Modulation of the Oxidative Burst in Mouse Macrophages and Human Neutrophils by Superlow Concentrations of GM1 Ganglioside

N. F. Avrova, V. P. Ivanova, V. A. Tyurin, I. A. Gamalei, I. V. Klyubin, I. A. Shchepetkin, E. V. Borunov, and Yu. Yu. Tyurina

UDC 577.352.38:612.112.9

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 1, pp. 44-46, January, 1994
Original article submitted June 24, 1993

It is shown that ganglioside GM1 in picomolar concentrations stimulates the phorbol-12-myristate-13-acetate (PMA)-induced generation of active forms of oxygen by neutrophils and peritoneal macrophages. GM1 (10^{-11} M) is found to enhance the luminol-dependent chemiluminescence induced by 10^{-8} M PMA in mouse macrophages in comparison with the effect of PMA alone.

Key Words: *gangliosides; phagocytes; oxidative burst*

Until recently, the involvement of the signal transduction systems in the regulation of free radical reactions has been shown solely for phagocytes [1]. Previously we found that gangliosides and phorbol-12-myristate-13-acetate (PMA) are capable of inhibiting lipid peroxidation (LPO) in brain synaptosomes, whereas a penetrating cAMP analog reduces and polymyxin B almost completely abolishes the inhibiting effect of these compounds [2]. These data suggest that, since these compounds act as transmitters in the signal transduction systems, the effect of gangliosides on free-radical processes is mediated through these systems. Gangliosides are membrane constituents not only in nervous but also in other tissues. They may be released from immune cells and affect the functional activity of lymphocytes [3-5]. However, there are no data on the regulatory effect of gangliosides on the function of phagocyting cells.

The objective of the present investigation was to study the effect of nano- and picomolar ganglioside concentrations on the generation of active forms of oxygen by mouse macrophages and human neutrophils in response to low concentrations of phorbol esters. Such data are of great interest for understanding the mechanism of the immunomodulatory effect of gangliosides and, probably, the mechanism of their neurotrophic effects [2].

MATERIALS AND METHODS

The experiments were carried out on primed peritoneal macrophages of BALB/c mice. The mice were injected intraperitoneally with 1 ml sterile 10% peptone in physiological saline. Forty-eight hours later the animals were killed, and the peritoneal cavity was washed with medium 199 containing 15% bovine serum, 5 U/ml heparin, 1000 U/ml penicillin, and 100 μg/ml streptomycin. The obtained exudate was placed in special measuring cuvettes (2×10^6 cells per cuvette). After a 2-hour

Laboratory of Comparative Biochemistry of the Nervous System, Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences; Laboratory of Cell Physiology, Institute of Cytology, St. Petersburg; Laboratory of Immunology, Institute of Oncology, Tomsk Research Center, Russian Academy of Medical Sciences. (Presented by A. N. Klimov, Member of the Russian Academy of Medical Sciences)

