IMMUNOLOGY AND MICROBIOLOGY

Suppressive Effect of Immature Erythroid Cells on the B-Cell Proliferation

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Immature erythroid cells suppress the proliferative response of preactivated B lymphocytes to lipopolysaccharide. The same effect is observed when recombinant human interleukin-2 or culture medium conditioned by preactivated T or B cells is added to cultured cells. Suppressive nucleate erythroid cells are resistant to leucine methyl ester.

Key Words: *erythroblast; natural immunosuppression; B-cell proliferation*

Natural suppressor cells (NSC) usually occur in zones of intense hemopoiesis [6]. These cells may be important for the tolerance to autoantigens and for restriction of potentially damaging immune reactions. The population of NSC is heterogeneous. Immunosuppressor activity was demonstrated for polypotent hemopoietic cells [6], immature monocytes [14], mast cells [15], and large granular lymphocytes [8].

Most NSC have a null phenotype, i.e., bear no markers of mature immunocompetent cells. After gradient centrifugation, NSC concentrate primarily in the fraction with a low floating density (1.068-1.076 g/liter) [6]. Previously, it was shown that most if not all cells with natural immunosuppressor activity are sensitive to the toxic action of leucine methyl ester (LME), a lysosomotropic agent [10,12,13].

The ability of nucleate erythroid cells (NEC) to suppress humoral immune response to both thymus-dependent and thymus-independent antigens was originally demonstrated at the Laboratory of Regulation of Immunopoiesis, Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences [1-5,9]. The immunosuppressive effect of NEC on B lymphocytes is at least partially mediated by secretion of soluble factor(s) [9].

In the present study we examined the effect of NEC on the proliferative activity of isolated preactivated B lymphocytes and evaluated their sensitivity to the cytotoxic effect of LME.

MATERIALS AND METHODS

Experiments were carried out on 6-9-month-old (CBA×C57Bl/6) F_1 (CBF_1) mice.

Phenylhydrazine (Sigma) was injected intraperitoneally in a dose of 1.2 mg/mouse. After 12 and 20 h, the compound was injected in a dose of 0.6 mg/mouse. After 48 h, spleens were isolated and used as a source of NEC.

For isolation of NEC 1 ml of splenocyte suspension (2×10^7 cells) from phenylhydrazine-treated mice was layered on Percoll (1.076 g/liter, Pharmacia) and centrifuged at 400*g* for 30 min. The cells from the interface ring were aspirated and washed three times with Dulbecco's buffer (Vektor, Russia). The cells ($10\text{-}15\times10^6$/ml) were incubated for 60 min at 4°C in serum-free RPMI-1640 medium supplemented with 20 mM HEPES in the presence of anti-Thy 1.2 monoclonal antibodies (5a-8 clone, IgG2b,

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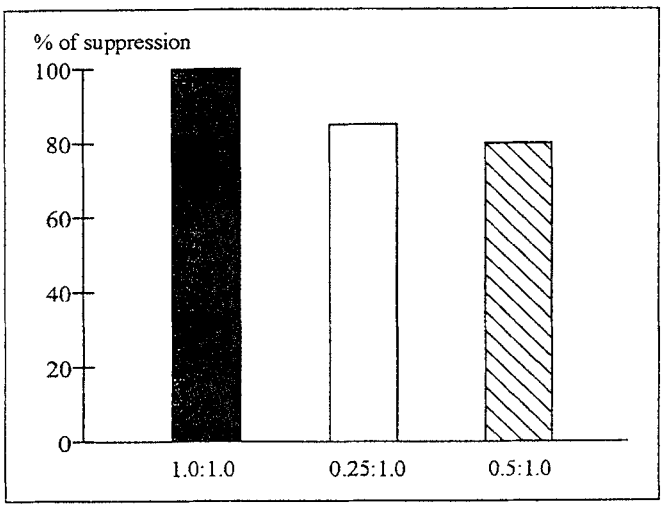

