Aspiration Cytology of Lymph Nodes in Leprosy[1]

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Besides being of diagnostic importance, studies of lymph node morphology in leprosy have been used to evaluate the immunological status ([8, 9]) and the response to treatment with antileprosy drugs by studying the bacteriological (BI) and morphological (MI) indexes ([3]).

Fine needle aspiration biopsy, a much easier and less traumatic technique than excision biopsy, is becoming increasingly popular in the cytological diagnosis of tumors with good diagnostic accuracy. The special advantages of the technique over excision biopsy are that multiple sites of enlarged lymph nodes can be studied, and the same lymph node is available for study and comparative evaluation during follow-up. We began using this technique in leprosy cases in order to determine if it could replace excision biopsies of lymph nodes for the study of the BI and MI ([3]).

This paper describes other interesting observations regarding the cytological smear patterns seen in the histological spectrum of leprosy.

MATERIALS AND METHODS

Sixty-seven untreated patients with leprosy with inguinal or femoral lymph node enlargement were subjected to aspiration biopsy. Forty-eight of the patients had polar lepromatous (LL) and 19 had borderline lepromatous (BL) disease. Five of the LL patients were experiencing erythema nodosum leprosum (ENL) reactions at the time they were studied. The technique of aspiration and the method of preparation of the slides were the same as those described by Franzen and Zajicek ([2]). Two smears were made from the thick, milky fluid aspirates. One was stained with May-Grünwald-Giemsa (MGG) stain for the study of cellular morphology, and the other was stained by the Ziehl-Neelsen method for leprosy bacilli, using a modified technique of Fite, *et al.* ([1]). The smears were evaluated blindly, without knowledge of the clinical or histopathological diagnoses. Histopathology of the lymph nodes was available for comparison in 48 (40 LL and eight BL) of the 67 cases.

Cell-mediated immune reactivity was assessed in 11 of the patients by counting the percentages of T-cells and by determining stimulation indexes to PHA and lepromin in *in vitro* lymphocyte cultures. The techniques used for the various immunological tests have been described earlier ([6]). The results were graded as good, borderline, and poor. The overall cell-mediated immune reactivity was considered good if two of the three parameters were graded good, borderline when two of the three parameters were borderline, and poor when two of the parameters were graded as poor. Normal ranges for our laboratory for each of these tests and their gradations are indicated in Table 1.

RESULTS

Of the 67 lymph node aspirates, five smears were unsatisfactory for evaluation because of degenerated cell morphology or scanty cell content. In an additional two instances the smears showed reactive lymphoid tissue without any granulomatous reaction. In the remaining 60 cases the smears were evaluated with respect to the nature and degree of histiocytic cell reaction, the number of lymphocytic cells, and the presence of mast cells, stimulated lymphocytes, plasma cells, and eosinophils. Based on those observations it was possible to group the smear patterns into the following types:

Type I. Thirty-one out of 60 patients (51.7%) showed this pattern. The charac-

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TABLE 1. *Normal ranges and gradations for our laboratory for immunologic tests.*

Test	Normal	Gradations		
		Poor	Borderline	Good
T-cell counting (%)	55.87 ± 7.38	≤37	38–47	≥48
PHA-Stimulation Index	2.37 ± 0.98	≤1.4	1.5–1.9	≥2.0
Lepromin-Stimulation Index	2.53 ± 0.38	≤1.4	1.5–1.9	≥2.0

teristic findings were a lymphedematous background stained pale violet, with numerous superimposed clear vacuoles (Fig. 1a) of variable sizes bordered by sparse aggregates of lymphoid tissue. The lymphocytic cell population consisted of cells with pale cleaved and noncleaved nuclei, indicating their follicular cell center origin. The findings of degenerated cell debris associated with mild polymorphonuclear cell reaction and occasional eosinophils were other interesting features. Appreciable numbers of mast cells (Fig. 1b) were seen in 20 of these 31 cases. The typical syncytial histiocytes or Virchow cells were only occasionally seen or, when present, had poorly defined cell outlines because of over distention of the cytoplasm by coarse vacuoles (Fig. 1c). Ziehl-Neelsen staining revealed globi of bacilli inside these vacuoles. Bacilli were also seen scattered haphazardly in between these globi.

