BIOPHYSICS AND BIOCHEMISTRY

Effect of Individual and Combination Treatment with Cytokines on Expression of Sialoadhesin by Bone Marrow Macrophages

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 8, pp. 160-162, August, 2003
Original article submitted April 3, 2002

We studied the effects of lipopolysaccharide, interferon-γ, interleukin-1, or tumor necrosis factor-α and combination treatment with tumor necrosis factor-α and interleukin-1 or interferon-γ on the expression of sialoadhesin receptors on the membrane of bone marrow macrophages, macrophage adherent ability, and production of nitric oxide by these cells. Sialoadhesin expression was evaluated by binding of macrophages to sheep erythrocytes labeled with radioactive ^{51}Cr. Treatment of bone marrow cells with interferon-γ improved adhesive properties of macrophages, but did not modulate expression of sialoadhesin. Interleukin-1 and tumor necrosis factor-α had no effect on the test parameters. Combination treatment with these cytokines enhanced binding of sheep erythrocytes to macrophages. Administration of lipopolysaccharide or combination treatment with interferon-γ and tumor necrosis factor-α increased the count of macrophages adhering to plastic and stimulated expression of sialoadhesin. Combination treatment with interferon-γ and tumor necrosis factor-α stimulated production of nitric oxide by bone marrow macrophages. Blockade of nitric oxide synthesis had no effect on adhesive properties of macrophages and expression of sialoadhesin.

Key Words: *sialoadhesin; macrophages; nitric oxide*

Mature macrophages (MP) of the bone marrow are the major components of the hemopoiesis-inducing microenvironment. These cells secrete cytokines and carry receptors responsible for maturation, differentiation, and proliferation of hemopoietic cells [1]. Cell-cell contacts realized via specific surface structures (adhesion molecules) determine maturation of hemopoietic cells. The count of cells with surface adhesion molecules and density of these molecules determine the intensity of hemopoiesis [5,6,8]. Expression of membrane receptors on bone marrow stromal cells is regulated by various cytokines. Here we studied the effects of lipopolysaccharide (LPS), interferon-γ (IFN-γ), interleukin-1 (IL-1), or tumor necrosis factor-α (TNF-α) and combination treatment with TNF-α and IL-1 or IFN-γ on the ability of bone marrow MP to form rosettes with sheep erythrocytes (SE), adhere, and produce nitric oxide (NO).

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MATERIALS AND METHODS

Experiments were performed on 10 male C57Bl/6JY mice aging 8 weeks and obtained from the collection of the Institute of Pharmacology. The animals were

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kept under standard conditions and received sour drinking water (pH 4).

Sialoadhesin expression was estimated by binding of ^{51}Cr-labeled SE to MP [4,7]. The cells were cultured in RPMI 1640 medium (Sigma) containing 10% fetal bovine serum (Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences), 20 mM HEPES (Sigma), 0.05 mM 2-mercaptoethanol (Sigma), 50 μg/ml gentamicin (Belmedpreparaty), and 2 mM L-glutamine (Flow Lab). Bone marrow cells ($0.5\text{-}1\times10^5$) were placed in 96-well flat-bottom plates and cultured at 37°C and 5% CO_2. Nonadherent cells were removed. The suspension of ^{51}Cr-labeled SE (50 μl, 4%) was added to each well. Control wells (adhesion to plastic) did not contain bone marrow cells. Incubation was performed at 37°C and 5% CO_2. Unbound erythrocytes were removed by the method of reverse sedimentation. Triton X-100 (50 μl) was added to each well. The intensity of sialoadhesin expression was estimated by label incorporation in 100 μl supernatant from each well on a γ-counter and expressed in counts per minute (cpm).

Adherent properties of cells were determined by the ability to adhere to plastic. The count of adherent cells was determined by crystal violet accumulation [3]. The cells were incubated in 96-well flat-bottom plates. After sedimentation 100 μl 0.1% crystal violet in 25% methanol was added to each well and stained at room temperature for 45 min. Unbound dye was thoroughly removed by washing in phosphate buffered saline. Triton X-100 (100 μl, 1%) was added to each well. Optical density of the supernatant was measured at 550 nm after 10 min.

For evaluation of the effect of cytokines binding of SE by bone marrow cells was performed in the presence of 400 U/ml IFN-γ, 500 U/ml TNF-α, 50 U/ml IL-1, and 10 μg/ml LPS (Sigma). L-Monomethylarginine (LMMA, Sigma) in a concentration of 0.5 μmol/ml was used as the NO synthesis blocker.