Fig. 1. Suppressive effect of nucleated erythroid cells (NEC) on proliferative activity of purified population of preactivated B lymphocytes. NEC were incubated for 48 hours with B cells (3×10^5/well) at the indicated ratios in the presence of lipopolysaccharide. Data of one of 3 experiments are shown. Here and in Fig. 2: standard deviation was not higher than 10%.

supernatant dilution 1:40, Cedarlane). After incubation, cells were washed and transferred in 90-mm plastic Petri dishes (Medpolimer, St. Petersburg) with immobilized rabbit anti-mouse immunoglobulin antibodies (Biosan, Novosibirsk). Panning was performed as described elsewhere [7]. In brief, the cells (no more than 4×10^7) were incubated for 2 h at 4°C in the dish containing RPMI-1640 medium supplemented with 20 mM HEPES and 5% fetal calf serum. In the middle of the incubation, the suspension was agitated for 20-30 sec to adsorb macrophages, B cells bearing surface immunoglobulin receptors, and T cell—anti-Thy 1.2 antibody complexes on plastic. After incubation, unattached cells were harvested and used in experiments. Morphological analysis of cells stained by the method of Nocht—Maksimov showed that the suspension contained no less that 97% NEC.

For determination of sensitivity to LME, NEC (10^7/ml) were incubated with 15 mM LME (Fluka) for 30 min at 22°C in serum-free RPMI-1640 containing 20 mM HEPES, washed three times with complete culture medium, and used in experiments. Cell viability was assessed by trypan blue exclusion test.

The cells were cultured in RPMI-1640 supplemented with 10% fetal calf serum, 20 mM HEPES, 2 mM L-glutamine (all reagents from Vektor) and antibiotics in a humidified atmosphere of 95% air/5% CO_2.

Preactivated B cells were prepared as follows: the splenocyte suspension (10 ml, 5×10^6/ml) was cultured for 24 h in the presence of lipopolysaccharide (LPS of *E. coli* 055:B5, Sigma) in a plastic flask (25 cm^2, Linbro). After washing, no more than 4×10^7 cells were incubated for 2 h at 4°C in a Petri dish containing the same culture medium. In the middle of the incubation, the cell suspension was agitated for 10 sec. After incubation, unattached cells were washed and panned against immobilized anti-immunoglobulin antibodies as described above. After the removal of unattached cells, the attached B cells were suspended with a rubber policeman (Costar), washed, and used in experiments. Immunofluorescent microscopy with FITC-labeled rabbit antibodies to mouse immunoglobulins (Cedarlane) showed that practically all cells isolated by positive panning were B lymphocytes.

The B-cell cytokines were obtained as follows: preactivated B cells ($3\text{-}4\times10^6$/ml) were cultured in 24-well plates (Linbro) for 24 h, centrifuged, and the supernatant was stored at -20°C. Special tests showed that in the presence of 12 μg/ml LPS the supernatant promoted proliferation of splenocytes seeded at the suboptimal concentration ($4\text{-}5\times10^4$) in a flat-bottom 96-well plate (data not shown).

Preactivated T lymphocytes were obtained as follows: splenocytes (10 ml, 5×10^6 cells/ml) were cultured for 24-h in the presence of concanavalin A (ConA, Pharmacia) in a plastic flask (25 cm^2, Linbro). After washing, no more than 4×10^7 cells were transferred to a 90-mm Petri dish (Medpolimer) and panned against immobilized anti-immunoglobulin antibodies as described above. Unattached cells were washed and incubated in the presence of anti-Thy 1.2 monoclonal antibodies (clone 5a-8, IgG2b, dilution 1:40) at 4°C for 60 min in serum-free RPMI-1640 containing 20 mM HEPES. After incubation, the cells were washed and transferred to a 90-mm Petri dish (Medpolimer) with immobilized anti-immunoglobulin antibodies (Biosan). After panning, attached T lymphocytes were suspended and used in experiments. Cytotoxic tests with anti-Thy 1.2 monoclonal antibodies and rabbit complement (Cedarlane) showed that practically all isolated cells were T lymphocytes.