0007-4888/94/0001-0045$12.50

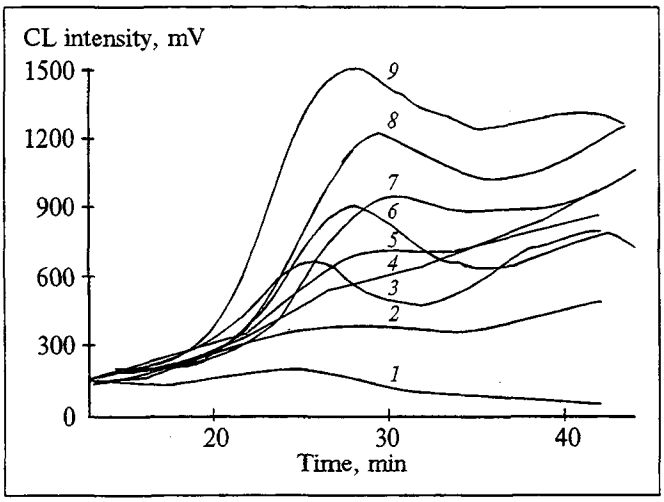

Fig. 1. Effect of different concentrations of ganglioside GM1 on luminol-dependent CL in PMA-activated human neutrophils. Neutrophils (5×10^5 cells) in a volume of 1 ml were preincubated for 30 min at 37°C with varying concen-trations of GM1 (in control samples GM1 was omitted), after which the CL response was induced by PMA (10^{-8} M). *1*) without GM1 or PMA; *2–9*) 10^{-8} M PMA; *3*) 3.3×10^{-7} M GM1; *4*) 1.65×10^{-7} M GM1; *5*) 3.3×10^{-8} M GM1; *6*) 3.3×10^{-9} M GM1; *7*) 1.65×10^{-9} M GM1; *8*) 3.3×10^{-10} M GM1; *9*) 3.3×10^{-11} M GM1.

incubation at 37°C the medium was replaced with a heparin-free medium. Ganglioside GM1 in final concentrations of 10^{-15} - 10^{-8} M was added to the medium 4 hours after seeding the cells. After a 40-min incubation the medium was replaced with Hanks solution, containing 50 μM luminol, 1 g/ml serum albumin, and 20 μM HEPES (pH 7.4). Spontaneous chemiluminescence (CL) was recorded during 5 min, after which phorbol ester (10^{-8} M) was added to the cuvette and the maximal level of induced CL was recorded. The CL response of GM1-preincubated cells was recorded in the presence of the test concentration of GM1 in Hanks solution. Statistical processing of the results was carried out using the Student *t* test. Neutrophils were isolated from heparinized blood of healthy volunteers as described earlier [6]. Production of active forms of oxygen by neutrophils (5×10^5 cells per vial) was measured with an L-1251 luminometer (Wallac, Finland) in the presence of luminol, as described elsewhere [6]. After recording of the spontaneous CL, 10 μl ganglioside GM1 were added to the samples to final concentrations of 3.3×10^{-11}-3.3×10^{-7} M, the samples were incubated for 30 min at 37°C, and the CL response of the cells was induced by adding PMA in a final concentration of 10^{-8} M. Gangliosides were isolated after Folch from bovine brain tissue [7]; GM1 ganglioside as well as other individual gangliosides were isolated by preparative column chromatography on silica gel, as described previously [2]. The purity of GM1 and other isolated gangliosides assayed by HPLC was no less than 98-99%.

RESULTS

Preincubation of human neutrophils with ganglioside GM1 markedly stimulated PMA-induced generation of active forms of oxygen. Figure 1 shows the results of one of three experiments studying the effect of the GM1 concentration in the samples on the intensity of luminol-dependent CL in human neutrophils induced by 10^{-8} M PMA. As is seen from the figure, the lowest concentrations of GM1 (3.3×10^{-11} - 3.3×10^{-10} M) increased the maximal magnitude of the PMA-induced cell response 5-7 fold in comparison with the same concentration of PMA alone (10^{-8}), while at higher concentrations the effect was less pronounced. Incubation of the neutrophils with GM1 alone (3.3×10^{-11} - 3.3×10^{-7} M) did not enhance the luminol-dependent CL. Synergism in the action of ganglioside and phorbol esters on the production of active forms of oxygen by neutrophils and the inverse concentration-response dependence for a GM1 concentration within 3.3×10^{-11} - 3.3×10^{-7} M were evaluated by measuring the integral CL over a 30-min period (data not shown).

TABLE 1. Effect of Different Concentrations of GM1 on Luminol-Dependent CL in PMA-Stimulated Mouse Peritoneal Macrophages ($M \pm m$)

Concentration of GM1, M	Intensity of CL, cpm		Number of experiments
	control	experiment	
10^{-8}	1467±318	1033±291	3
10^{-9}	2475±758	1325±384	4
10^{-10}	2700±545*	1120±185	5
10^{-11}	3083±961*	900±163	6
10^{-12}	3100±771*	1050±206	4
10^{-13}	2550±236*	1350±366	4
10^{-14}	2567±491	2500±608	3
10^{-15}	2367±578	2500±608	3

Note. Control – incubation without ganglioside, * denotes reliable differences ($p<0.05$) in comparison with the control.