Type II. Smears from 15 patients (25%) showed this pattern, which was characterized by large numbers of typical multi-nucleated syncytial histiocytes with better defined cell outlines. The cytoplasm showed fine to coarse vacuolation (Figs. 2a and 2b). The surrounding cell population consisted of mononuclear cells. There were clear vacuoles of a similar nature to those seen in Type I smears but comparatively much smaller and of more uniform size. The lymphocytic cell population consisted of mature lymphocytes interspersed with occasional transformed lymphocytes and plasma cells. Mast cells were seen in nine of the 15 cases. Acid-fast staining revealed numerous leprosy bacilli both intra- and extracellularly.

Type III. This pattern was observed in ten patients (16.8%). Smears of this type showed singly scattered, well defined mono- or multinucleated histiocytes surrounded by abundant lymphoid cells (Fig. 3a). The histiocytes were slightly oval to polygonal in shape with small eccentrically placed nuclei and abundant cytoplasm. The cytoplasm contained elongated needle-like clefts or discrete rounded fine vacuoles (Fig. 3b). The lymphoid cell population consisted of mature lymphocytes with many transformed cells and also plasma cells. An occasional mast cell was found in four of the ten cases. Leprosy bacilli were found singly or in small groups and were mostly intracellular.

Type IV. Four patients (6.7%) had this picture. The smears showed clusters of epithelioid histiocytes (Fig. 4) and a variable mixture of lymphoid tissue consisting of mature lymphocytes, transformed lymphocytes, and plasma cells. These epithelioid histiocytes were usually seen in groups with poorly defined cell outlines and lightly stained, eosinophilic cytoplasm. The nuclei were elliptical and eccentrically placed. No vacuolation of the cytoplasm was noticed, and only occasional leprosy bacilli were found. An occasional mast cell was noticed in one case.

Table 2 shows the correlations among the different smear types, the clinical classification of the patients, and the histopathology of the lymph nodes available in 48 of the cases. Of the 31 patients with Type I smear patterns, all 28 cases studied histologically had typical histological lesions of LL. Two were clinically classified as having BL disease and the remaining 29 as LL.

Fifteen patients had Type II smear patterns. Thirteen of these had histopathologic studies of their lymph nodes, and 11 of them were histologically classified as LL, one as BL, and one as BB. On clinical grounds, eight patients were classified as LL and seven as BL.

Of the ten patients with Type III smear patterns, three had histopathologic evaluations, being classified as BL in one case and

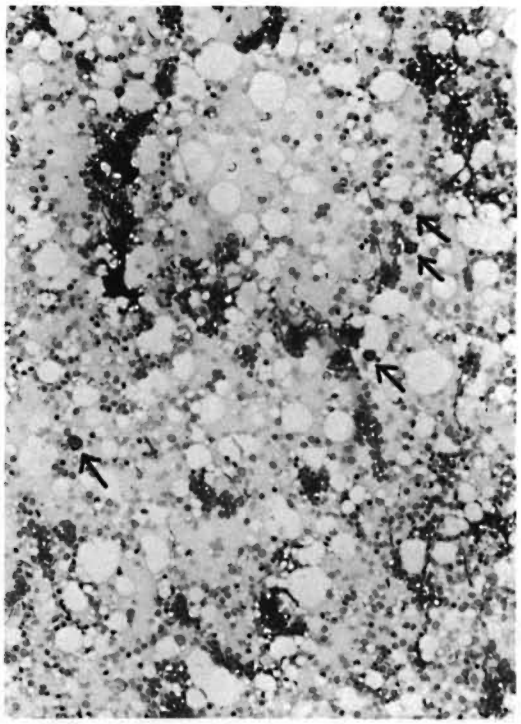

FIG. 1a. Type I smear pattern. Arrows indicate number of mast cells in this field (MGG ×110).

