EFFECT OF MICROBIAL SENSITIZATION AND DELAYED-TYPE HYPERSENSITIVITY REACTIONS ON INTERLEUKIN-1 PRODUCTION AND CYTOTOXICITY GENERATION BY MONOCYTES AND NATURAL KILLER CELLS

S. M. Gordienko

UDC 616-056.43-022.7-092:612.112.95.017.4

KEY WORDS: monocytes, natural killer cells, delayed-type hypersensitivity reaction, interleukin I

The connection between allergy and immunity, since the classical experiments of Robert Koch on tuberculosis, has been discussed in many publications because of the enormous theoretical and practical importance of this problem, but the nature of this connection still remains debatable [1, 2, 7]. The writer previously showed [3, 6] that injection of specific antigen, namely old tuberculin or staphylococcal phage lysate into animals sensitized with BCG vaccine or staphylococci respectively, inducing a reaction of delayed-type hypersensitivity (DTH), leads to an increase in resistance to tumors and infection, which is connected with potentiation of the effector functions of macrophages and killer cells.

The aim of this investigation was to study the mechanisms of potentiation of cytotoxicity of mononuclear phagocytes and natural killer (NK) cells during the DTH reaction.

EXPERIMENTAL METHOD

Immunocompetent cells utilized were from 86 healthy subjects aged 20-34 years with positive (TB^+) and negative (TB^-) tuberculin tests. To obtain monocytes from blood, mononuclear cells were first isolated on a one-step Ficoll–Verografin gradient (d = 1.077). Further enrichment with monocytes was carried out by making use of their adhesive properties [10] in FB-4 plastic dishes (Linbro, England), treated with embryonic calf serum (ECS). The purity of the monocytes when stained for nonspecific esterase varied from 87 to 98% and viability, according to trypan blue uptake, was not below 95%. To obtain large granular lymphocytes (LGL) mononuclears were isolated in the first stage on a Ficoll–Verografin gradient, adherent cells were isolated on columns with nylon wadding in the second stage, and they were layered on a two-step Percoll gradient (Pharmacia, Sweden) in the third stage. An enriched population (70-90%) of LGL was obtained on the boundary between 42.5 and 47.5% Percoll by centrifugation for 30 min at 500g.

The cytotoxicity of monocytes and LGL was tested by the writer's method [4], using K-562 cells as target cells. The ratio of effectors to targets for monocytes was always 15:1 and for LGL 10:1; the time of the cytotoxic test was 4 h with activation of effectors in vivo, and 16 h in vitro. The number of monocytes in the blood was counted in films, the number of LGL in centrifuge preparations of concentrated leukocytes stained by the Romanovsky–Giemsa method [12].

To determine interleukin-1 (IL-1) production by monocytes or LGL, these cells in a concentration of 10^6/ml in nutrient medium RPMI-1640 (Flow Laboratories, England) with 10% ECS 10 mM HEPES, 200 μg/ml L-glutamine, and 80 μg/ml gentamicin, were treated for 24 h with 20 μg/ml of lipopolysaccharide (LPS) from E. coli 0127:B8 (Difco, USA) or 20 μg/ml of tuberculin PPD in 24-well plates (Costar). Activity of the supernatants in a final dilution of 1:4 was estimated on the basis of their commitogenic effect on thymocytes (5×10^6 cells in 1 ml) of 5-10 week C3H/He mice in the presence of a suboptimal dose (2 μg/ml) of phytohemagglutinin P (Difco) in flat-bottomed 96-well Micro Test II 3040 plates (Falcon Plastics, USA) with

City Immunologic Laboratory, Factory Hospital, Altai Automobile Construction Combine, Barnaul. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 9, pp. 324-327, September, 1986. Original article submitted June 12, 1985.

 0007-4888/86/0009-1244$12.50

TABLE 1. Affinity of Effector Cells of TB^+ and TB^- Individuals After Injection of Tuberculin PPD or BCG Vaccine

Group of subjects tested	Injected with	Cytoxicity, % of lysis of K-562 cells	
		monocytes	effector cells (LGL)
TB^- individuals	PPD	21±1,9 24—3,3	34±2,7 38—3,4
	BCG	22±1,5 26±3,1	37±2,5 42±3,1
TB^+ individuals	PPD	25±2,0 42±3,6*	36±3,0 68±5,6*
	BCG	23±1,7 48±3,9*	38±3,2 81±5,8*

Legend. For each value the upper series of numbers indicates activity of effector cells before injection of PPD or BCG, the lower series the same, 24 h after injection. *) Significance of differences within groups before and after injection of preparations at $P < 0.001$ level.

