The spectrum of cells expressing an antigen revealed by ICO-11 MCAB, blockade of NKC activity in the lymphocyte blast transformation reaction to PHA, and the molecular weight of the antigen thus indicate that ICO-11 are directed against the α-chain of human lymphocytic functionally-associated antigen (LFA-1).

LITERATURE CITED

1. A. Yu. Baryshnikov, Byull. Éksp. Biol. Med., No. 9, 324 (1984).
2. A. Yu. Baryshnikov, L. P. Trubcheninova, E. V. Savel'eva, et al., Éksp. Onkol., No. 3, 34 (1985).
3. L. J. Cosgrove, M. S. Sandrin, P. Rajasekarish, and I. F. McKenzie, Proc. Natl. Acad. Sci. USA, 83, 752 (1986).
4. J. E. K. Hildreth and J. I. August, J. Immunol., 134, 3272 (1985).
5. A. M. Krensky, F. Sanchez-Madrid, E. Robbins, et al., J. Immunol., 131, 611 (1983).
6. A. M. Krensky, E. Robbins, T. A. Springer, and S. J. Burakoff, J. Immunol., 132, 2180 (1984).
7. U. K. Laemmli, Nature, 226, 680 (1970).
8. F. Sanchez-Madrid, J. A. Nagy, E. Robbins, et al., J. Exp. Med., 158, 1785 (1983).
9. H. Towbin, T. Staechelin, and J. Gordon, Proc. Natl. Acad. Sci. USA, 76, 4350 (1979).

ACTIVATION OF MACROPHAGES BY A SYNTHETIC ANTIOXIDANT

I. S. Freidlin, N. K. Artemenko,
T. S. Freidlin, I. G. Shcherbak,
Yu. O. Danilevskii, V. I. Kuz'min,
and L. D. Smirnov

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Much research in recent years has been devoted to the general rule that acitvation of macrophages (MPh) is associated with a metabolic (oxidative) burst, with activation of the glucose monophosphate shunt (GMPS), with the production and secretion of highly active unstable products of oxygen reduction, namely superoxide anions O_2^-, hydrogen peroxide (H_2O_2), OH^- radicals, and singlet oxygen (O_2) [5].

The excess of toxic superoxide radicals formed under these circumstances, and also lipoperoxides accumulating in phagosomes of MPH during phagocytosis, may be responsible for oxidative damage to cell membranes and associated depression of MPh functions. An intrinsic system of antioxidative protection, including superoxide dismutase, which removes the excess of superoxide radicals, and also glutathione peroxidase and NADP-dependent glutathione reductase, which neutralize lipoperoxides [2, 8], has been described in MPh. However, if endogenous antioxidants are deficient, disturbances of MPh function may arise. It was shown previously [4] that alkyl-substituted derivatives of 3-hydroxypyridine (3-HP), which has moderate antioxidative action, are effective inhibitors of free-radical reactions and can be used to protect against the destructive effect of free radicals.

The aim of this investigation was to study the effect of synthetic antioxidants of MPh functions. Of all the various synthetic derivatives of 3-HP we chose 2-tert-butyl-3-hydroxypyridine (TBHP), whose ability to stabilize erythrocyte membranes was described previously [3].

EXPERIMENTAL METHOD

Mouse peritoneal MPh, removed by intensive irrigation of the peritoneal cavity of mice with Hanks's solution containing heparin (5 U/ml), were used as the target cells. The re-

I. P. Pavlov First Leningrad Medical Institute. Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow.

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TABLE 1. Parameters of MPh Activation during Incubation with Preparations in Vitro (M ± m)

Preparation	Increase in GPDH activity of MPh compared with control, after incubation with prep. for 30 min		Increase in frac. of MPh spreading over glass (comp. with control) after incubation w/prep., %	
	Δ %	multiplicity of increase	2h	24h
TBHP (10 μg/ml)	19,25±2,79 ($n=9$)	2,89±0,44 ($n=9$)	36,4±6,0 ($n=5$)	26,5±3,6 ($n=6$)
LPS (1 μg/ml)	12,03±3,82 ($n=4$)	2,10±0,47 ($n=4$)	19,0±6,0 ($n=5$)	45,5±4,8 ($n=5$)

Legend. Here and in Table 2, n denotes number of determinations.