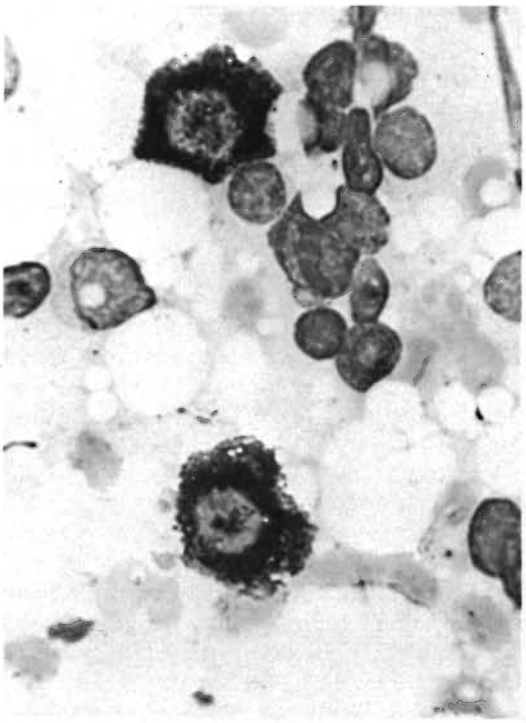

FIG. 1b. Two mast cells. The cells show coarse deep basophilic granules in the cytoplasm and pale nuclei (MGG ×440).

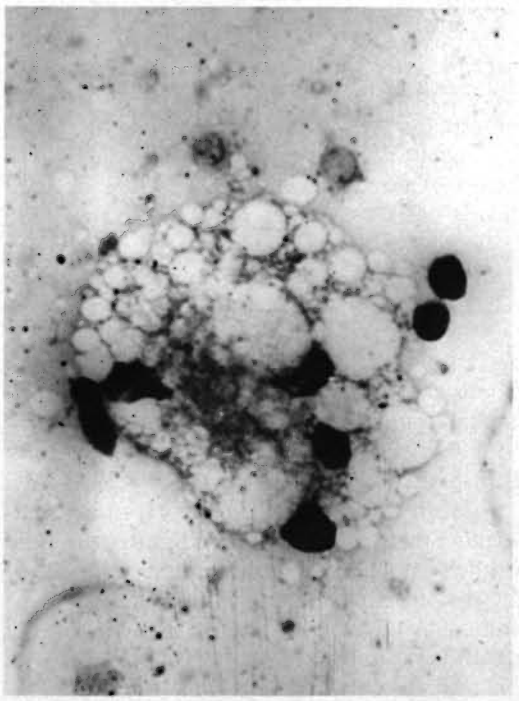

FIG. 1c. Syncytial histiocyte showing distention of the cytoplasm by coarse vacuoles. Many of the vacuoles are lying free in the periphery (MGG ×440).

BB in the other two. Five of these patients were clinically classified as LL and five as BL.

All four patients showing Type IV smear patterns were evaluated histopathologically. The histology was LL in one, BB in two, and BT in one. Two of these patients were clinically classified as LL and two as BL.

Correlations of cytology smear patterns with clinical classifications, cell-mediated immune reactivities, and lymph node histology in 11 of the cases are given in Table 3. Cell-mediated immunity was graded as poor in all five of the LL cases with Type I smears. Three cases with Type II smear patterns were tested. In two, the immunological reactivity was graded as borderline, and in one it was graded as good. In the one case with good immunological reactivity, the lymph node histology showed BB leprosy. Of the three cases tested with Type IV smear patterns, cell-mediated immune reactivity was graded as borderline in one and good in two. The case with borderline immune reactivity showed a lymph

FIG. 2a. Type II smear pattern with a number of clearly defined syncytial histiocytes (MGG ×110).

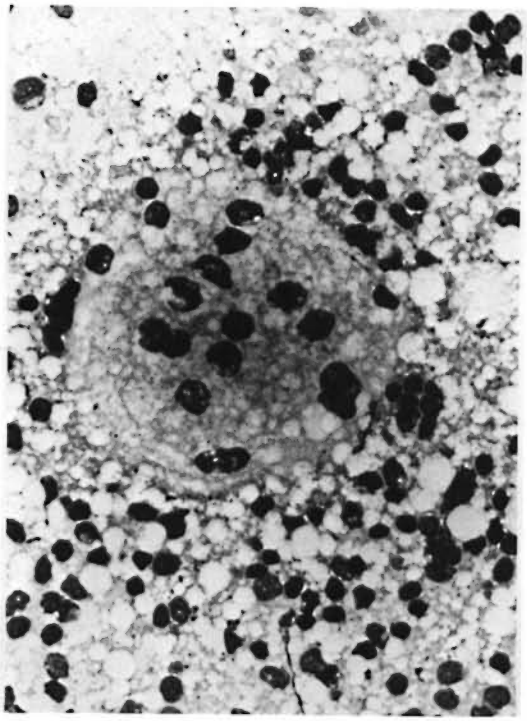

FIG. 2b. One of the syncytial histiocytes (MGG ×440).

