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NBT Responses of Neutrophils and Monocytes in Leprosy[1, 2]

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Polymorphonucleocytes are involved in phagocytosis during infectious processes and undergo metabolic changes by which they can be identified in this altered physiological state by their increased capacity to reduce nitroblue tetrazolium (NBT) dye. Park *et al* ([14]) have used this NBT test as a useful diagnostic aid in differentiating bacterial diseases from nonbacterial diseases in which the NBT test is negative ([5, 12, 13]). This NBT test is negative in chronic granulomatous disease of childhood. In this derangement there is a dysfunction of neutrophils in that they are unable to digest invading microbacteria ([1, 10]). Subsequently, Park *et al* have reported an improved test (stimulated NBT) which can differentiate true negative results (absence of bacterial infection) from false negative results (defective phagocytic function) ([15]).

In leprosy, it is generally believed the defense against *Mycobacterium leprae* is largely mediated through cellular immunity. The function of the human macrophages in the presence of *Mycobacterium leprae* has been studied *in vivo* and in tissue culture ([2]) and it has been found that the macrophages of certain persons were able to digest the bacilli, whereas macrophages from others were not. This result led these authors to separate people into two groups according to the capacity of their macrophages to digest *Mycobacterium leprae* ([2, 3, 7, 8]). Godal and Rees ([6]) failed to repeat the above finding in their study of blood-derived macrophages. The present study was undertaken to evaluate with the NBT tests the phagocytic function of neutrophils and monocytes in order to determine the relative roles of these cells in the bodily defense of patients with leprosy.

MATERIALS AND METHODS

Subjects. Thirty-six patients with leprosy were studied. Of these, 25 had lepromatous leprosy and 11 tuberculoid leprosy. Most of the patients had received antileprosy chemotherapy with diaminodiphenyl sulfone in varying doses and for varying time periods. However, four of the lepromatous patients and one of the tuberculoid patients had received no chemotherapy at the time of the study. All patients were checked by a complete physical examination and by routine laboratory tests as well as clinical, histologic, bacteriologic and immunologic examinations to establish the diagnosis of leprosy. None of the patients had concomitant bacterial infections at the time of the performance of the NBT tests. These patients were seen in the Dermatology Clinic, Seoul National University Hospital, Seoul, Korea. Twenty healthy volunteers were used as control subjects. They were selected from the clinical research laboratory and university hospital personnel. Their ages ranged from 20 to 60 years. All volunteers also had a complete physical examination and routine laboratory tests in order to check for possible concomitant bacterial infection.

Method. The NBT tests (unstimulated and stimulated tests) were performed and calculated as described by Park *et al* ([14, 15]). In addition, we counted the number of monocytes per hematologic smear, usually 10-20 monocytes per slide. The percentage NBT-positive monocytes was calculated from the number of NBT positive monocytes per slide divided by the total number of monocytes X 100. The neutrophil and monocyte determinations were done on the same hematologic smears. The NBT tests were completed

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within one to two hours after the drawing of heparinized blood from the patients.

RESULTS

Table 1 summarizes the absolute number and percentage of positive NBT neutrophils in the unstimulated and stimulated NBT tests. Table 2 shows the percentage of NBT positive monocytes in the unstimulated and stimulated NBT tests. Table 1 shows no apparent significant results in both tests with respect to neutrophil function. The stimulated NBT test shows that neutrophils of leprosy patients respond as normal controls. Table 2 shows significant reduction of nitroblue tetrazolium by monocytes of patients with both types of leprosy (lepromatous and tuberculoid) in the unstimulated and stimulated tests ($P < 0.01$ and 0.05). The normal control values of neutrophils reported by us are in agreement with those reported by Park *et al* ([14, 15]).

DISCUSSION

The role of cellular immunity in the pathogenesis of leprosy has emphasized the tissue cellular response against *M. leprae*. Little attention has been given to the function of the circulating white cells in the vascular compartment. Recent evidence suggests that there is a constant bacteremia present in patients with leprosy ([4]). *Mycobacterium leprae* has been demonstrated intracellularly in both circulating neutrophils and monocytes with the latter white cells having many times the number of intracellular organisms that are seen in neutrophils. Since both white cells have these intracellular organisms, the question must be asked as to the relative importance of the phagocytic activity in each population.

