Effect of Configuration of Muramyl Dipeptide Glycoside Bond and Structure of Glycoside Aglycon on Their Capacity to Stimulate Production of Interleukin-1 and Tumor Necrosis Factor by Macrophages

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Effects of 11 original glycoside derivatives of muramyl dipeptide on the production of interleukin-1 and tumor necrosis factor by mouse peritoneal macrophages were studied. The relationship between macrophage activation evaluated by induction of these cytokines and configuration of glycoside bond and aglycon structure of MDP was revealed.

Key Words: *muramyl dipeptide glycosides; interleukin-1; tumor necrosis factor*

We previously showed that modification of N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) and creation of O-glycosides improved biological activity of this glycopeptide [3]. It was shown that β-glycosylation improved MDP capacity to stimulate lymphocyte proliferation and production of interleukin-2 (IL-2) by these cells *in vitro* [2]. α-Glycosides had no advantages in comparison with nonmodified MDP in this respect. We studied the relationship between configuration of the glycoside bond and aglycon structure of MDP glycosides and their macrophage-activating effect, evaluated by induction of interleukin-1 (IL-1) and tumor necrosis factor (TNF) production *in vitro*.

MATERIALS AND METHODS

MDP and its derivatives were synthesized and given by Prof. A. E. Zemlyakov (V. I. Vernadskii Tavrian National University, Simferopol) [1]. Two α-glycoside derivatives of MDP were used: MDP α-heptylglycoside (α-C_7MDP) and MDP α-cyclohexylglycoside (α-cyclMDP) and nine MDP β-glycosides: MDP β-butylglycoside (β-C_4MDP), MDP β-hexylglycoside (β-C_6MDP), MDP β-heptylglycoside (β-C_7MDP), MDP β-octylglycoside (β-C_8MDP), MDP β-cyclohexylglycoside (β-cycl MDP), MDP β-adamantylglycoside (β-adaMDP), MDP β-phenylglycoside (β-phenMDP), MDP β-phenethylglycoside (β-phenethMDP), and MDP β-naphthylglycoside (β-naphthMDP). Ultrasonic treatment was carried out in order to improve the dispersion and dissolving of MDP derivatives [7]. Cells in functional tests were cultured in RPMI 1640 (Flow Lab.) with 5% inactivated fetal calf 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) at 37°C in humid atmosphere with 5% CO_2.

The production of IL-1 and TNF was induced as described previously [10]. The producer cells were peptone-activated peritoneal macrophages of 6-8-week-old C57Bl/6 mice (Experimental Animal Breeding Center, Kryukovo). The effects of MDP glycosides on the production of IL-1 and TNF were evaluated as described previously [7]. The original MDP served as the reference control. The activity of IL-1 was evaluated in biological test with murine (C57Bl/6) thymocytes as indicator cells [11]. TNF was evaluated

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by the method based on the lysis of TNF-sensitive L-929 cells [6].

RESULTS

Production of cytokines, initiating and regulating many biological processes, including the defense reactions to introduction of infective agents and tumor transformation of cells is an important function of macrophages. IL-1 and TNF-α are the key and the most pleiotropic cytokines secreted by monocytes/macrophages [4].

We therefore investigated the capacity of MDP glycosides to regulate the production of these monokines by peritoneal macrophages *in vitro*. Since MDP derivatives induce the production of IL-1 and TNF synergistically with LPS [5], in some experiments LPS in the suboptimal concentration (20 ng/ml) was added into culture medium with test muramyl peptides.

The production of IL-1 was induced by all tested agents in concentrations 1-100 μg/ml (Table 1). MDP β-alkylglycosides (β-C_6MDP, β-C_7MDP, β-C_8MDP) and β-cyclMDP possessed the highest activity. Their stimulating effect on the production of IL-1 was higher than that of MDP in the majority of cases. β-AdaMDP showed activity higher than that of MDP only in a concentration of 1 μg/ml, while for β-phenethMDP the highest effective concentration superior to that of MDP was 100 μg/ml. Other β-glycosides did not notably differ from the reference control by their biological effect.

β-C_4MDP, β-C_6MDP, β-C_7MDP, β-C_8MDP, β-cyclMDP, β-adaMDP, and β-phenMDP in combination with LPS were more potent activators of IL-1 than MDP (Table 1). β-PhenethMDP and β-napnthMDP stimulated the cytokine production more actively than the reference control only in a high concentration (100 μg/ml).

α-Glycosides were less active than MDP both alone and in combination with LPS.

Study of muramyl peptide-stimulated TNF production did not change our concepts on their macrophage-activating effect observed with respect to IL-1 production. The most potent stimulators of TNF secretion were β-C_7MDP, β-C_8MDP, β-adaMDP, and β-phenMDP, which in many cases were significantly more effective ($p<0.05$) than the original MDP (Table 2). In combination with LPS, apart from the above-listed agents, β-C_6MDP (1 μg/ml) and β-cyclMDP (10 μg/ml) were more effective than MDP+LPS. The capacity of α-C_7MDP and α-cyclMDP to stimulate TNF production was lower than that of MDP.