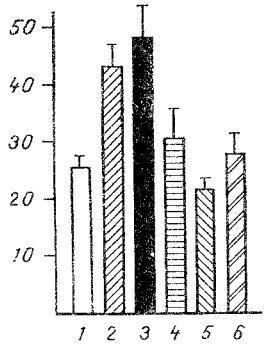

Fig. 1

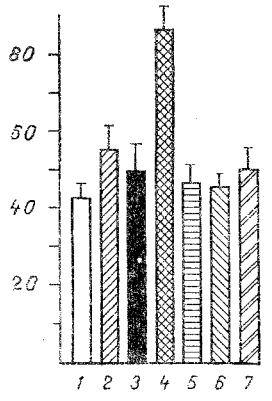

Fig. 2

Fig. 1. Role of Ia structures in cytotoxic activation of monocytes by preparations inducing DTH reaction in TB^+ individuals. Ordinate, percentage of lysis of K-562 target cells by monocytes. 1) Control with no treatment of effectors; 2-6) treatment in vitro; 2) with PPD, 3) with BCG, 4) with PPD + anti-Ia-antibodies, 5) with PPD + anti-Ia-antibodies + complement, 6) with PPD + UV irradiation.

Fig. 2. Effect of monocytes and their Ia structures on cytotoxic activation of LGL from TB^+ individuals by preparations inducing DTH reaction. Ordinate, percentage lysis of K-562 target cells LGL. 1) Control; 2-7) treatment in vitro: 2) with PPD, 3) with BCG, 4) with PPD + 10% monocytes, 5) with PPD + 10% monocytes treated with anti-Ia-antibodies, 6) with PPD + 10% monocytes treated with anti-Ia-antibodies and complement, 7) with PPD + 10% monocytes, treated by UV-irradiation.

the above-mentioned nutrient medium with the addition of 2×10^{-5} M 2-mercaptoethanol [11]. To act on the Ia structures of the macrophages, these cells were treated with IKO-1 monoclonal antibodies (generously provided by I. N. Blokhina and V. V. Novikov, Gor'kii Research Institute of Epidemiology and Microbiology) in a concentration of 10 μg/ml for 1 h at 37°C, and after washing twice, some of the macrophages were additionally treated with low-toxicity rabbit complement in a final dilution of 1:10. In parallel experiments, for the same purpose, a monolayer of macrophages in LUX Sci No. 5220 plastic dishes were subjected to UV irradiation on an OKN-11 source in a dose of 200 J/m^2.

EXPERIMENTAL RESULTS

A single intradermal injection of tuberculin PPD (2 TU) or of BCG vaccine (0.05 mg) caused marked potentiation of cytotoxic activity of monocytes and LGL after 24 h in TB^+ individuals, whereas in TB^- subjects this activity was unchanged (Table 1). Individual analysis revealed good correlation between the intensity of the DTH reaction to PPD, as reflected in the results of skin tests, and potentiation of cytotoxicity of macrophages ($r = 0.88$, $P < 0.01$) and LGL ($r = 0.91$, $P < 0.001$). The skin test with PPD became positive in some TB^- individuals 1 month after injection of BCG, i.e., sensitization to BCG appeared, but the level of cytotoxicity of the monocytes and LGL did not differ significantly from that before sensitization. Injection of PPD into one such group of subjects with a positive tuberculin test now caused activation of the effector cells. Thus activation of natural cytotoxicity of human monocytes and LGL arises, not in response to microbial sensitization, but only in the course of the DTH reaction, confirming results obtained previously in animals [3]. Besides potentiation of effector cell function, the number of these cells was increased in the group of subjects with a positive Mantoux test 24 h after injection of PPD (the number of monocytes from 7.6 ± 0.9 to 11.2 ± 1.0%, the number of LGL from 5.4 ± 0.3 to 9.2 ± 0.8%, with an increase in the number of leukocytes from 4.8×10^6 to 7.3×10^6/ml), whereas in subjects with a negative test these changes were at a low level of significance.

In experiments in vitro to elucidate some mechanisms of activation of effectors during the DTH reaction it was found that addition of specific antigens, namely BCG vaccine (25 μg/ml) or tuberculin PPD (20 μg/ml) to an enriched population of monocytes (95.6 ± 4.2%) from sensitized TB^+ individuals led to a marked increase in their cytotoxicity (Fig. 1). Similar addition of these same antigens to monocytes of TB^- individuals had no significant effect on the result of the cytotoxic test (data not given). Incubation of PPD with monocytes from TB^+ individuals, treated with monoclonal anti-Ia-antibodies (especially after additional treatment with complement) or by UV irradiation, did not lead to potentiation of monocytic cytotoxicity.

