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Peripheral Blood Monocyte Function in Leprosy Patients[1]

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It has been shown that lepromatous patients are anergic to lepromin and that the anergy observed *in vivo* is paralleled by the inability of these patients' lymphocytes to respond to *Mycobacterium leprae* antigens ([13]) and also by their failure to produce macrophage inhibitory factor (MIF) in response to these antigens ([9]). There is growing evidence that the basic defect is a specific one and that nonspecific abnormalities in cellular immunity are due to an inability to eliminate the *M. leprae* ([7, 16]).

The mononuclear phagocyte system plays an important role in several manifestations of cellular immunity, including granuloma formation, immune complex disease, and, particularly, in infection by intracellular parasites ([12, 17, 18]). Conceivably, the depressed delayed hypersensitivity in leprosy is caused by defective macrophage function.

There is evidence that tissue macrophages are derived from peripheral blood monocytes. Since this cell is readily obtainable as a mononuclear phagocyte, our present studies were designed to investigate monocyte function in patients with lepromatous leprosy and to compare the findings with those observed in tuberculoid leprosy patients and normal controls.

MATERIALS AND METHODS

Subjects. Fifty-seven leprosy patients and 20 normal controls were studied. The lepromatous group consisted of 32 patients, ten females and 22 males, ranging from 18–55 years in age (average 32) and disease duration ranging from 2–10 years (average seven). The tuberculoid group consisted of 25 patients, ten females and 15 males, ranging from 19–50 years (average 31) and disease duration ranging from 2–12 years (average five). These patients were classified as lepromatous (LL) or tuberculoid (TT) leprosy by clinical, histological, and bacteriological methods. Most of the patients in the TT group had single or few lesions, often self-healing. Patients with LL had progressive and disseminated disease. Samples were collected before treatment was started.

Peripheral blood monocytes. Monocytes were isolated from peripheral blood mononuclear cells obtained by passage on Ficoll-Hypaque gradients with attachment of monocytes onto coverslips in Leighton tubes. The mononuclear cell suspension was kept for 60 min in medium containing 10% human AB serum, and nonadherent cells were removed by washing with excess medium.

Monocyte receptor activity. This was assayed by determining the avidity of binding of sheep erythrocytes coated with specific rabbit IgG antibody (EA) or with human erythrocytes coated with rabbit IgM antibody and complement (HEAC). Both reagents were prepared as previously described in Hanks' balanced salt solution ([15]). The binding of EA and HEAC to Fc and C3 receptor sites was determined by layering of 0.5% EA or HEAC over monocytes attached to coverslips for 30 min at 37°C. The coverslips were washed and stained with Leishman's solution. The binding of EA and HEAC was assessed by counting the number of these complexes adhered to or ingested by the monocytes. The results were expressed as the percentage of monocytes that bind sensitized red cells multiplied by the mean number of red cells attached and/or ingested per monocyte, this being termed the "attachment index." Adherence and uptake of red cells without antibody or complement were used

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as controls. Determinations for each patient were made in duplicate, and the results represent the arithmetic mean of these two determinations. The individual variability of this test was previously shown by us to be within an acceptable range.

Nitroblue tetrazolium test (NBT). Reduction of the NBT dye by monocytes was tested according to the technique described by Park and Good with minor modifications ([11]). Heparinized venous peripheral blood was obtained, and the leukocyte rich plasma (3×10^6 cells) was added to 0.4 ml of a solution of 0.1% NBT in phosphate-buffered saline (PBS), pH 7.2 (NBT without previous stimulation). In the stimulated NBT assay 10 μg of *Escherichia coli* endotoxin (Difco Laboratories, Detroit, Michigan, U.S.A.) was added to the system.

The incubation of cells and the dye was carried out for 15 min at 37°C, followed by an additional 15 min at room temperature. Afterwards, smears were made on a glass slide, fixed, and stained with Leishman's solution. The percentage and absolute number of monocytes that contained formazan precipitates in their cytoplasm were evaluated using a light microscope, under oil immersion.

Glucosamine incorporation. It has been previously shown that the uptake of labeled glucosamine in peripheral blood mononuclear cells is made preferentially by monocytes[10]. We have recently confirmed this finding in our laboratory (unpublished observations). In this study we used this assay to determine monocyte function in leprosy patients. Culture tubes containing 2×10^6 mononuclear cells in Minimum Essential Medium with 10% human AB serum were incubated for 24 hr at 37°C with 1 μCi of tritium-labeled glucosamine (New England Nuclear, Boston, Massachusetts, U.S.A.) in a 5% CO_2 incubator. After this period, the tubes were washed, centrifuged at 200 × *g*, and the radioactivity associated with the cell pellet was determined by precipitation with 5% trichloroacetic acid followed by solubilization with Protosol® and counted in a liquid scintillation counter (Model LS-100C, Beckman Instruments, Inc.). The results were expressed as counts per minute. The proportion of monocytes in the mononuclear cell suspensions obtained by Ficoll-Hypaque gradients ranged from 2 to 25% esterase positive cells as determined by the technique described by Yam, *et al.* ([20]). In our hands, glucosamine incorporation by lymphoid cells is negligible.

