MECHANISM OF ACTION OF LEVAMISOLE ON CELLS OF THE MONONUCLEAR PHAGOCYTIC SYSTEM

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UDC 615.276.4.015.45:612.112.3

KEY WORDS: macrophages; membrane permeability; lysosomes; levamisole.

Much attention is currently being paid to the study of factors affecting the state of immunocompetent cells. Levamisole is an agent with immunostimulant action. Most workers have studied the effect of levamisole on lymphocyte function. Levamisole has been shown to stimulate proliferation of lymphocytes [3], to increase the number of E-rosette-forming cells [10, 11], to promote maturation of immunologically immature cells and, in particular, to promote maturation of some thymocytes [8]. There is evidence that levamisole affects certain functions of macrophages [5, 7], chemotaxis in leukocytes [9], and lymphokine production [12]. It has been suggested that levamisole exerts its action on intracellular cyclic nucleotide levels [6].

The object of this investigation was to study the effect of levamisole on the phagocytic function of cells of the mononuclear phagocytic system (macrophages of peritoneal exudate and spleen), and also on the response of the lysosomal apparatus and its membranes.

EXPERIMENTAL METHOD

Experiments to study the phagocytic activity of macrophages were conducted on mice of line A. Sheep's erythrocytes (SE), in a dose of $3.5 \cdot 10^8$ cells, labeled with ^{51}Cr, were used as the antigen. Peritoneal exudate cells (PEC) were obtained on the 4th day after intraperitoneal injection of nutrient broth (NB) into mice. A macrophage monolayer was obtained by culturing PEC ($1 \cdot 10^7$ cells in 5 ml medium No. 199) at 37°C for 1 h, followed by careful washing to remove nonadherent cells and free antigen. To obtain splenic macrophages, spleens were homogenized in medium No. 199 with heparin, the cell concentration was adjusted to $2 \cdot 10^6$/ml, and they were cultured in the same way as PEC.

Depending on the experimental conditions, the antigen simultaneously with levamisole (0.07 mg per mouse or culture) was injected *in vivo* or added to the macrophage culture in the above-mentioned doses. Ingestion of SE-^{51}Cr by macrophages was determined by means of a Gamma Spectrometer (Nuclear Chicago).

To study the effect of levamisole on the state of the lysosomal apparatus in PEC and spleen cells, the activity of cathepsin, a proteolytic lysosomal enzyme, was determined. This series of experiments was conducted on CBA mice. Cathepsin activity was determined by Anson's method [4] in the cytoplasmic fraction of the cells (fraction 1) and in the lysosomes themselves after lysis with distilled water (fraction 2). A 2.5% solution of hemoglobin in an acid medium was used as the substrate. The results were expressed in μg tyrosine/mg protein. Sterility of the lysosomal membranes was judged by the ratio of enzyme activity in the cytoplasmic fraction to its activity in the lysosomes (fraction 1:fraction 2).

To study the effect of levamisole on the ability of PEC and splenic macrophages to adhere to the glass, a cell monolayer was obtained, carefully washed to remove nonadherent cells, fixed in methanol, and stained in a 1% solution of eosin and methylene blue. The number of adherent cells in 50 fields of vision was counted.

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 90, No. 10, pp. 456-458, October, 1980. Original article submitted December 17, 1979,

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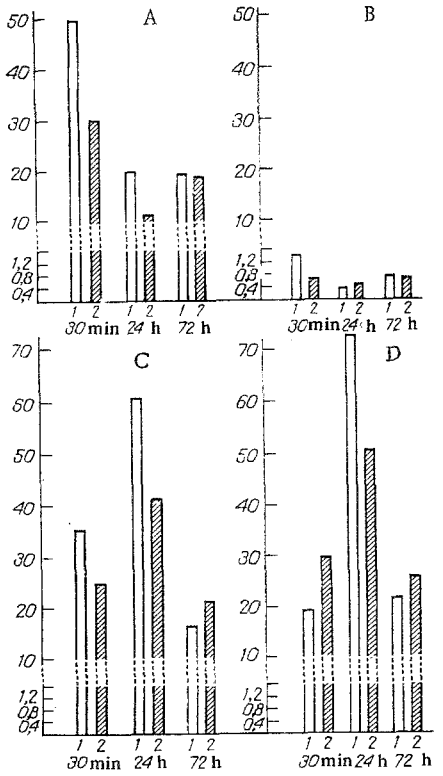