The phagocytosis of gram positive and gram negative bacteria in bacterial infections leads to increased formation of cytoplasmic enzymes and striking metabolic changes

TABLE 1. *Mean and range of absolute number and percentage of NBT-positive neutrophils per cu mm.*

Group	No. of subjects	Unstimulated		Stimulated	
		%	Absolute	%	Absolute
Control	20	8.3 ± 2.7 (3 - 13)	421.2 ± 227.2 (103 - 685)	30.8 ± 9.7 (21 - 58)	1358.0 ± 973.4 (759 - 4001)
L-type	25	10.3 ± 5.0 (1 - 21)	482.1 ± 224.2 (38 - 942)	32.4 ± 10.6 (17 - 59)	1507.9 ± 590.1 (640 - 829)
T-type	11	9.5 ± 4.5 (3 - 19)	503.0 ± 379.2 (156 - 1328)	37.2 ± 9.2 (27 - 62)	1971.0 ± 1263.6 (959 - 4336)

TABLE 2. *Mean and range of absolute number and percentage of NBT-positive monocytes per cu mm.*

Group	No. of subjects	Unstimulated		Stimulated	
		%	Absolute	%	Absolute
Control	20	5.7 ± 6.1 (0 - 12.5)	13.3 ± 16.2 (0 - 39.8)	9.3 ± 3.7 (0 - 15.3)	19.8 ± 14.2 (0 - 53.9)
L-type	25	32.9 ± 14.4[a] (10 - 50)	130.5 ± 86.1[a] (13.2 - 321)	40.2 ± 14.2[a] (18.1 - 66.7)	150.0 ± 95.1[a] (28 - 291)
T-type	11	22.2 ± 9.5[b] (10 - 40)	80.0 ± 20.9[b] (40 - 155.2)	25.0 ± 6.9[b] (8.3 - 54.5)	96.2 ± 26.5[b] (24.9 - 207.9)

[a] Indicates significant difference from normal value: $P < 0.05$.
[b] Indicates significant difference from normal value: $P < 0.01$.

necessary to the intracellular digestion of these bacterial organisms. This change in metabolic activity can be identified in neutrophils by the NBT tests. But, at present the biochemical mechanism of reduction of NBT-dye has not been delineated fully. It is assumed that cell membrane be altered to permit the dye to penetrate to the cytoplasm. The NBT diaphorase, which is responsible for the transfer of hydrogen ion from reduced pyridine nucleotide to NBT dye, seems to be localized in a certain subpopulation of lysosomal granules. Thus, the release of the NBT diaphorase from the granule is required for the NBT dye to be reduced. These changes are probably induced not only by actual phagocytosis of bacteria or latex particles, but also by the influence of bacterial products such as endotoxin, staphylococcal protein-A, and streptolysin-0. Our results with these two tests indicate that the neutrophil does not participate very extensively in phagocytosis of *M. leprae,* whereas the monocytes seem to be so actively engaged. On the other hand, the neutrophils in patients with extensive *M. leprae* infections are not generally depressed since they respond vigorously after stimulation with endotoxin.

Our data shows clearly a vigorous response of circulating monocytes to a peripheral bacteremia, in this instance caused by *M. leprae.* In addition, the use of endotoxin in the stimulated NBT test does not enhance the function of the monocyte as it does the function of the neutrophil in these patients. If one can assume that the reduction of nitroblue tetrazolium by monocytes has the same basis as NBT positivity in neutrophils in other forms of bacteremia, then the NBT positivity of monocytes in leprosy patients indicates the predominant importance of these cells in the defenses of the body in *M. leprae* infection.

SUMMARY

A role of cellular defense against infection of *Mycobacterium leprae* was studied with 36 leprosy patients. Using the NBT test, we found no significant difference in the proportion and absolute number of NBT positive neutrophils. However, these indices were markedly increased in the monocytes. Thus our results confirm that the monocytes are of prime importance in the defense against *Mycobacterium leprae,* but neutrophils are not. Neutrophils, however, do respond well against test endotoxin in this disease.

RESUMEN

Se estudió uno de los roles de la defensa celular contra la infección por *Mycobacterium leprae* en 36 pacientes con lepra. Utilizando la prueba de NBT, nuestros resultados confirmaron que el monocito es responsable de la defensa celular, pero que los neutrófilos no lo son. Los neutrófilos sin embargo, sí responden bien contra pruebas con endotoxina en esta enfermedad.

RÉSUMÉ

Chez 36 malades de la lèpre, on a étudié le rôle de défense cellulaire contre l'infection par *Mycobacterium leprae.* On utilisant l'épreuve NBT, nos résultats ont pu confirmer que le monocyte est responsable de cette défense cellulaire, alors que les neutrophiles n'y jouent pas de rôle. Toutefois, les neutrophiles répondent bien à l'administration d'endotoxine dans cette affection.

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