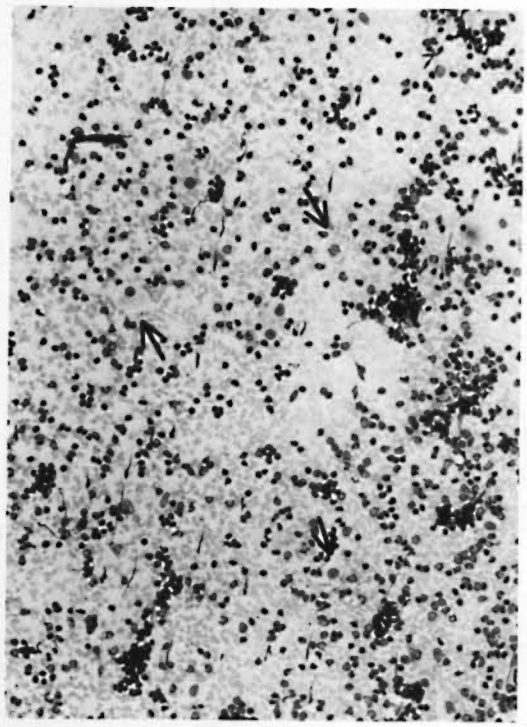

FIG. 3a. Type III smear pattern. Arrows point out the pale histiocytes (MGG ×110).

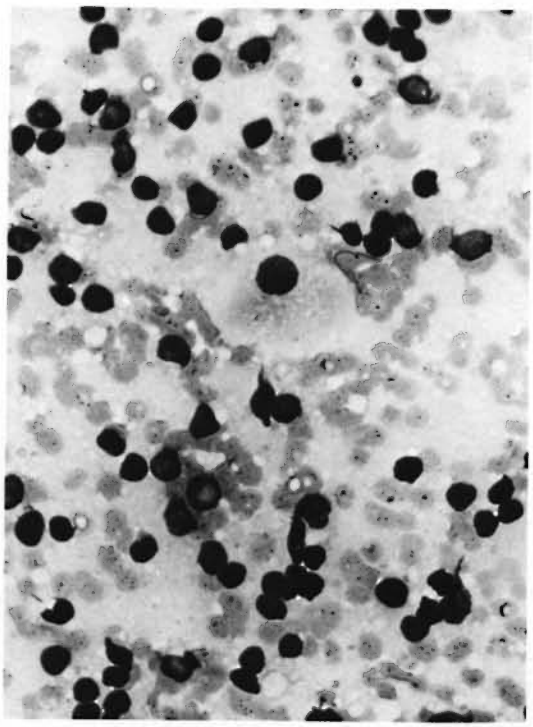

FIG. 3b. One of the mononucleated histiocytes. The cytoplasm shows numerous needle-like clefts and fine vacuoles (MGG ×440).

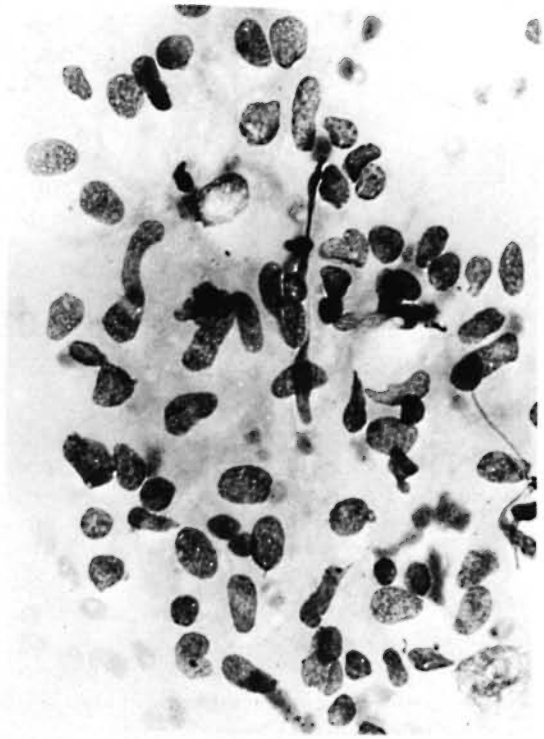

FIG. 4. Collection of epithelioid histiocytes with oval or elliptical eccentric nuclei and indistinct cell outlines (MGG ×440).