TABLE 1. *C_3b and Fc receptor sites in peripheral blood monocytes of lepromatous and tuberculoid leprosy patients and normal controls. Data presented as mean attachment index ± standard deviation.*

Subjects	N	Receptor sites	
		C_3b	Fc
Lepromatous	15	558 ± 135[a]	529 ± 110[b]
Tuberculoid	10	481 ± 102[a]	406 ± 137[b]
Normals	20	245 ± 70	270 ± 105

[a] $p < 0.02$ compared to normals.
[b] $p < 0.02$ compared to normals.

Preparation of chemotactic factor for monocytes. Lymphocyte derived chemotactic factor (LDCF) was used to evaluate monocyte chemotaxis. This was obtained by culturing 2×10^6 cells from normal donors (LDCF-N) in 1 ml of Minimum Essential Medium (MEM) with 2 μg of Concanavalin A in a 5% CO_2 incubator for 48 hr at 37°C. At the end of the incubation period, the culture tubes were centrifuged at 200 × *g* for 15 min and the supernatant, harvested under sterile conditions, was stored at −20°C until the assay was performed.

Chemotactic assay. The assay for monocyte chemotaxis was performed as follows. Briefly, 0.2 ml of the mononuclear cell suspension at a concentration of 3×10^6 mononuclear cells/ml was placed in the upper compartment and the lymphocyte derived chemotactic factor obtained from normal donors (LDCF-N) in the lower compartment of a chemotactic chamber. After 90 min of incubation at 37°C, the filter (Nucleopore®, 5 μ pore size) was removed, stained, and quantitated by counting the number of monocytes that had migrated through the filter in 20 high power fields. The spontaneous migration activity of the various cell preparations was assessed by determining the number of cells which had migrated into the medium alone. This value was subtracted from the response of the cell suspension to the chemotactic factor activity.

Studies on circulating inhibitors of chemotaxis. To test for the presence of plasma

TABLE 2. *Spontaneous and stimulated reduction of the NBT dye by peripheral blood monocytes of lepromatous and tuberculoid leprosy patients and normal controls. Data presented as mean percentage and absolute numbers per mm³ of NBT positive monocytes ± standard deviation.*

Subjects	N	Monocyte/mm³ (range)	NBT test			
			Spontaneous		Stimulated	
			Percent	Absolute value	Percent	Absolute value
Lepromatous	17	381 (89–400)	21 ± 12[a]	79 ± 14[a]	25 ± 11[b]	92 ± 19[b]
Tuberculoid	15	383 (94–693)	18 ± 9[a]	70 ± 12[a]	21 ± 15[b]	82 ± 20[b]
Normals	20	282 (100–480)	6 ± 2	16 ± 4	15 ± 9	15 ± 8

[a] $p < 0.002$ compared to normals.
[b] $p < 0.02$ compared to normals.

factors inhibiting cell migration, 3×10^6 cells from normal donors were incubated with plasma from lepromatous or tuberculoid patients and normal controls at a 10% concentration in MEM for 60 min at 37°C. After this period, the cells were washed and added to the upper compartment of a chemotactic chamber. Monocyte chemotactic factor obtained from lymphocytes of normal donors (LDCF-N) was placed in the lower compartment. The assay was performed as described for monocyte chemotaxis. The results were expressed as the percentage inhibition of the chemotactic activity obtained with normal monocytes incubated with normal plasma.

Statistical analysis. Comparisons among the various groups in the receptor cell assay, NBT test, and glucosamine incorporation were performed by the Kruskal-Wallis analysis of variance. Comparisons in the stimulated and non-stimulated NBT test were performed by the Wilcoxon's test. Other comparisons were performed according to the Mann-Whitney test. The null hypothesis was rejected at $p = 0.05$.