TABLE 2. Multiplicity of Increase in Number of Peritoneal MPh in Mice and also in Number of MPh Spreading over Glass and Engaging in Phagocytosis in Cultures of Cells Obtained from Mice 1 h after Intraperitoneal Injection of Preparations (M ± m)

Preparation	Multiplicity of inc. of parameters reflecting No. and functional activity of MPh after injection of preparations (M ± m)			
	all MPh	large MPh	spreading MPh	phagocytosing MPh
TBHP (50 mg/kg)	2,10±0,58 ($n=23$)	1,86±0,42 ($n=12$)	2,07±0,39 ($n=10$)	1,64±0,49 ($n=7$)
LPS (5 mg/kg)	1,50±0,36 ($n=6$)	1,72±0,52 ($n=6$)	1,40±0,30 ($n=6$)	2,00±0,52 ($n=6$)

sulting cell suspension ($5 \cdot 10^6$ cells/ml) contained 60-70% of MPh. The cells were incubated in the presence of the test preparation either in suspension for 30 min in siliconized test tubes, or, after adhesion of the cells to glass, in a monolayer for 2 and 24 h. In parallel tests the cells were incubated in Hanks's solution (control) or in the presence of the standard MPh activator: bacterial lipopolysaccharide (LPS) from E. coli 055:B5 (from Calbiochem, USA).

In conformity with our system of screening, to select and study biologically active preparations acting at the level of the mononuclear phagocytic system, in the first stage of the investigation in vitro we used two orienting tests: metabolic – activity of the key enzyme of GMPS, namely glucose-6-phosphate dehydrogenase (GPDH) [7] – and functional – the intensity of spreading of MPh over glass [9]. GPDH activity was determined by a spectrophotometric method after disintegration of the cells by freezing and thawing 3 times, on the basis of increased absorbance at 340 nm due to $NADPH_2$ formation. Two parameters were chosen as criteria of the stimulating action of the preparations on GPDH activity. The first, the multiplicity of increase, was calculated as the ratio of GPDH activity of the experimental sample to activity of the control sample during freezing to −12°C (a mixture of ice and salt). The second parameter was calculated as follows. To begin with the ratio, in %, of GPDH activity during freezing in ice and salt to its activity during freezing in liquid nitrogen was calculated for both control and experimental samples. The difference between the percentages thus obtained for the experimental and control samples (Δ%) was then found. After intraperitoneal injection of the test preparation, the number of MPh in the peritoneal cavity was counted at intervals of time, their ability to spread and to carry out phagocytosis of Candida albicans cells in vitro was estimated, and clearance of the peritoneal cavity of the mice from injected Salmonella typhimurium cells also was evaluated [6].

The results were subjected to statistical analysis by parametric and nonparametric methods [1].

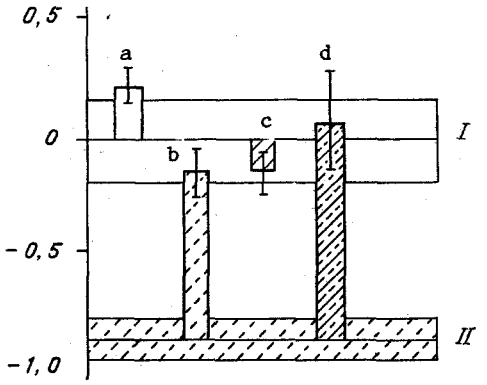

Fig. 1. Clearance of mouse peritoneal cavity from injected bacteria, after injection of test preparations. Ordinate, mean values of differences between logarithms of concentration of bacteria in peritoneal cavity (M ± m). I) Confidence interval for control mice; II) confidence interval for mice 4 days after injection of medium with thioglycollate. a) 1 h after injection of TBHP; b) 1 h after injection of TBHP combined with injection of medium with thioglycollate; c) 24 h after injection of LPS; d) 24 h after injection of LPS combined with injection of medium with thioglycollate.

EXPERIMENTAL RESULTS

The results of the study of the direct effect of TBHP compared with that of LPS on peritoneal MPh are given in Table 1. Incubation of the cells with TBHP for 30 min was sufficient to activate GPDH ($p < 0.01$). To judge by the increase in enzyme activity, the effect of TBHP was similar to that of the standard activator – bacterial LPS. The fraction of MP which spread increased compared with the control as early as after 2 h of incubation with the test preparations. At the early stages (2 h) the effect of TBHP was more marked than that of the standard activator, LPS ($p < 0.05$). In the later stages of culture (24 h) the activating effect of LPS continued to increase, whereas the proportion of MPh which spread over the glass, under the influence of TBHP showed a tendency to fall compared with the early times, but it still remained significantly higher compared with the gradually rising control level ($p < 0.01$).

As early as 1 h after intraperitoneal injection of TBHP it caused a distinct rise in the number of cells in the peritoneal cavity on account of MPh, with the accumulation mainly of large MPh; this effect, moreover, was similar to that of LPS (Table 2).