For preparation of the T-cell supernatant, preactivated T cells ($3\text{-}4\times10^6$/ml) were cultured in a 24-well plate (Linbro) for 24 h. The culture medium was separated by centrifugation and stored at -20°C. In special tests the supernatant enhanced the antitumor cytostatic activity of bone marrow cells and activated macrophages in the presence of LPS (data not shown).

For determination of the suppressor activity NEC were cultured for 48 h (the last 4-6 h in the presence of ^{3}H-thymidine) with preactivated B cells (3×10^5/well) in the above-mentioned ratio and LPS (12 μg/ml) in a 96-well round-bottom plate (BDSF) in a volume of 0.15 ml/well. In the control samples, B cells were incubated with LPS and without NEC.

Cell proliferation was assessed by ^{3}H-thymidine incorporation. Suppression was calculated from the following formula: $(1-A/B)\times 100\%$, where A and B are radioactivities (cpm) of B cells cultured with and without NEC, respectively. Each sample was processed in triplicate (three parallel cultures).

Proliferation of preactivated B cells in response to LPS varied from 16 to 30×10^3 cpm.

The data were analyzed using the Student's t test. The differences were regarded as significant at $p<0.05$.

RESULTS

Preliminary experiments showed that LPS did not stimulate proliferation of normal B cells isolated from intact spleen by positive panning (data not shown). Therefore, we have hypothesized that initiation of B-cell proliferation depends on functional activity of the satellite cells. Our protocol including LPS-induced activation of B cells in the total splenocyte population with subsequent isolation of preactivated B cells by positive panning made it possible to obtain pure B-cell population responding to LPS in the absence of satellite cells. This approach allowed us to study the immediate effect of NEC on proliferative activity of B cells. As Fig. 1 shows, NEC markedly suppressed the LPS-induced proliferation of preactivated B cells in a dose-dependent manner.

Previously, it was demonstrated that inhibition of lymphokine production (particularly of interleukin-2) may play an important role in the immunosuppressive activity of NSC [11]. Neither 25% cultured medium conditioned by preactivated T cells nor recombinant human interleukin-2 (100 units/ml, Biogen) abolished the suppressive effect of NEC on B-cell proliferation (Fig. 2). The effect was also observed after the addition of 25% cultured medium conditioned by preactivated B cells to the culture. Thus, our findings suggest that immunostimulating lymphokines do not abolish the suppressive effect of NEC on proliferative activity of B lymphocytes.

Treatment with LME had no effect on the suppressor activity of NEC. In parallel experiments, analogous LME treatment of bone marrow cells markedly reduced their suppressor effect on ConA-induced lymphoblastogenesis (data not shown). This suggests that NSC are heterogeneous in terms of sensitivity to LME.

It was found that NEC directly suppress LPS-induced proliferation of pure population of preactivated B lymphocytes (Fig. 1), implying that immediate interaction between NEC and B cells plays a crucial role in the realization of suppressive effect of NEC on humoral immune reactions. This hypothesis was supported by the finding that neither T- or B cell-derived lymphokines nor human recombinant interleukin-2 abolish direct suppression of B-cell proliferation by NEC (Fig. 2). Thus, our data suggest that the presence of both T and B cell lymphokines has no substantial influence on NEC-induced suppression of B-cell proliferation. However, the effect of NEC on secretion of lymphokines regulating B-cell lymphopoiesis cannot be ruled out.