TABLE 3. *Glucosamine incorporation by peripheral blood monocytes of lepromatous and tuberculoid leprosy patients and normal controls. Data presented as mean counts per minute ± standard deviation.*

Subjects	N	Glucosamine incorporation
Lepromatous	8	3344 ± 1002
Tuberculoid	9	2098 ± 1312
Normals	14	2363 ± 800

RESULTS

Monocytes from patients with lepromatous and tuberculoid leprosy revealed a significant increase in the extent of attachment and/or ingestion of EA and HEAC as compared to normals. No differences were observed in the attachment index of these complexes between LL and TT patients (Table 1).

The results of the monocyte counts and the NBT test on leprosy patients are shown

TABLE 4. *The chemotaxis of monocytes from lepromatous and tuberculoid leprosy patients and normal controls. Results expressed as mean number of leukocytes which completely migrated through the filter in 20 high power microscopic fields ± standard deviation.*

Subjects	N	Monocyte migration	
		Medium	LDCF-N[a]
Lepromatous	15	14 ± 5[b]	24 ± 8[c]
Tuberculoid	10	16 ± 6[b]	28 ± 10[c]
Normals	20	30 ± 15	92 ± 25

[a] LDCF-N = lymphocyte derived chemotactic factor obtained from normal controls.
[b] $p < 0.05$ compared to normal controls.
[c] $p < 0.002$ compared to normal controls.

in Table 2. The spontaneous and the stimulated reductions of the NBT dye were significantly elevated in LL and TT when compared to normal controls; no differences were observed between the two polar forms of leprosy. Analysis of the data either by percentages or absolute values did not change the overall interpretation of the results. Monocyte counts in LL, TT, and controls were within the normal range.

Glucosamine incorporation into peripheral blood monocytes was also evaluated in leprosy patients (Table 3). No marked differences were observed between LL, TT, and normal controls.

The chemotaxis of monocytes from lepromatous and tuberculoid leprosy patients and normal controls is shown in Table 4. Monocytes from LL and TT patients migrated to medium alone and to LDCF-N significantly less than monocytes from normal controls. There were no differences in monocyte migration between LL and TT patients.

The effect of leprosy plasma on the chemotactic activity of normal monocytes is shown in Table 5. A marked inhibition of the chemotactic activity of normal monocytes was observed by treatment with plasma from LL patients when compared to plasma from TT patients (Table 5).

TABLE 5. *Effect of plasma from lepromatous and tuberculoid leprosy patients on the chemotactic activity of normal monocytes to lymphocyte derived chemotactic factor obtained from normal controls (LDCF-N). Results are expressed as the mean percentage inhibition of the chemotactic activity obtained with normal monocytes treated with homologous normal plasma.*

Source of plasma	N	Inhibition of chemotactic activity (range)
Lepromatous	5	61[a] (38–96)
Tuberculoid	5	30 (11–46)

[a] $p < 0.03$ compared to the degree of inhibition seen with tuberculoid plasma.

DISCUSSION

It is well known that patients with leprosy have defects in the expression of delayed hypersensitivity. Several theories have been offered to explain defective cellular immunity in leprosy. Genetic defects, impairment of thymus dependent lymphocyte function, and the presence of serum inhibitors that can interfere with lymphocyte function have all been implicated. Although the monocyte-macrophage system has been shown to play an important role in the relationship between facultative or obligate intracellular parasites and their hosts, this relationship in leprosy is poorly understood ([13]). The problems of macrophage function in lepromatous leprosy have been previously pinpointed by Beiguelman, who described a failure of lepromatous macrophages to lyse autoclaved *M. leprae in vitro* ([2]). However, other studies have failed to confirm these observations ([3, 5, 6]). Macrophages from leprosy patients do not support the survival and growth of *M. leprae in vitro* any better than macrophages from tuberculoid or healthy subjects, and they show a normal capacity to kill other microorganisms.

The increased attachment and/or ingestion of EA or HEAC by LL and TT monocytes when compared to normals is consistent with an augmented density of Fc and C_3b receptor sites on these cells. Douglas, *et al.* ([4]) have shown increased receptor activity in monocytes from sarcoidosis patients, apparently reflecting monocyte activation. Our results are similar although we were unable to demonstrate a difference in monocyte receptor activity between LL and TT patients. If we consider the presence of different bacterial loads known to be present in the two polar forms of the disease, we could expect a different degree of macrophage activation between LL and TT patients. It should be pointed out, however, that our studies were performed on newly-formed blood monocytes, and their activities in relation to the activities of tissue macrophages associated with lepromatous lesions can only be circumstantial at the present time.

Evaluation of the intracellular metabolism of monocytes as it relates to the generation of superoxide was determined

by the reduction of NBT. The spontaneous and stimulated reductions of the dye in LL and TT were greater than that observed in normals. Analogous to the results with monocyte receptor activity, no significant differences could be detected between the two polar forms of the disease. These results are similar to those obtained by Lim, *et al.* [11]. The unstimulated reduction of NBT appears higher in patients than in controls, implying that the patients' monocytes behave as though they are already stimulated.