node histology of LL while the two patients with good immune reactivity showed a lymph node histology of BB disease.

DISCUSSION

Aspiration cytology is well accepted and widely employed in the diagnosis of malignant lesions. On the other hand, it can only be of help in the diagnosis of inflammatory lesions if specific infective organisms can be demonstrated on smear or culture along with specific cytological reactions. The easy stainability of leprosy bacilli, which were usually seen in large numbers, made the diagnosis of leprosy fairly easy in these lymph node aspiration smears from multibacillary cases.

Depending upon the nature and degree of the histiocytic cell reaction and the associated depletion or proliferation of lymphoid cells, it may also be possible for aspiration cytology to reflect the histological spectrum of leprosy, which in turn reflects the cell-mediated immune status of the patient. Turk and Waters (9) discussed the immunological significance of histological changes in lymph nodes in leprosy. In lepromatous leprosy, the paracortical areas are enlarged and replaced by undifferentiated histiocytes. Increasing numbers of lymphocytes appear in this area as the disease spectrum crosses from the lepromatous to the tuberculoid pole. Similarly, the typical foamy and syncytial histiocytes of lepromatous leprosy become more and more atypical and discrete in the borderline lesions of leprosy and finally become epithelioid in appearance in patients towards the tuberculoid end of the spectrum. From the above description the Type I smear pattern in the present study could be taken as classical of lepromatous leprosy and so also the Type II smears. However, the presence of well defined histiocytes in Type II smears could be reflecting earlier lesions of lepromatous leprosy. The paucity of these classical foamy histiocytes in Type I smears could be accounted for by the rup-

TABLE 2. *Correlations among cytology smear patterns, clinical classifications, and histopathological diagnoses of lymph nodes.*

Cytology smear pattern	Clinical classification		No. of patients studied for:		Histopathology of lymph node[a]			
	LL	BL	Cytology	Histo-pathology	LL	BL	BB	BT
Type I	29	2	31[b]	28	28	—	—	—
Type II	8	7	15[c]	13	11	1	1	—
Type III	5	5	10	3	—	1	2	—
Type IV	2	2	4	4	1	—	2	1

[a] LL (lepromatous leprosy), BL (borderline lepromatous), BB (mid-borderline), BT (borderline tuberculoid).
[b] Three patients were experiencing ENL reaction.
[c] Two patients were experiencing ENL reaction.

TABLE 3. *Correlations among smear patterns, clinical classifications, cell-mediated immunity, and lymph node histology.*

Cytology smear pattern	No. of patients studied	Clinical classification		Cell-mediated immune reactivity		
		BL	LL	Poor	Borderline	Good
Type I	5	5	—	5[a]	—	—
Type II	3	2	1	—	2[c]	1[b]
Type III	—	—	—	—	—	—
Type IV	3	1	2	—	1[a]	2[b]

[a] All diagnosed as LL on lymph node histology.
[b] All diagnosed as BB on lymph node histology.
[c] One diagnosed as BL and one as LL on lymph node histology.

ture of these cells because of increasing distention by globi with the advance of the disease. This might explain the finding of dispersed clear vacuoles of variable sizes without any identifiable cell structures in smears of this type. Fig. 1c is illustrative of how the cell boundary is disrupted by distention of the vacuoles, some of which are liberated into the surrounding areas. Type III smears may be taken to be corresponding to borderline lesions towards the lepromatous end of the spectrum, and Type IV smears possibly reflect borderline lesions more towards the tuberculoid end of the spectrum.