These results are in line with previous data on the effect of MDP glycosides on lymphocyte proliferation and their production of IL-2 [2]. We know that some stages of lymphocyte activation depend on macrophages. The products of these cells are essential for optimal expression of *c-myc* genes, IL-2 receptor and IL-2 [8]. Therefore more potent inductors of production of IL-1 and some other first phase immune response monokines *a priori* should cause more active production of IL-2 and stimulate its reception by lympho-

TABLE 1. Effects of MDP Derivatives on IL-1 Production by Mouse Peritoneal Macrophages (*M*±*m*, *n*=3)

Experimental series	Concentration, μg/ml					
	1		10		100	
	without LPS	+LPS	without LPS	+LPS	without LPS	+LPS
Control	2.3±0.6	3.1±0.6	—	—	—	—
MDP	6.7±0.9*	8.8±1.1*	8.4±1.0*	11.3±1.4*	3.8±0.5*	4.9±0.7*
β-C_4MDP	9.2±1.4*	12.3±1.5*+	10.5±1.8*	15.3±1.7*+	5.0±0.6*	10.1±0.9*+
β-C_6MDP	11.1±1.2*+	11.9±0.9*+	11.6±1.1*+	15.7±1.5*+	6.5±1.2*+	8.2±1.1*+
β-C_7MDP	9.9±1.7*+	12.8±1.4*+	12.4±1.2*+	17.4±2.1*+	6.9±1.0*+	9.8±1.3*+
β-C_8MDP	10.6±1.9*+	13.2±1.6*+	11.3±1.4*+	17.2±1.9*+	4.3±0.7*	10.2±1.9*+
α-C_7MDP	3.4±0.8+	4.6±0.9+	5.4±1.1*+	7.3±1.2*+	3.3±0.8	6.9±1.2*
β-adaMDP	10.1±1.0*+	14.0±1.5*+	9.4±0.9*	18.1±2.3*+	2.9±0.9	8.7±0.8*+
β-cyclMDP	10.9±0.9*+	12.4±1.1*+	12.3±1.4*+	16.3±1.8*+	3.7±1.1	6.7±1.4*
α-cyclMDP	5.2±1.1*	6.4±1.0*+	5.3±0.9*+	8.9±1.2*	4.1±0.7*	4.1±0.7*
β-phenMDP	6.8±0.8*	11.8±1.7*	7.6±1.8*	15.9±1.9*+	5.2±0.8*	9.5±1.7*+
β-phenethMDP	7.3±1.4*	7.9±1.5*	9.5±1.6*	11.4±1.0*	6.1±1.2*+	9.7±1.6*+
β-naphthMDP	6.9±0.6*	8.1±0.9*	6.8±1.4*	9.8±2.1*	4.8±0.7*	8.1±1.4*+

Note. Here and in Table 2: $p<0.05$: *compared to the control; +compared to MDP.

TABLE 2. Effects of MDP Derivatives on TNF Production by Mouse Peritoneal Macrophages ($M \pm m$, n=3)

Experimental series	Concentration, μg/ml					
	1		10		100	
	without LPS	+LPS	without LPS	+LPS	without LPS	+LPS
Control	7.8±3.9	14.2±4.3	—	—	—	-
MDP	19.8±4.3*	24.7±5.0	24.9±4.1*	32.3±4.4*	17.1±3.7*	19.9±3.1
β-C_4MDP	22.6±5.6*	28.3±2.9*	29.8±3.9*	35.7±4.2*	19.1±4.4*	19.3±4.0
β-C_6MDP	25.8±4.7*	35.8±3.2*+	33.2±3.5*	39.8±3.6*	16.3±3.3*	20.7±3.3
β-C_7MDP	29.3±5.0*	39.0±5.1*+	34.5±4.2*+	46.5±5.3*+	19.9±2.7*	22.5±3.8
β-C_8MDP	28.3±3.6*	39.3±4.7*+	36.3±5.4*+	43.2±4.8*+	21.3±4.2*	21.9±3.1
α-C_7MDP	12.3±4.1	19.9±3.5	16.9±3.2*	21.5±3.7+	14.8±3.1	17.8±3.4
β-adaMDP	31.2±5.4*+	41.1±5.8*+	35.4±5.8*	49.3±6.6*+	22.3±2.7*	20.9±3.2
β-cyclMDP	27.7±4.3*	29.6±4.8*	33.1±5.5*	43.9±5.1*+	19.6±4.4*	23.3±2.7*
α-cyclMDP	14.2±3.0	18.3±2.6	19.2±2.9*	23.2±2.0*+	14.3±3.7	18.4±3.2
β-phenMDP	29.3±4.1*	39.1±5.1*+	35.3±3.3*+	44.3±4.2*+	23.5±4.9*	22.7±3.2
β-phenethMDP	28.5±3.7*	33.5±4.6*	29.1±1.0*	39.4±5.4*	19.6±3.0*	22.0±3.2
β-naphthMDP	25.3±2.9*	25.1±3.7*	23.9±4.6*	31.0±4.0*	20.6±4.2*	18.6±4.1

cytes. This explains (at least partly) the similarity of the results of evaluation of the effects of MDP derivatives on of macrophage and T lymphocyte functions. Of course, both T and B cells are direct targets of the biological effect of muramyl peptide [9]. Obviously, some characteristics of the mechanisms of lymphocyte function stimulation by MDP derivatives and the macrophage activation mechanisms are common.

Our findings confirm the assumption that structural modification of MDP molecule with fixed configuration of C_1 in β-position results in a higher biological activity of this glycoprotein, while α-glycosylation reduces it [2]. Presumably, the compound with the anomeric hydroxyl in β-position is the active MDP form, forming a mixture of α- and β-anomers in aqueous solutions at the expense of free configuration of the glycoside hydroxyl in C_1. As for the aglycon structure, the most pronounced potentiation of the activating effect was attained after addition of aliphatic (β-C_6MDP, β-C_7MDP, β-C_8MDP), adamantile (β-adaMDP), phenol (β-phenMDP), and carbocyclic (β-cyclMDP) modifying components to the MDP glycoside center.

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