Besides monocytes, LGL of TB^+ individuals were activated by the additon of specific antigens, but the potentiation of cytotoxicity was relatively weaker and the soluble PPD antigen led to more marked activation than the corpuscular, in the form of BCG mycobacteria (Fig. 2). Addition of 10% of monocytes to the enriched LGL population, together with PPD, led to a sharp increase in their cytotoxicity, which indicates the need for cooperative interaction of monocytes and LGL in order that the cytotoxicity of the latter be increased. Monocytes treated with anti-Ia-antibodies with or without complement or with UV irradiation did not significantly change the cytotoxicity of LGL.

Monocytes from TB^+ donors (divided into two subgroups), some of whom had received an injection of BCG vaccine 4 weeks previously ($1TB^+$), whereas others had not received such an injection ($2TB^+$), produced a high level of IL-1 in the presence of tuberculin PPD, whereas monocytes from TB^- individuals produced a low level of IL-1, comparable with a culture of unstimulated monocytes (Fig. 3). Stimulation of monocytes of TB^+ individuals, especially $1TB^+$, by LPS also led to higher production of IL-1 than by TB^- monocytes. Addition of indomethacin (from Merck, West Germany), an inhibitor of prostaglandin synthesis, in a final concentration of 10^{-6} M to the monocyte culture simultaneously with LPS led to increased production of IL-1, but by a relatively greater degree in TB^- than in TB^+ individuals (especially in $1TB^+$). Stimulation of monocytes of TB^+ individuals by LPS led to greater production of IL-1 and to less production of prostaglandins than by monocytes from TB^- individuals, with, consequently, a greater activating effect on thymocyte proliferation. Treatment of monocytes of TB^+ individuals with anti-Ia-antibodies abolished the activating effect of LPS or PPD, added in vitro. The 24-h culture medium of monocytes from all groups of subjects, untreated in vitro with LPS or PPD, possessed low interleukin activity, but

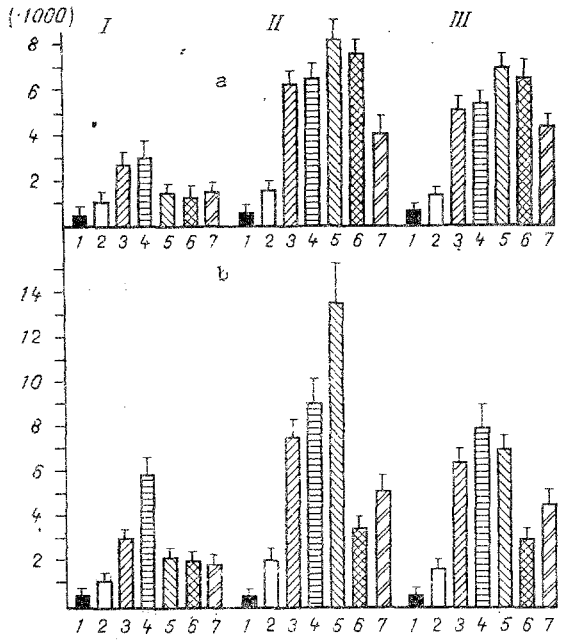

Fig. 3. Differences in IL-1 production (in cpm) by LGL (a) and monocytes (b) of TB^+ and TB^- individuals. 1) Thymocyte control; 2-7) medium from macrophages or LGL: 2) without stimulation, 3) treatment with LPS in vitro, 4) LPS + indomethacin, 5) PPD, 6) PPD + anti-Ia-antibodies, 7) without stimulation in vitro from subjects with skin test to PPD. I) TB^- donors II) $1TB^+$ donors, III) $2TB^+$ donors.

monocytes isolated from TB^+ individuals, injected with PPD in vivo 24 h previously, i.e., from subjects with a DTH reactions, spontaneously produced increased quantities of IL-1.

Similar but weaker activation of IL-1 production was observed in TB^+ individuals when LGL were tested also, the difference being that indomethacin and anti-Ia-antibodies did not affect production of IL-1 by LGL. This result can be taken as evidence against the possibility that LGL secretes prostaglandins, although sensitivity of LGL to the inhibitory action of prostaglandins on IL-1 production has been demonstrated [9], and also against the participation of Ia structures in the regulation of IL-1 production by these cells.

Previously we obtained similar data on stimulation of IL-1 production by peritoneal macrophages of BCG-sensitized mice under the influence of PPD and LPS [5]. It was later shown [8] that some allergens inducing atophy can induce IL-1 and prostaglandin production in a culture of human monocytes, but no difference was found in the production of these mediators in healthy individuals and patients with allergy.

The results indicate an important role of IL-1, and also of the Ia structures of macrophages in activation of the natural (nonimmune) cytotoxicity of macrophages and effector cells in the DTH reaction caused by injection of a specific antigen into a previously sensitized organism, and they also shed light on the causes of successful or unsuccessful use of microbial immunomodulators, such as BCG vaccine, in the treatment of tumors.