Fig. 1. Effect of levamisole on uptake and elimination of SE-^{51}Cr. A) Peritoneal exudate macrophages *in vivo*; B) splenic macrophages *in vivo*; C) peritoneal exudate macrophages *in vitro*; D) splenic macrophages *in vitro*. 1) SE-^{51}Cr 2) SE-^{51}Cr + levamisole. Abscissa, time of determination; ordinate, phagocytic activity ofmacrophages (in cpm × 1000/mg protein).

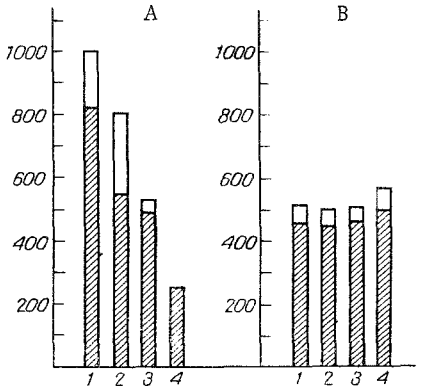

Fig. 2. Cathepsin activity after injection of levamisole. A) Peritoneal exudate cells; B) spleen cells; 1) intact animals; 2) levamisole; 3) SE; 4) levamisole + SE. Ordinate, cathepsin activity (in μg tyrosine/mg protein). Shaded part of columns represents cathepsin activity in lysosomal fraction; unshaded part of columns represents cathepsin activity in cytoplasmic fraction.

TABLE 1. Permeability of Lysosomal Membranes after Injection of Levamisole

Treatment of mice	Number of mice	Permeability of membranes (fraction 1 : fraction 2)		
		PEC	*P*	spleen cells
Intact	25	0,23	—	0,13
Levamisole	25	0,47	<0,001	0,11
SE $3,5 \cdot 10^8$	25	0,07	<0,001	0,11
Levamisole + SE $3,5 \cdot 10^8$	25	0,002	<0,001	0,12

EXPERIMENTAL RESULTS

Investigation of the effect of levamisole on the phagocytic activity of the macrophages in experiments *in vivo* showed (Fig. 1) that 30 min after injection of antigen the peritoneal macrophages of mice treated with levamisole and receiving simultaneous intraperitoneal injection of SE-^{51}Cr contained only half as many erythrocytes as in animals not treated with levamisole. By 24 h, 50% of the antigen relative to the amount of it detected 30 min after injection was still present in macrophages of both groups. After 72 h, under the influence of levamisole 53% of the antigen still remained in the peritoneal macrophages, compared with only 31% of antigen in the macrophages in the control group after 72 h compared with the quantity detected after 30 min.

The same pattern was observed when uptake of SE-^{51}Cr by splenic macrophages from mice treated with levamisole was studied (Fig. 1).

In the experiments *in vitro*, after contact for 24 h between peritoneal exudate macrophages and SE-^{51}Cr and levamisole, a delay in the entry of antigen into the macrophages was found under the influence of levamisole (Fig. 1). In the macrophage cultures of the experimental groups 50% of the antigen still remained after the end of contact of the macrophages with SE and levamisole, compared with only 24% in the controls. Similar results also were obtained when uptake of SE-^{51}Cr by splenic macrophages was studied (Fig. 1).