The maximal effect of GM1 on mouse peritoneal macrophages was attained with concentrations of 10^{-12}-10^{-11} M, while at both higher (10^{-10} M) and lower (10^{-13} M) concentrations this effect was less pronounced but still reliable, but ultimately it was virtually absent at concentrations of 10^{-15}-10^{-14} M (Table 1).

For instance, the luminol-dependent CL induced by 10^{-8} M PMA was 3.4-fold stronger in mouse macrophages preincubated with 10^{-11} M GM1 in comparison with the cells incubated with PMA alone (3083±961 vs. 900±163 cpm, n=6, p<0.05).

It has previously been shown that individual gangliosides in nanomolar concentrations are capable of inhibiting LPO induced in synaptosome membranes and of preserving lipid fatty acids, receptors, and enzymes from oxidative destruction [2,8]. The data reported here provide additional evidence that low concentrations of gangliosides exert a modulatory effect on the free-radical processes. Gangliosides apparently can affect the processes of extracellular cytotoxicity and phagocytosis which play a crucial role in the nonspecific resistance of the organism, by stimulating the production of superoxide anion and other active forms of oxygen.

It is now thought that gangliosides possess immunomodulatory properties [3-5]. The data obtained by us suggest that, in very low concentrations (which, for instance, may arise in the blood due to a loss of membrane fragments by lymphocytes or other blood cells [4]), gangliosides can modulate the metabolic processes and functional activity of phagocytes. Since the effect of ganglioside GM1 on free-radical reactions was similar in human neutrophils and mouse macrophages, it may be assumed that the ability of gangliosides to activate free-radical processes in mammalian phagocytes is independent of the type of cells and of their species specificity.

Activation of protein kinase C is a key step in the activation of the NADPH-oxidase complex in neutrophils and macrophages; however, other protein kinases seem to affect these processes as well. It should be noted that gangliosides are modulators of specific ganglioside-dependent [9,10] and other protein kinases, and, in particular, they exert a calmodulin-like effect [11]. Since phagocytes are the only cells where the role of signal transduction systems in the regulation of the production of active forms of oxygen has been systematically studied, they represent the most promising model for elucidating the mechanism of the effect of gangliosides on free-radical reactions.

REFERENCES

1. V. A. Tyurin, L. A. Kuznetsova, Yu. Yu. Tyurina, *et al.*, *Byull. Eksp. Biol. Med.*, **111**, № 6, 597-599 (1991).
2. K. F. G. Chan, *J. Biol. Chem.*, **262**, 5248-5255 (1987).
3. N. O. Christiansen, L. C. Schade, and V. Esmann, *Biochim. Biophys. Acta*, **25**, 317-324 (1988).
4. E. V. Dyatlovitskaya, E. V. Sinitsyna, K. Jung, *et al.*, *Europ. J. Biochem.*, **131**, № 3, 601-605 (1983).
5. J. Folch, M. Lees, and G. H. Sloane-Stanley, *J. Biol. Chem.*, **226**, 497-509 (1957).
6. K. Fukunaga, E. Miyamoto, and T. R. Soderling, *J. Neurochem.*, **54**, 102-109 (1990).
7. Y. Nagai and S. Tsuji, in: *New Trends in Ganglioside Research: Neurochemical and Neuroregenerative Aspects* (Ed. R. W. Leen et al.) Padua (1988), pp. 329-350.
8. H. Offner, T. Thieme, and A. A. Vandenbark., *J. Immunol.*, **139**, № 10, 3295-3305 (1987).
9. I. A. Schepetkin, N. V. Cherdentseva, and E. V. Borunov, *Cancer Res.*, **117**, 172-174 (1991).
10. F. J. Sharom and T. E. Ross, *Biochim. Biophys. Acta*, **854**, № 2, 287-297 (1986).
11. V. A. Tyurin, Yu. Yu. Tyurina, and N. F. Avrova, *Neurochem. Int.*, **20**, № 3, 401-407 (1992).