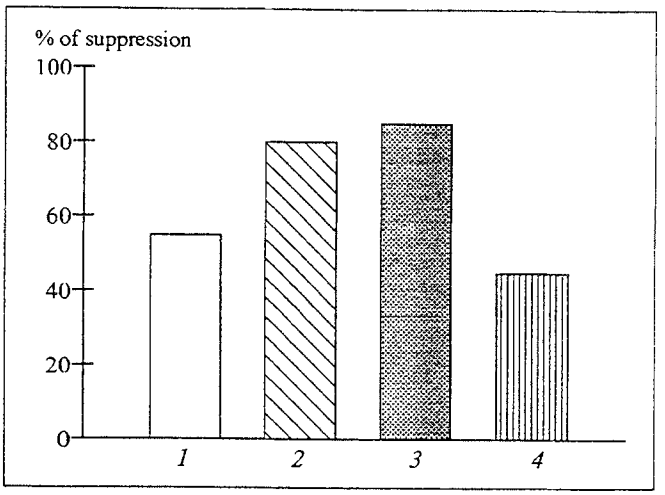

Fig. 2. Effect of lymphokines on the suppression of B-cell proliferation induced by nucleate erythroid cells (NEC). NEC were cultured for 48 h with B cells (3×10^5/well, 1:1) in the presence of lipopolysaccharide (*1*), 25% T-cell supernatant (*2*), interleukin-2 (100 units/ml, *3*), or 25% B-cell supernatant (*4*). Data of one of 2 experiments are shown.

It has been previously showed that LME is a lysosomotropic agent selectively affecting granulocytes, macrophages, natural killer cells, cytotoxic T lymphocytes, and large granular lymphocytes [12,13]. Natural suppressor cells occurring in the sites of active hemopoiesis are highly sensitive to this agent [10]. Our experiments showed that the treatment of erythroid cells with LME has no pronounced effect on their immunosuppressive activity. Presumably, the resistance to LME is characteristic of NEC, which distinguishes them from other NSC.

The proliferative responses of B cells are more sensitive to the suppressive effect of NEC than those of T cells [3,4]. The mechanism of this phenomenon remains unclear and requires further investigations.

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Immunotropic Action of Nonlaser Monochromatic Radiation

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It is shown that infrared or monochromatic red light from a novel device stimulates immune processes and nonspecific resistance in mice.

Key Words: *infrared light and monochromatic red light; immune system*

Low-intensity helium-neon lasers [1-3] (for example, semiconductor *Uzor* lasers [4]) have found wide application in clinical practice. In recent years, monochromatic radiation has been successfully used in the treatment of some diseases. Unfortunately, its effects have not been studied in sufficient detail in experiments, and clinical application in most cases remains empirical. Here we report the data on the effects of infrared light (IRL) and monochromatic red light (RL) generated in a KRIK-ED-57 apparatus on the immune system and nonspecific resistance of mice. Red light has a wavelength of 0.66-0.68 μ and penetrates deeper (1.5 cm) into living tissues than does the light from a helium-neon laser (0.5 cm). The wavelength of IRL (0.89 μ) is the same as that of the radiation from an Uzor laser, but its mean power (not less than 150 mW), which determines the absorbed radiation dose, is five times as high as that from an Uzor laser (30 mW).

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MATERIALS AND METHODS

The study was conducted on (CBA×C57Bl/6) F_1 mice and noninbred mice weighing 16-18 g. A KRIK-ED-57 apparatus provided monochromatic infrared and red light at a power flux density of 10 mW/cm^2 for IRL and 1 mW/cm^2 for RL.

In order to assess the effects of IRL and RL on the humoral immune responses the area of the sternum projection was irradiated for 1 or 2 min once or three times, after which splenic antibody-producing cells were identified by local hemolysis in gel [5]; serum titers of hemagglutinins and hemolysins were determined by conventional methods and expressed as natural logarithm.

The effects of IRL and RL on cell-mediated immunity were assessed using the delayed hypersensitivity test [6]. The thymic area was irradiated for 1 min once or three times within a 24-h period before sensitization or once before challenge with sheep erythrocytes. Intact mice served as the control.

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