The object of the next series of experiments was to study the effect of levamisole on the state of the lysosomal apparatus of the macrophages. As Fig. 2 shows, preliminary injection (24 h before injection of the cells) of levamisole into the animals led to a decrease in total cathepsin activity (fraction 1 + fraction 2) in the peritoneal macrophages. Under these circumstances cathepsin activity detected in their lysosomal fraction was much lower, whereas in the cytoplasmic fraction it was higher than the corresponding values in cells of the intact control mice.

Injection of antigen without levamisole also led to a decrease in cathepsin activity. Simultaneous injection of SE and levamisole led to a further decrease both in total activity and in cathepsin activity in the lysosomal and cytoplasmic fractions.

It is important to note that lysosomes of PEC and of spleen cells reacted differently to injection of levamisole. Whereas PEC reacted sensitively to treatment of the mice with levamisole, the enzyme activity of the spleen cells of these animals was virtually indistinguishable from the control level (Fig. 2). Similar results were obtained by the writers previously after injecting substances of different nature into animals (SE, albumin, typhoid vaccine, antilysosomal sera [2]).

The characteristics of stability of the lysosomal membranes, reflected in the ratio of the enzyme level in the cytoplasmic fraction to that in the lysosomal fraction (membrane permeability), are of great interest.

In the group of intact CBA mice membrane permeability for PEC was 0.23 (Table 1). Preliminary injection of levamisole alone led to an increase in permeability of the lysosomal membranes of the peritoneal macrophages. Injection of the antigen alone led to a sharp decrease in membrane permeability. Simultaneous injection of levamisole and SE into the animals led to stabilization of the lysosomal membranes of the peritoneal macrophages. The permeability of the lysosomal membranes of the spleen cells was virtually unchanged after all forms of treatment.

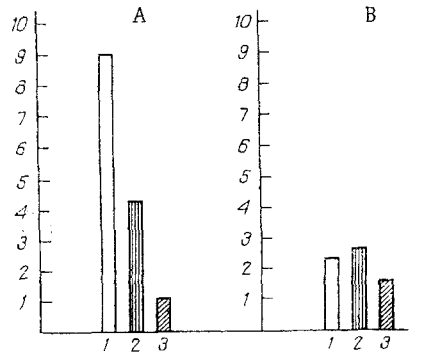

Fig. 3. Effect of levamisole on ability of macrophages to adhere to glass. A) Peritoneal exudate macrophages; B) splenic macrophages. 1) Intact animals; 2) SE; 3) levamisole + SE, Ordinate – number of adherent cells (in thousands).

A study of the ability of peritoneal and splenic macrophages to adhere to the slide showed that only 26% of peritoneal macrophages taken from animals receiving SE and levamisole simultaneously 30 min beforehand adhered to the glass compared with the number of peritoneal macrophages from animals receiving SE alone. The corresponding figure for splenic macrophages was 60% (Fig. 3).

The results of these experiments indicate that uptake and catabolism of antigen in the macrophages are modified by levamisole. In all experiments, both *in vivo* and *in vitro*, treatment of the macrophages with levamisole led to reduced uptake of antigen. Levamisole also delayed elimination of the ingested antigen. It can be tentatively suggested that, by delaying the entry of SE into the macrophages and also their elimination, levamisole regulates catabolism of the antigen and so promotes its more complete assimilation. The data on delay of elimination of SE-^{51}Cr from peritoneal macrophages and stabilization of their lysosomal membranes under the combined influence of antigen and levamisole suggest that levamisole exerts an adjuvant action. A similar effect of certain adjuvants on the lysosomal apparatus ofmacrophages was described previously by Bubashvili [1].

Changes in cathepsin activity observed under the influence of levamisole were not of the nature of a true decline, which usually takes place as the result of utilization of enzymes in antigen catabolism and is expressed as a decrease in cathepsin activity in the lysosomal fraction and a reduction in permeability of the lysosomal membranes (the ratio of fraction 1:fraction 2 was 0.47 compared with 0.23 in the control), to a decrease in cathepsin activity in the lysosomal fraction, and to a simultaneous and proportional increase in its activity in the cytoplasmic fraction of the PEC. Under these circumstances the total enzyme activity in PEC (fractions 1 + 2) fell, probably as a result of increased permeability of the cell membranes also.