The findings were also supported by the histopathological study (Table 2). The increasing amount of lymphocytic cell population from Type II to IV smears with changing appearance of foamy histiocytes to atypical and epithelioid appearance also correlated with the degree of cell-mediated immune reactivity of these patients (Table 3). Thus the immune status of the patient could also be assessed from aspiration cytology smears. However, large numbers of cases need to be evaluated for final acceptance of this statement.

Another interesting observation was the finding of an appreciable number of mast cells. These were found in a higher percentage of Type I and Type II smears (64%) than in Type III and IV smears (36%). The significance of the finding of mast cells in lepromatous leprosy has been discussed ([4, 5, 7, 10]).

The utility of the aspiration biopsy technique in the evaluation of treatment by grading the BI and MI during followup of patients has been discussed ([3]). The aspiration biopsy technique gave almost identical information about the BI and MI as that obtained from the more time consuming and traumatic procedure of excision biopsy impression smears.

SUMMARY

Lymph node aspiration cytology smear patterns were analyzed in 60 cases of leprosy. A Type I smear pattern was found in 51.7%, Type II in 25%, Type III in 16.6%, and Type IV in 6.7% of the cases. Comparing with the histopathological diagnosis, Type I and Type II smears corresponded to LL lesions. Type III smears corresponded to BB lesions toward the lepromatous end of the spectrum, and Type IV smears corresponded to BB lesions toward the tuberculoid end of the spectrum. An increasing degree of lymphocytic cell admixture was noticed from Type I to Type IV smears with a changing appearance from classical foamy histiocytes in Type I and Type II smears to atypical histiocytes in Type III smears and epithelioid histiocytes in Type IV cases. These findings correlated with the better cell-mediated immune reactivity observed in these patients as the disease spectrum crosses from Type I and Type II smears to Type III and Type IV smears. A larger number of cases needs to be studied for definite assessment of these criteria.

RESUMEN

Se analizaron los patrones citológicos en extendidos de aspirados de ganglios linfáticos obtenidos de 60 casos de lepra. En el 51.7% de los casos se encontró un patrón del tipo I, el tipo II en el 25%, el tipo III en el 16.6%, y el tipo IV en el 6.7% de los casos. Comparando con el diagnóstico histopatológico, los tipos I y II correspondieron a lesiones LL, el tipo III a lesiones BB cercanas al extremo lepromatoso, y el tipo IV a lesiones BB proximas al extremo tuberculoide del es-

pectro. Llendo de los extendidos del tipo I a los del tipo IV, se observó un grado creciente de infiltración linfocítica y un cambio en la apariencia de los histiocitos, desde los histiocitos espumosos clásicos en los extendidos de los tipos I y II, a los histiocitos atípicos en el tipo III, hasta los histiocitos epitelioides en el tipo IV. Estos hallazgos citológicos en los extendidos correlacionaron bien con la mejor reactividad inmune celular observada en los pacientes conforme el espectro de la enfermedad cruzó de los tipos I y II a los tipos III y IV. Se requiere del estudio de un mayor número de casos para el establecimiento definitivo de estos criterios.

RÉSUMÉ

Chez 60 cas de lèpre, on a étudié les profils des frottis cytolologiques obtenus par aspirations de ganglions lymphatiques. Un profil de type I a été observé chez 51,7 % des cas, de type II chez 25%, de type III chez 16,6%, et de type IV chez 6,7% des cas. Lorsqu'on compare le diagnostic histopathologique, les frottis de type I et II correspondent aux lésions LL. Les frottis de type III correspondent aux lésions BB qui se situent vers le volet lépromateux du spectre, et les frottis de type IV correspondent aux lésions BB qui se situent vers le volet tuberculoïde de ce spectre. On a observé une augmentation progressive du degré d'absorption des cellules lymphocytaires, depuis les frottis de type I jusqu'aux frottis de type IV; l'aspect se modifiait depuis l'image classique d'histiocytes spumeux dans les frottis de type I et II, jusqu'à présenter un aspect d'histiocytes atypiques dans les frottis de type III et des histiocytes epithelioides dans les cas de type IV. Ces observations peuvent être mises en relation avec une amélioration de la réactivité immunitaire à médiation cellulaire observée chez ces malades, lorsque le spectre de la maladie passe des types I et II, aux types III et IV. Il serait nécessaire d'étudier un nombre plus grand de cas pour évaluer de manière définitive ces critères.

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