cases of multibacillary leprosy were analysed. The macrophages accounting at the most for 3.5% of marrow cells were observed in 5 cases of paucibacillary and 43 cases of multibacillary leprosy with a maximum incidence being observed in patients with ENL (16/17). The lipid present in the cytoplasm of these cells could be derived from the lipid of the cell wall of *Mycobacterium leprae*. To the best of our knowledge, these cells have not been reported in leprosy so far.

## Introduction

A variety of cytomorphological changes have been described in bone marrow (BM) of leprosy patients. These include megaloblastic erythropoiesis, increased percentage of plasma cells, large reticulum cells and epitheloid granulomas and histiocytes with lepra bacilli in ghost areas.<sup>1-4</sup> We observed large sudanophilic macrophages which showed on Romanowsky stains ample sky blue cytoplasm and small central nuclei in the bone marrow of these patients. While collections of lipid containing macrophages are seen in sphingolipidoses (Gaucher's disease, Niemann–Pick's and Sea-blue histiocyte syndrome), cells with similar morphology have been described in many other conditions including chronic granulocytic leukemia (CML), thalassaemia major, vitamin E deficiency, idiopathic thrombocytopenic purpura (ITP), hyperlipoproteinemia and sickle cell anemia.<sup>5</sup> To the best of our knowledge, so far, their presence has not been described in leprosy. The present study was planned to observe the occurrence of these macrophages in various types of leprosy and their morphological characterization.

## Material and methods

We studied 120 cases of leprosy in patients attending the leprosy clinic, irrespective of age and sex. While 48 patients had paucibacillary leprosy (PB), 72 patients had multibacillary (MB). In the former group 15 patients were new and in the latter, 17. All the other patients were receiving multidrug therapy (MDT) for a variable period. Erythema nodosum leprosum (ENL) was observed in 17 out of 55 MB patients receiving MDT. These patients were also receiving steroids to control the reactional state. The bone marrow (BM) was aspirated from the sternum/posterior superior iliac spine taking precaution to avoid contamination by the lepra bacilli present in the overlying skin and several smears were prepared. The BM smears were stained with Ramanowsky's stains including May– Grunwald–Giemsa (MGG), and Leishman, Ziehl–Neelsen's, Sudan black B, Prussian blue and periodic acid Schiff reaction (PAS).

# Observations

Lipid containing macrophages were observed in the BM smears of 48 patients out of the 120 patients investigated. The percentage varied from occasional to 3.5% of all the marrow cells. The size of these cells varied from 20 to  $50 \mu$ . The nuclei were vesicular and central in location. The cytoplasm was ample and sky blue to deep blue in colour on MGG staining. Cytoplasmic granularity and foamy appearance as observed in sea-blue histiocytes<sup>5</sup> were noted in some of these cells. Cytoplasm was strongly positive for Sudan black B staining and a variable, generally weak positivity was observed for Prussian blue and PAS stains. Ziehl–Neelsen's staining revealed a diffuse faint pink coloration of the



Figure 1. Bone marrow aspirate Leishman stain  $400 \times$ . Centre of the field shows big macrophage, cytoplasm shows coarse granularity.



Figure 2. Bone marrow aspirate PAS stain 400×. The macrophage shows PAS positivity.

cytoplasm in some of the macrophages. However, definite structures identified as lepra bacilli were not observed in any of these macrophages. In this regard, it is interesting to note that such faint diffuse acid fastness may also have been seen with lipofuscin, the age pigment which is also derived from lipids.<sup>6</sup>

These macrophages could be differentiated from the foamy macrophages on the basis of their distinct sky blue to deep blue, and at times granular, ample amount of cytoplasm



Figure 3. Bone marrow aspirate Sudan black stain  $280 \times$ . Lipid-laden macrophage in the centre shows cytoplasmic positivity for Sudan black.

in Romanowsky stains that showed prominent bluish-black granularity on Sudan black staining, whereas the foamy macrophages did not show Sudan black positive granules and the foamy appearance was imparted by the presence of lepra bacilli with all of its morphological features demonstrated on Z–N staining. Similarly, the histiocytes with lepra bacilli in ghost areas may be considered a variant of foamy macrophages and can be differentiated from lipid-laden macrophages.<sup>4</sup>

As shown in Table 1 these macrophages were observed with a greater frequency in MB leprosy than PB. In both groups a higher frequency was noted in those receiving treatment compared with new patients, while 94% of 17 patients with ENL had these macrophages in bone marrow. Table 2 depicts the relationship of these macrophages with the bacillary load in bone marrow. It was observed that with an increasing bacillary load, an increasing percentage of patients had macrophages in the bone marrow.

Associated cytomorphological changes in bone marrow are shown in Table 3. The marrow with lipid-laden macrophages revealed a higher incidence of acid-fast bacilli, megaloblastic erythropoiesis, collections of foamy macrophages, collections of epithe-lioid cells and collections of plasma cells compared with the marrow which did not reveal lipid-laden macrophages.

