NITRO-BT REDUCTION TEST AS A CIRCULATING LEUKOCYTE FUNCTION TEST IN MICE WITH EXPERIMENTAL MALARIA

E. Gilpin-Jackson and B. P. Shadrin

UDC 616.936-092.9-07:616.155.3-008.13:
616.155.3-008.931-074

KEY WORDS: malaria, leukocytes, phagocytosis, demonstration of activity

Once the agent of malaria has penetrated into the host, it abandons its harmful action on phagocytes and the humoral factors of natural resistance. Later, during multiplication in the blood, it causes the development of secondary immunologic insufficiency and pathophysiological disturbances of a general character, similar to those observed in acute inflammation [8, 11, 14]. The main sources of mediators of inflammation and of endogenous immunodepressants are known to be granulocytes and cells of the macrophage system [7, 9, 10]. Information on the functional state of these cells in malaria is contradictory. Some workers have observed increased phagocytic activity of monocytes relative to the parasites [6, 11], whereas others have observed more intensive production of macrophages with reduced bactericidal activity [12, 13]. The latter points to a disturbance of enzyme systems responsible for digestion of the phagocytosed material. To assess activity of these enzymes in phagocytic cells in bacterial infections the nitroblue-tetrazolium (nitro-BT) reduction test in various modifications is used widely.

The aim of this investigation was to determine the ability of circulating monocytes and neutrophils to reduce nitro-BT in mice infected with the agents of two strains of experimental malaria differing in pathogenicity and in sensitivity to antimalarial preparations.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL/6j)F_1 hybrid mice weighing 18-20 g. Animals of two groups (20 mice in each group) were infected by a single intraperitoneal injection of 100,000 syngeneic erythrocytes containing blood forms of *Plasmodium berghei* strain N (strain 1) with normal sensitivity or *P. berghei* strain LNK-65 (strain 2) with reduced sensitivity to antimalarial preparations, and with somewhat weaker pathogenic properties. The dose, method of injection of the infective material, and the body weight of the mice were determined in preliminary experiments. After infection with agents of the two strains, solitary parasites were found in blood films obtained from the mice after the second or third day. However, subsequent growth of parasitemia in animals infected with strain 1 was more than twice as rapid as in animals infected with strain 2. The majority of mice infected with strain 1 died after 7-9 days, compared with 15-20 days for those infected with strain 2. On the day before injection and every 24 h thereafter, blood was taken from the tip of the mouse's tail for testing. The relative number of erythrocytes invaded by parasites and of individual forms of leukocytes was determined in blood films fixed with methanol and stained with azure II-eosin. The ability of neutrophils and monocytes to produce nitro-BT was determined by methods described previously [1, 3], in the writer's modification. According to this, 20 µl of heparinized blood, 20 µl of a 0.1% solution of nitro-BT (Reanal, Hungary) in isotonic phosphate buffer (pH 7.2), and 10 µl of a 0.83% suspension of polystyrene latex, with particle size of 0.8 µ, in the same buffer were applied to a defatted slide by means of a microdose dispenser. In the spontaneous version of the test, 10 µl of buffer was added instead of the latex. After the ingredients had been mixed on the same slide films were prepared from the cell suspension and incubated in a humid chamber at 37°C for 30 min. The films were then dried in air and stained with a 0.1% aqueous solution of neutral red. During analysis of the results the number of intact cells, containing large or small crystals or formazan in their cytoplasm, was counted among 100-200 neutrophils and monocytes. The signif-

E. I. Martsinovskii Institute of Medical Parasitology and Tropical Medicine, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 2, pp. 166-168, February, 1988. Original article submitted April 14, 1987.