Simultaneous administration of levamisole and SE to the mice led to a further decline in cathepsin activity in the lysosomes, but by contrast with the action of levamisole alone, the combined effect of antigen and levamisole led to stabilization of the lysosomal membranes of the peritoneal macrophages.

Considering that levamisole modifies permeability of the lysosomal membranes and also affects adhesion of macrophages to a surface, it can be postulated that one mechanism of its action on macrophages is through the change it produces in both the outer and the inner cell membranes.

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ANTIBODIES AGAINST AN INTERSPECIFIC ERYTHROKARYOCYTE ANTIGEN IN PATIENTS WITH PARTIAL RED CELL APLASIA OF THE BONE MARROW

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UDC 616.419-007.23-07: 616.155.14-097.5-078

KEY WORDS: partial red cell aplasia of bone marrow; erythrokaryocytes; interspecific antigen.

Partial or "pure" red cell aplasia (PRCA) of bone marrow is a disease characterized by selective damage to the erythroid branch of hematopoiesis with a reduction in the number of bone-marrow erythrokaryocytes which may amount to total disappearance, reticulocytopenia, and severe normochromic anemia. Antibodies with a selective cytotoxic action on nucleated red cells in the bone marrow have been found in the plasma of some patients with PRCA [7, 8].

A previous investigation showed that an antigen immunologically similar to mouse erythroblast antigen (EB-AG) described perviously [2], is present on the surface of erythrokaryocytes in adult human bone marrow and embryonic human liver.

In the present investigation an attempt was made to identify the antigenic specificity of antibodies from patients with PRCA by testing the patients' sera in the *in vitro* cytotoxicity test (CTT) and the indirect immunofluorescence test (IFT) against target cells of different types containing surface interspecific mammalian erythrokaryocytic antigen, by the use of absorption tests, and by blocking the IFT by serum against mouse EB-AG.

EXPERIMENTAL METHOD

Sera from 19 patients with PRCA and from 14 patients with autoimmune hemolytic anemia (AIHA), with incomplete thermoagglutinins against mature peripheral erythrocyte antigen (control), and pools of 10-20 healthy donors' sera were inactivated by heating to 56°C for 30 min. Before the test with human cells the sera were exhausted twice with an equal volume of peripheral erythrocytes and, in some cases, by a freeze-dried preparation of human amniotic fluid. In tests with mouse cells the sera were first absorbed with equal volumes of erythrocytes (twice) and thymocytes of BALB/c mice. Spleen cells of mice with Rauscher leukemia, erythrokaryocytes from mouse and human embryonic liver, normoblasts from bone marrow of patients with microspherocytosis and from the spleen of a patient with H hemoglobinopathy, with a high content of erythrokaryocytes, and also bone marrow cells from a patient with acute erytheoleukemia, were used as target cells for the IFT and CTT. All suspensions contained about 80% of erythrokaryocytes. The methods of preparation of the cell suspensions, of conducting the IFT, and of obtaining sera and monospecific antibodies against EB-AG were described previously [3]. The CTT was performed by a modified method of Gorer and O'Gorman, using the indirect variant with additional treatment of the cells with serum against human IgG [5]. IFT blockade was carried out by incubating the cells with monospecific antibodies

Department of Virus Preparations Control, L. A. Tarasevich Research Institute for Standardization and Control of Medical Biological Preparations; Laboratory of Immunochemistry and Diagnosis of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR; Department of Hematology, Central Postgraduate Medical Institute, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 90, No. 10, pp. 459-461, October, 1980. Original article submitted July 26, 1979.

 0007-4888/80/9010-1410$07.50 © 1981 Plenum Publishing Corporation