	No.	No. of marrow smears showing macrophages
Paucibacillary		
Total	48	5 (10.4%)
New cases	15	1 (6.6%)
Cases on treatment	33	4 (12.1%)
Multibacillary		
Total	72	43 (59.7%)
New cases	17	6 (35.2%)
Cases on treatment	55	37 (67.2%)
Cases with ENL	17	16 (94.1%)

 Table 1. Incidence of lipid-containing macrophages in bone marrow

Table 2. Relationship of lipid containing macrophages with bacillary load in bone marrow

Bacillary load in bone marrow	Paucibacillary		Multibacillary	
	No. of patients	No. of patients showing lipid macrophages in bone marrow	No. of patients	No. of patients showing lipid macrophages in bone marrow
Negative	40	1 (2.5%)		
1-2+	8	4 (50%)	12	2 (16.6%)
3-4+			46	28 (60.8%)
5-6+			14	13 (92.8%)
Total	48	5 (10·4%)	72	43 (59.7%)

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Bone marrow with lipid macrophages (48)	Bone marrow without lipic macrophages (72)
45 (93.7%)	13 (18%)
38 (79.1%)	5 (6.9%)
12 (25%)	2 (2.7%)
4 (8.3%)	6 (8.3%)
21 (43.7%)	8 (11.1%)
	Bone marrow with lipid macrophages (48) 45 (93·7%) 38 (79·1%) 12 (25%) 4 (8·3%) 21 (43·7%)

Table 3. Associated changes in bone marrow

#### Discussion

Inherited enzymatic deficiencies seen in sphingolipidoses (Gaucher's disease, Niemann-Pick's disease and sea-blue histiocyte syndrome) result in storage of lipids in macrophages and their accumulation in reticuloendothelial (RE) organs.<sup>7</sup> However, the appearance of these cells is nonspecific and similar histiocytes in smaller numbers have been described in the spleen or BM in many different hereditary and acquired conditions without any primary defect in enzymes.<sup>8,9</sup> These conditions include CML, thalassaemia, major ITP, hyperlipoproteinemia and sickle cell anaemia.<sup>5</sup> Such cells have been referred to as Gaucher-like cells and if the cytoplasm is blue, granular and foamy appearing on a Giemsa stain, as sea-blue histiocytes.<sup>8,9</sup> The lipids which accumulate in these cells are sphingolipids that have major structural functions in many cells and are found in cell membranes, including those of erythrocytes, leukocytes and platelets amongst others.<sup>7</sup> The blue-black staining of granules on Sudan black B suggests the presence of phosphatide or cerebroside.<sup>10</sup> In the hereditary lipoidoses, clear-cut deficiencies in specific catabolic enzymes have been well documented.<sup>11</sup> On the other hand, in the acquired conditions, overloading of normal lipid catabolic mechanisms seems likely, however, the mechanism of presence of these cells in secondary conditions is not precisely defined. In CML and thalassemia major, increased catabolism of myeloid and erythroid cells giving rise to glucocerebroside accumulation may be responsible.<sup>7</sup> In ITP, the accumulation of these cells may be related to the therapeutic use of steroids or the breakdown of platelets in macrophages.<sup>12</sup> Steroids facilitate the effect of adipokinetic agents in eliciting lipolysis of triglycerides of the adipose tissues.13

We observed these cells in all types of leprosy patients. The incidence was higher in MB leprosy compared with PB. In both groups the macrophages were discovered in a higher proportion of patients who were receiving treatment compared with new patients. The incidence was highest in patients with ENL who were receiving steroids as well. The percentage of such cells was in a range which indicated that these cells did not cause a significant reticuloendothelial overload. The associated findings in bone marrow included a higher incidence of demonstration of lepra bacilli, megaloblastic changes, collections of foamy macrophages, and collections of plasma cells in the marrow that showed these macrophages compared with negative marrow smears of such cells.

The observations suggest that lipid-laden macrophages in the bone marrow of leprosy patients may appear as a result of one or more of the following mechanisms:

1 Incomplete metabolism and degradation of lipids, phosphatides and lipopolysaccharides which are present in the cell wall of lepra bacilli.<sup>14</sup>

- 2 Treatment with steroids for a long period in patients having ENL.
- 3 Rapid cell turnover associated with megaloblastic erythropoiesis.

A high incidence of megaloblastic erythropoiesis in lepromatous leprosy has been documented.<sup>15</sup> Also, in the present study megaloblastic erythropoiesis was observed in association with these macrophages in most of the marrow samples.

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## Les macrophages chargés en lipides dans la moelle osseuse des lépromateux

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*Résumé* Au cours d'une étude des changements cytomorphologiques de la moelle osseuse dans le cas de la lèpre multibacillaire, on observa des macrophages chargés en lipides semblables à ceux qui avaient été notés dans les sphingolipidoses. La présente étude fut réalisée pour observer l'apparition et les caractéristiques morphologiques de ces macrophages dans différents types de lèpre. Les résultats de l'examen de la moelle osseuse de 48 lépreux paucibacillaires et de 72 lépreux multibacillaire furent analysés. Les macrophages représentant tout au plus 3,5% des cellules de la moelle osseuse, furent observés chez 5 lépreux paucibacillaires et chez 43 lépreux multibacillaires. La plus forte incidence fut observée chez les patients souffrant d'ENL (16/17). Les lipides dans le cytoplasme de ces cellules pourraient provenir des lipides de la paroi du *Mycobacterium leprae*. Pour autant que nous le sachions, jusqu'à présent, ces cellules n'ont pas été rapportées dans le cas de la lèpre.

#### Los macrofagos cargados con lipidos en la medula osea de los pacientes leprosos

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*Resumen* Durante un estudio en la que se observaban cambios citomorfológicos de la médula ósea, se notaron macrófagos cargados con lípidos como las que se observan en casos de esfingolipidosis. El estudio actual fue planificado para observar la occurencia y caracterización morfológica de estos macrófagos en various tipos de lepra. Se analizó la información sobre las médulas óseas de 48 casos de lepra paucibacilar y 72 casos de lepra multibacilar. Los macrófagos, que representaban un máximo del 3,5% de las células óseas, fueron observados en 5 casos de lepra paucibacilar y en 43 casos de lepra multibacilar, con una incidencia máxima en los pacientes con ENL (16/17). Es posible que el lípido presente en el citoplasma de estas células derive del lípido de la pared celular de *Mycobacterium leprae*. Somos del parecer que hasta ahora no se ha informado este hecho en conexión con la lepra.

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