Search for Potent Modulators of Cytokine Production by Macrophages

A. A. Nikitin*, M. T. Abidov, E. O. Kovalevskaya*, and O. V. Kalyuzhin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 9, pp. 293-295, September, 2004
Original article submitted April 13, 2004

We compared the effects of Tamerit, Polyoxidony, and Licopid on spontaneous and lipopolysaccharide-stimulated production of interleukin-1 and tumor necrosis factor by mouse peritoneal macrophages *in vitro*. The test preparations were equally potent in stimulating nonactivated cells. Licopid produced a costimulatory effect on macrophages primed with endotoxin. Tamerit in different doses suppressed cytokine production by cells. Polyoxidony in low doses activated, but in high doses suppressed this process.

Key Words: *Tamerit; Polyoxidony; Licopid; lipopolysaccharide; macrophages*

Monocytes and macrophages are the main target of biological activity of lipopolysaccharide (LPS) from gram-negative bacteria. LPS binds to specific receptors (CD14 and TLR-4) and activates macrophages and other cells. It results in the secretion of various inflammatory mediators, including cytokines (tumor necrosis factor-α, TNF-α; interleukin-1, IL-1; IL-6; IL-18; and interferon-α), prostaglandins, and nitrocompounds. Biological activity of LPS determines pathogenesis of various inflammatory diseases. For example, endotoxins of bacteria colonizing the subgingival area (*Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, and *Fusobacterium nucleatum*) mediate the stimulatory effect of microflora on macrophages, fibroblasts, lymphocytes, and osteoblasts. It results in chronic inflammatory and destructive changes underlying the pathogenesis of periodontitis (common inflammation in humans) [8].

This work was designed to reveal the most potent regulators of macrophage function that *in vitro* suppress the secretion of proinflammatory cytokines by LPS-activated cells in model test systems.

Institute of Human Morphology, Russian Academy of Medical Sciences; *M. V. Vladimirsky Moscow Regional Research and Clinical Institute, Moscow. ***Address for correspondence:*** info@abidov.ru. M. T. Abidov

We studied three synthetic immunotropic preparations of different chemical groups that modulate functions of monocytes and macrophages: Licopid (analog of natural glucosaminylmuramyl dipeptide, GMDP) [2,3], Polyoxidony (polyethylene piperazine N-oxide) [4], and Tamerit (aminophthalhydrazide derivative) [1].

MATERIALS AND METHODS

Functional tests were performed with cells cultured in RPMI-1640 medium (Flow Lab.), containing 5% inactivated fetal bovine serum (Flow Lab.), 2 mM L-glutamine, 10 mM HEPES (Flow Lab.), 5×10^{-5} M 2-mercaptoethanol (Serva), and 50 μg/ml gentamicin (Unique). Culturing was performed in moistened air at 37°C and 5% CO_2. Secretion of IL-1 and TNF was induced as described elsewhere [6]. Cytokines were produced by peptone-activated peritoneal macrophages from 6-8-week-old C57Bl/6 mice. The animals were obtained from the Central Nursery of Experimental Animals (Kryukovo). We compared the effects of Tamerit, Polyoxidony, and GMDP in equigram concentrations of 1, 10, and 100 μg/ml on the secretion of IL-1 and TNF. For evaluation of the effect of immunomodulators on cytokine production by LPS-stimulated macrophages, the cell monolayer was incuba-

0007-4888/04/1383-0259

ted in a medium containing 1 μg/ml *E. coli* LPS (Difco) for 2 or 3 h before addition of the test preparation. IL-1 activity was assayed in the biological test with thymocytes from C57Bl/6 mice (indicator cells) [7]. TNF was studied by the method based on lysis of TNF-sensitive L-929 cells [5].

RESULTS

First, we compared the effects of immunomodulators on the production of IL-1 and TNF-α by nonactivated macrophages.

The test preparations stimulated cytokine production by nonactivated macrophages (Table 1). Tamerit in a concentration of 1 μg/ml was most potent in this respect. Licopid was superior to Polyoxidony in inducing the release of IL-1, but ranked below Polyoxidony in the ability to stimulate TNF-α secretion. These preparations in high concentrations had a less pronounced activating effect. Tamerit in high concentration slightly inhibited baseline secretion of cytokines.

Then we studied the effects of Licopid, Polyoxidony, and Tamerit on the production of IL-1 and TNF-α by macrophages preactivated with bacterial LPS. The concentration of endotoxin and time of exposure required for priming macrophages were estimated in preliminary experiments, where we evaluated the optimum time of macrophage preincubation with LPS and determined the suboptimal concentration of endotoxin inducing the release of IL-1 and TNF (data not shown). The use of LPS in suboptimal doses allowed us to study the activating and inhibitory effect of the test preparations in the model system.