It will be clear from Table 2 that MPh removed from the peritoneal cavity of the mice 1 h after intraperitoneal injection of TBHP were distinguished by an increased tendency to spread compared with MPh of the control animals. Judging by the intensity of ingestion of Candida albicans cells by them, the phagocytic activity of these same MPh was increased. Under these experimental conditions TBHP activated spreading by a greater degree than the standard activator, bacterial LPS, whereas it increased phagocytic activity by a lesser degree. At the later stages after injection of the test preparation (1.5-24 h) no further increase in the number of MPh in the peritoneal cavity and in their functional activity was observed. By contrast, after injection of LPS the number of MPh in the peritoneal cavity and their functional activity did not reach their peak until after 24 h.

On account of the differences discovered in the times of the stimulating effects when the action of the preparations on the intensity of clearance of the mouse peritoneal cavity from injected S. typhimurium cells was studied, LPS was injected 24 h before, and TBHP 1 h before infection. To assess clearance, the mean differences of the logarithms of concentration of the bacteria 1 h after infection were calculated in mice of the control and experimental groups. The intensity of clearance in mice receiving 1 ml of medium with thioglycollate 4 days before infection was found to be considerably lower than in the control (Fig. 1).

It will be clear from Fig. 1 that neither TBHP nor the standard MPh activator bacterial LPS had any effect on the intensity of clearance of the peritoneal cavity from injected bacteria. However, against the background of the deficiency of bactericidal activity of MPh induced by preliminary injection of medium with thioglycollate, the two preparations increased the initially depressed intensity of clearance of the mouse peritoneal cavity by an equal degree. Under the influence of TBHP, just as of bacterial LPS, normalization of the level of clearance of the peritoneal cavity was observed, i.e., the experimentally created deficiency of bactericidal activity of MPh was corrected.

The synthetic antioxidant TBHP was thus found to activate mouse peritoneal MPh by its direct action on them in vitro. After intraperitoneal injection of the same preparation an increase in the number of MPh was found in the peritoneal cavity, and their functional activity also was increased. In mice with a defect of peritoneal clearance induced previously, TBHP led to restoration of the normal level of antibacterial protection. In all tests of activating action on MPh which were studied, this antioxidant was not inferior to the standard MPh activator – bacterial LPS. When TBHP was injected into mice, earlier manifestations of MPh activation were observed than when LPS was injected.

LITERATURE CITED

1. I. P. Ashmarin, N. N. Vasil'ev, and V. A. Ambrosov, Rapid Methods of Statistical Analysis and Planning of Experiments [in Russian], Leningrad (1975).
2. D. N. Mayanskii, The Kupffer Cells and System of Mononuclear Phagocytes [in Russian], Novosibirsk (1981).
3. L. K. Obukhova, A. B. Tsipin, V. I. Kuz'min, and L. D. Smirnov, Izv. Akad. Nauk SSSR, Ser. Biol., No. 4, 548 (1979).
4. L. D. Smirnov and K. M. Dyumaev, Khim.-Farm. Zh., No. 4, 28 (1982).
5. I. S. Freidlin, The System of Mononuclear Phagocytes [in Russian], Moscow (1984).
6. D. Briles, J. Lehmeyer, and C. Fennan, Infect. Immun., 33, No. 2, 380 (1981).
7. A. Kornberg and B. Horecker, Meth. Enzymol., 1, 323 (1955).
8. M. Rister and R. Baehner, J. Cell Physiol., 87, No. 3, 345 (1976).
9. J. S. Sundsmo and O. Gotze, Cell. Immunol., 52, No. 1, 1 (1980).

IMMUNOGENETIC INVESTIGATION OF FAMILIES WITH CLOSE INTERMARRIAGE

L. P. Alekseev, N. M. Khaitova,
N. G. Dmitrieva, M. N. Boldyreva,
R. T. Kadyrova, V. V. Yazdovskii,
and O. I. Istamova

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The prospects for the study of genetic control of the human immune response are linked first and foremost with investigation of the HLA-D region of the principal human histocompatibility complex [2]. In order to study the HLA-D region it is necessary to have HLA-D homozygous typing cells (HTC), by means of which not only can HLA-D antigens be detected, but monoclonal typing reagents for class II antigens of the HLA system can also be obtained [2, 3]. The main source from which HTC can be obtained is donors arising from close intermarriages. Although in the USSR no HTC as such are available, on the territory of the Soviet Union there are regions in which offspring of close intermarriages live. One such region is Samarkand. It is worth noting that in the settlements (kishlaks) where such families live, there is a permanently increased prevalence of chronic infectious diseases, so that a genetic basis can be postulated for the disturbance of immunoreactivity found in these patients.

Institute of Immunology, Ministry of Health of the USSR, Moscow.

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