LITERATURE CITED

1. A. D. Ado, General Allergology [in Russian], Moscow (1978).
2. B. D. Brondz, Usp. Sovrem. Biol., 59, No. 2, 257 (1965).
3. S. M. Gordienko, "Antitumor and antiinfectious activity of macrophages and killer cells in the delayed-type hypersensitivity reaction," Author's Abstract of Dissertation for the Degree of Candidate of Medical Sciences, Novosibirsk (1983).
4. S. M. Gordienko, Lab. Delo, No. 9, 45 (1983).
5. S. M. Gordienko, in: Mediators of the Immune Response in Experimental and Clinical Medicine [in Russian], Moscow (1983), p. 42.

6. B. V. Polushkin and S. M. Gordienko, Vopr. Onkol., No. 7, 51 (1980).
7. V. I. Pytskii, Immunologiya, No. 3, 82 (1980).
8. J. M. Cavaillon, J. P. Dandeu, S. Mecheri, et al., Int. Arch. Allergy, 75, 113 (1984).
9. J. Herman and A. R. Rabson, Clin. Exp. Immunol., 57, 380 (1984).
10. K. Kumagi, K. Itoh, S. Hinuma, et al., J. Immunol. Metho, 19, 17 (1979).
11. S. G. Mizel and D. Mizel, J. Immunol., 126, 834 (1981).
12. T. Timonen, J. R. Ortaldo, and R. B. Herberman, J. Exp. Med., 153, 569 (1981).

STIMULATING EFFECT OF A POLYION OF CYTOLYTIC T LYMPHOCYTE PRODUCTION IN CELL CULTURE IN VITRO

I. G. Derevyanchenko, S. N. Bykovskaya, R. I. Ataullakhanov, and R. M. Khaitov

UDC 612.112.94.017.1.014.46: 615.276.4/-084

KEY WORDS: immunostimulator, cytolytic T lymphocytes, proliferation of lymphocytes.

The immunostimulating effect of polyions on antibody formation has been studied in detail [2, 3, 6]. The cellular and molecular mechanisms of this phenomenon have been investigated during synthesis of protective antibodies in response to microbial [7] or viral [8] antigens under natural conditions in vitro, and also in cultures of lymphoid cells in model experiments in vitro [5, 9]. The action of polyions potentiating T-cell responses has been studied [7]. On the whole, however, the effect of polyionic immunostimulators on immunity mediated by effector T lymphocytes has been inadequately studied. Yet as we know, these cells are one of the most important factors in antitumor immunity.

The aim of this investigation was to study the action of the polyionic agent vegetan on the formation and functioning of cytolytic T lymphocytes (CTL) in cell culture.

EXPERIMENTAL METHOD

Mice of lines BALB/c ($H\text{-}2^d$), C3H ($H\text{-}2^k$), and (CBA × C57BL)F_1 hybrids aged 8-12 weeks were studied. Mouse splenocytes were suspended in medium RPMI 1640, to which were added 10% embryonic calf serum, 2 mM L-glutamine, 5 mM HEPES, 30 μM 2-cercaptoethanol, and 100 U each of penicillin and streptomycin. The cells were cultured in 96-well plastic plates (Falcon Plastics, USA) at 37°C in an atmosphere of 5% CO_2 for 3-5 days.

CTL were obtained in mixed lymphocyte culture (MLC) by the method in [10]. For this purpose, 2×10^6 splenocytes from BALB/c mice were incubated for 5 days with 10^6 or 10^5 C3H mouse spleen cells, previously irradiated in a dose of 1000 R.

The cytolytic activity of cells from MLC was determined by using L-fibroblasts of the $H\text{-}2^k$ haplotype from mice syngeneic with C3H as the target cells. A suspension of 10^6 L-cells in 1 ml was incubated in the presence of ^{51}Cr (100 μCi/ml) for 45 min at 37°C. The L cells were then washed 3 times by centrifugation in Hanks' solution and introduced at 37°C into flat-bottomed plates (Falcon Plastics) at the rate of 4×10^4 cells per well in a volume of 100 μl. Lymphocytes from a 5-day MLC were added for 3 h with lymphocyte–target ratios of 10:1, 5:1, and 2:1 respectively. The degree of cytolysis was estimated from the specific outflow of ^{51}Cr from the target cells into the incubation medium. For this purpose the cell-free fluid was transferred into plastic ampules for measurement of radioactivity in a γ-spectrometer (Nuclear Chicago, USA). The percentage of specific outflow of chromium was calculated by the equation:

$$\text{Cytolysis} = \frac{\text{Experiment} - \text{control}}{\text{Total lysis} - \text{control}} \times 100\%.$$

All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'nois Biologii i Meditsiny, Vol. 102, No. 9, pp. 327-329, September, 1986. Original article submitted February 14, 1986.

 0007-4888/86/0009-1248$12.50