Increased glucosamine incorporation by activated macrophages has been previously shown in experimental animals [8, 19] and more recently in humans. In contrast to the results obtained with the monocyte receptor activity and NBT tests, we could not observe significant enhancement of glucosamine incorporation in monocytes from LL and TT patients when compared to normals. The fact that monocytes from LL and TT patients were capable of incorporating glucosamine (a lymphokine dependent assay) seems to confirm observations made by others that lepromatous macrophages appear to be capable of responding physiologically to T cell dependent activation and mobilization [7].

In the present study we have found that monocyte migration is impaired in both forms of leprosy, i.e., lepromatous and tuberculoid, when compared to healthy controls. This reduced chemotaxis may be due to the presence of an inhibitory plasma factor since we have found that leprosy plasma depresses the chemotactic response of normal monocytes. The plasma inhibition of normal monocyte chemotaxis was the only major difference between LL and TT patients. These observations as well as the effect of plasma from LL and TT patients on other monocyte-macrophage functions are presently under investigation in our laboratory.

Although LL and TT patients clearly differ in their capacity to clear *M. leprae* from tissues, our results point to a state of monocyte activation in both LL and TT patients. Whether this difference is a reflection of an intrinsic cellular abnormality in monocytes-macrophages or a defective interaction between these cells and lymphocytes [1] in LL cannot be determined at this time. We tend to favor the second hypothesis since LL patients' monocytes showed augmented receptor activities and augmented NBT reduction capacities when compared to those from normals. Defects in amplification of the immune response by serum factors or lymphokines may be responsible for the inability of LL patients to eliminate *M. leprae*. These possibilities should be the subjects of future investigations.

SUMMARY

The activities of monocytes from lepromatous (LL) and tuberculoid (TT) leprosy patients were studied in a variety of *in vitro* systems. In assays of receptor activity, increased densities of Fc and C_3b receptor sites were observed in monocytes of LL and TT patients as compared to normals. No differences were observed between the polar forms of the disease. Similar results were obtained in the nitroblue tetrazolium (NBT) reduction test. Glucosamine incorporation by monocytes from LL and TT patients was not significantly different from that by monocytes from normal controls. A diminished monocyte spontaneous migration and chemotactic activity to lymphocyte derived chemotactic factor (LDCF) was found in both forms of the disease. Plasma inhibitory factor of monocyte chemotaxis was more evident in the lepromatous form of the disease.

RESUMEN

Se estudiaron las actividades de los monocitos de pacientes con lepra lepromatosa (LL) o lepra tuberculoide (TT) en una variedad de sistemas *in vitro*. En los ensayos para medir la actividad de receptor, se observaron incrementos en las densidades de los receptores para Fc y C_3b en los monocitos de los pacientes LL y TT, en comparación con los normales. No se encontraron diferencias entre las formas polares de la enfermedad. En la prueba de la reducción del nitroazul de tetrazolio (NBT) se observaron resultados similares. La incorporación de glucosamina por los monocitos de los pacientes LL y TT no fue significativamente diferente de la mostrada por los controles normales. En ambas formas de la enfermedad se encontró disminuída la migración espontánea de los monocitos y su quimiotaxis en presencia de un factor quimiotáctico derivado de linfocitos. El factor plasmático inhibitorio de la quimiotaxis de los monocitos fue más evidente en la forma lepromatosa de la enfermedad.

RÉSUMÉ

Dans une variété du système *in vitro*, on a étudié l'activité de monocytes provenant de malades atteints de lèpre lépromateuse (LL) et tuberculoïde (TT). Lors d'essais d'évaluation de l'activité du récepteur, on a observé une augmentation de la densité des sites de réception Fc et C_3b, dans les monocytes provenant de malades LL et TT, par comparaison avec des monocytes provenant de sujets normaux. Des résultats analogues ont été obtenus par l'epreuve de réduction du nitroblue tetrazolium (NBT). L'incorporation de glucosamine par les monocytes provenant de malades LL et TT n'était pas significativement différente de celle observée pour des monocytes obtenus chez des témoins normaux. Une diminution de la migration spontanée des monocytes, de même qu'une diminution de l'activité chimiotactique pour le facteur chimiotactique dérivé des lymphocytes (LDCF) a été observée dans les deux formes de maladie. Le facteur inhibiteur du plasma de la chimiotaxie des monocytes était plus facilement mis en évidence dans la forme lépromateuse de la maladie.

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