 0007-4888/88/0002-0186$12.50

TABLE 1. Parameters of Activity of Nitro-BT Reduction by Circulating Lymphocytes during Experimental Malaria in Mice

Time of infection, days	Intensity of parasitemia, %		Percentage of nitro-BT-positive cells (X ± S)		p
	strain 1	strain 2	strain 1	strain 2	
1	0	0	48,0±1,8 (56,0±2,9)	32,0±0,9 (34,0±0,9)	<0,01 <0,01
2	0	0	48,8±0,9 (54,0±3,3)	26,0±1,3 (32,0±2,6)	<0,01 <0,01
3	4	1	44,4±2,0 (57,5±3,2)	24,0±0,4 (30,0±1,5)	<0,01 <0,01
6	42	15	33,5±1,4 (33,8±1,4)	28,0±1,2 (40,0±0,6)	<0,01 <0,01

Legend. Data on nitro-BT activity of leukocytes in the stimulated version of the test given in parentheses.

icance of differences in the parameters studied was estimated by Van der Waerden's nonparametric test. This modification greatly simplified the conduct of the nitro-BT test. The results were identical ($p < 0.01$) compared with those determined in parallel tests using a modification of the method described in the literature [1, 3].

EXPERIMENTAL RESULTS

The number of nitro-BT-positive cells in the uninfected mice was 74 ± 6.2% (68-88%) among neutrophils and monocytes in the spontaneous version and 85 ± 3.3% (84-94%) on the addition of latex. After infection (irrespective of the strain of the parasite), starting with 25 h, the number of nitro-BT-positive cells in all mice decreased, when detected in both versions of the test (Table 1). In animals infected with strain 2 the number of nitro-BT-positive cells was significantly smaller.

The decrease in the number of neutrophils and monocytes capable of reducing nitro-BT may indicate the ability of malaria parasites, by contrast with agents of bacterial infections [2, 5], to specifically depress activity of oxygen-dependent enzyme systems in these cells, and their phagocytic function. This view is confirmed by the development of the changes specified above in the early stages after infection, before the appearance of parasites in the blood, and also the presence of differences between strains as regards the degree of inhibition of nitro-BT activity of the leukocytes.

There are thus good grounds for using the nitro-BT test to study the pathogenesis of the immunologic disturbances in malaria, and also to use it in the differential diagnosis between malaria and other infections.

LITERATURE CITED

1. Yu. I. Bazhora, V. N. Timoshevskii, P. Z. Protchenko, and A. N. Golovchenko, Lab. Delo, No. 4, 198 (1981).
2. T. K. Bumagina and E. I. Shmelev, Lab. Delo, No. 4, 220 (1981).
3. S. M. Gordienko, Lab. Delo, No. 2, 21 (1983).
4. V. M. Kovalenko and V. M. Rassokhin, Lab. Delo, No. 4, 8 (1983).
5. B. S. Nagoev, Lab. Delo, No. 4, 195 (1981).
6. S. C. Avala and C. E. Varela, Rev. Inst. Med. Trop. Sao Paulo, 17, No. 4, 253 (1975).
7. R. S. B. Cordiero, F. Q. Cunha, J. H. Filho, et al., Ann. Trop. Med. Parasit., 77, No. 5, 455 (1983).
8. M. D. Danforth, M. Aikawa, A. H. Cochrane, and R. S. Nussenzweig, J. Protozool., 27, No. 2, 193 (1980).
9. B. M. Greenwood, A. J. Odulaju, and D. Stratton, Trans. R. Soc. Trop. Med. Hyg., 71, No. 5, 408 (1977).
10. B. M. Greenwood, A. J. Odulaju, and T. A. F. Platts-Mills, Trans. R. Soc. Trop. Med. Hyg., 73, No, 2, 178 (1979).

11. V. Houba, P. M. Lambert, L. J. Mackey, and P. H. Miescher, Immunopathology, 2, No. 4, 359 (1980).
12. T. K. Humphry, Infect. Immun., 31, No. 1, 396 (1981).
13. A. U. Orjih, A. H. Cochrane, and R. S. Nussenzweig, Nature, 291, No. 5813,331 (1981).
14. D. W. Taylor and W. A. Siddique, Am. J. Trop. Med. Immunol., 28, No. 2, 502 (1980).
15. A. Vernes, Lancet, 2, No. 8207, 1297 (1980).