TABLE 1. Effect of MDP Derivatives on the Production of IL-1 and TNF-α by Peritoneal Macrophages from C57Bl/6 Mice (*M±m*)

Immunomodulator	Concentration, μg/ml		
	1	10	100
IL-1 production			
Control	2.1±0.5	2.1±0.5	2.1±0.5
Licopid	5.6±0.7*	6.4±1.2*	4.5±0.6*
Polyoxidony	4.2±0.7*	4.9±0.8*	3.9±0.3*
Tamerit	8.9±1.7*	5.3±1.0*	1.7±0.4
TNF production			
Control	8.5±3.8	—	—
Licopid	16.4±3.8*	20.2±3.9*	14.1±3.0
Polyoxidony	18.2±5.6*	25.2±5.1*	15.9±5.2
Tamerit	26.8±5.7*	23.5±4.7*	4.9±3.4

Note. Here and in Table 2: results of 3 independent experiments. Biological activity of cytokines is characterized by the index of thymocyte proliferation after stimulation with IL-1 (arb. units) and index of TNF cytotoxicity (%). *$p<0.05$ compared to the control.

To evaluate the effect of immunomodulators on IL-1 production, macrophages were incubated with LPS in a concentration of 1 μg/ml for 2 h before administration of the test preparations into the culture medium. The study of TNF production was performed with cells exposed to LPS in the same concentration for 3 h. Preincubation of macrophages with 1 μg/ml LPS stimulated the production of IL-1 and TNF. The intensity of cytokine production was 30-50% of the level observed under the influence of bacterial endotoxin in optimal doses.

TABLE 2. Effect of Licopid, Polyoxidony, and Tamerit on Production of IL-1 and TNF-α by Macrophages from C57Bl/6 Mice (*M±m*)

Immunomodulator	Concentration, μg/ml		
	1	10	100
IL-1 secretion			
Baseline IL-1 secretion	2.2±0.4	2.2±0.4	2.2±0.4
Control (1 μg/ml LPS)	8.2±0.7	8.2±0.7	8.2±0.7
Licopid	15.6±0.9*	9.8±1.3	7.6±0.9
Polyoxidony	10.1±1.2	7.5±0.8	4.7±0.5*
Tamerit	6.9±1.6	3.1±0.7*	2.4±0.4*
TNF secretion			
Baseline TNF secretion	6.4±3.4	6.4±3.4	6.4±3.4
Control (1 μg/ml LPS)	33.5±5.9	33.5±5.9	33.5±5.9
Licopid	49.9±4.7*	43.2±6.1	24.9±6.4
Polyoxidony	38.4±4.1	27.2±5.3	18.7±5.0*
Tamerit	21.0±3.2*	13.5±4.7*	7.9±3.7*

As differentiated from nonactivated macrophages, immunomodulators had different effects on the production of IL-1 and TNF by mononuclear phagocytes primed with LPS in suboptimal doses (Table 2).

Licopid in concentrations of 1 and 10 μg/ml produced a costimulating effect on proinflammatory cytokine production. However, Licopid in a concentration of 100 μg/ml slightly suppressed cytokine production.

Polyoxidony in low concentration (1 μg/ml) slightly stimulated LPS-induced production of IL-1 and TNF. Functional activity of activated macrophages tended to decrease with increase in the concentration of Polyoxidony. Polyoxidony in high concentration inhibited the production of proinflammatory cytokines.

As differentiated from Licopid and Polyoxidony, Tamerit in different doses significantly suppressed production of IL-1 and TNF by macrophages primed with LPS. Tamerit in concentrations of 10 and 100 μg/ml decreased the intensity of cytokine production to the baseline level (Table 2).

Our results indicate that these immunomodulators regulate the production of cytokines by macrophages. Licopid, Polyoxidony, and Tamerit are the drugs of choice for patients with low functional activity of monocytes and macrophages. Tamerit should be used for the therapy of disorders accompanied by excessive release of proinflammatory cytokines from mononuclear phagocytes (including that produced by bacterial agents). Tamerit in different doses can suppress overproduction of macrophageal mediators that determine the severity of local and systemic inflammation.

REFERENCES

1. M. T. Abidov, *Terra Medika Nova*, No. 1, 28-30 (2001).
2. V. T. Ivanov, T. M. Andronova, V. A. Nesmeyanov, *et al.*, *Klin. Med.*, No. 3, 11-15 (1997).
3. V. T. Ivanov, R. M. Khaitov, T. M. Andronova, and B. V. Pinegin, *Immunologiya*, No. 2, 4-6 (1996).
4. R. V. Petrov, R. M. Khaitov, A. V. Nekrasov, *et al.*, *Allergiya Astma Klinicheskaya Immunologiya*, No. 3, 3-6 (1999).
5. H. Fisch and G. E. Gifford, *Int. J. Cancer*, **32**, 105-112 (1983).
6. D. Lovett, B. Kozan, M. Hadam, *et al.*, *J. Immunol.*, **136**, 340-347 (1986).
7. T. Uede, Y. Kohda, Y. Ibayashi, *et al.*, *Ibid.*, **135**, No. 5, 3243-3251 (1985).
8. M. Wilson, *Sci. Prog.*, **78**, No. 1, 19-34 (1995).