NO production was evaluated by the content of nitrites in supernatants using Griess reagent [2]. The reagent (0.1 ml) was mixed with an equivalent volume of the supernatant. Absorption was measured at 550 nm. Nitrite concentration was determined by the calibration curve constructed using sodium nitrite.

The results were analyzed by Student's *t* test. The differences were significant at $p<0.05$.

TABLE 1. Adhesive Properties of Bone Marrow MP and Binding of SE after 24-hTreatment with Cytokines for 24 h ($X\pm m$)

MP and cytokine	Optical density, ε	Label incorporation, cpm
+SE (control)	0.41±0.14	159.3±67.0
+IFN-γ	0.82±0.27*	424.3±211.9
+IL-1	0.57±0.05	442.0±149.4
+TNF-α	0.59±0.20	493.0±205.3
+(IL-1+TNF-α)	0.47±0.07	586.0±98.7*
+(IFN-γ+TNF-α)	1.03±0.01*	1317.3±297.1*

Note. *$p<0.05$ compared to the control.

RESULTS

Preincubation of bone marrow MP with IL-1 or TNF-α for 24 h practically did not enhance cell adhesion to plastic (Table 1), the only exception was IFN-γ, which 2-fold increased MP adhesion ($p<0.05$). However, IFN-γ did not stimulate binding of SE. Combination treatment of the bone marrow with IFN-γ and TNF-α markedly increased the count of cells adhering to plastic (252%, $p<0.05$). The number of bound SE increa-

TABLE 2. Adhesive Properties of Bone Marrow MP and Binding of SE after 24-h Treatment with Cytokines against the Background of NO Synthesis Blockade ($X\pm m$)

Preincubation	MP			
	+SE (control)	+IFN-γ	+LPS	+(IFN-γ+TNF-α)
Without LMMA				
NO, μM	<2	<2	<2	28.2±2.1*
optical density, ε	0.75±0.20	0.93±0.13	1.78±0.11*	1.88±0.11*
incorporation of label, cpm	129.5±49.2	228.8±161.2	797.20±267.41*	1539.2±166.5*
With LMMA				
NO, μM	<2	<2	<2	<2
optical density, ε	0.88±0.23	1.42±0.11*+	1.57±0.33*	1.92±0.05*
incorporation of label, cpm	394.5±135.3	449.5±280.7	714.8±131.9*	1483.5±111.9*

Note. $p<0.05$: *compared to the control; +compared to preincubation without LMMA.

sed by 8 times compared to the control ($p<0.05$). Combination treatment with IL-1 and TNF-α had no effect on the number of adherent cells, but 4-fold increased the intensity of SE binding. It should be emphasized that binding of SE to MP was high after combination treatment with INF-γ+TNF-α or IL-1+TNF-α (327 and 306%, respectively, compared to the control, $p<0.05$).

Several cytokines, including IFN-γ and TNF-α, activate inducible NO synthase in MP. We studied the effects of cytokines on these properties of MP in the presence of the NO synthesis blocker LMMA. Preincubation with IFN-γ and TNF-α markedly increased the content of NO (Table 2), stimulated binding of SE (by 12 times, $p<0.05$), and enhanced adhesive properties of MP (by 2.5 times, $p<0.05$). After addition of LMMA to cultured cells the concentration of NO decreased to zero, but adhesion of MP to plastic and binding of SE remained high (258 and 1145%, respectively, compared to the control, $p<0.05$, Table 2). As differentiated from IFN-γ, preincubation of bone marrow MP with IFN-γ and LMMA increased the number of cells adhering to plastic.

Preincubation of bone marrow MP with LPS enhanced binding of SE and increased adhesive properties by 258%. NO concentration in the medium remained unchanged during incubation of cells with LPS or LMMA. Under these conditions binding of SE and adhesive properties of MP remained high and surpassed the control by 262%.

Our results indicate that IFN-γ enhances adhesive properties of bone marrow cells, but does not modulate expression of sialoadhesin. IL-1 and TNF-α have no effect on the test parameters. Combination treatment with these cytokines markedly increases binding of SE to MP. Administration of LPS and combination treatment with IFN-γ+TNF-α increase the count of MP adhering to plastic and stimulate expression of sialoadhesin. Combination treatment of bone marrow MP with IFN-γ and TNF-α intensifies production of NO. Blockade of NO synthesis with LMMA has no effect on adhesive properties of MP and expression of sialoadhesin. Moreover, IFN-γ increases these parameters in MP preincubated with LMMA.

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