MICROBIOLOGY AND IMMUNOLOGY

Effect of Adriamycin and Its Complexes with Transition Metals on Induction of Immune Response of Human Lymphocytes in Culture

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Effect of adriamycin on effector cells of humoral and cell immune response can be enhanced or supressed by its complexation with Fe^{3+}, Cu^{2+} and Co^{2+}.

Key Words: *adriamycin; transition metal ions; lymphocytes; immune response in culture*

Cell and molecular mechanisms of induction and regulation of immune response in cultured human lymphocytes is the key problem of modern immunology.

Earlier, we used the system of humoral immune response induction under the conditions of adaptive reactions in lymphocyte culture for elaboration of the methods of these cells immunization [1,3]. It was shown that cytotoxic drugs adriamycin (ADR) and ethidium bromide potentiated both constitutive immunoglobulin (Ig) synthesis by B cell hybridoma and humoral immune response of antigen- and mitogen-stimulated human lymphocytes in culture [2,3]. Examination of the mechanisms of stimulatory effect of ADR on Ig synthesis revealed the role of intracellular iron ions and free radicals in B lymphocyte activation [5]. This effect of ADR can partly depend on ADR comlexation with transition metals, which can participate in redox reactions and modify ADR molecule [7,8]. Complexation with Fe^{3+} stimulates free radical processes and induces cyclic rearrangements and formation of transition ADR modifications accompanied by reduction of Fe^{3+} to Fe^{2+} [7,8]. When studying the effect of ADR and its complexes with Fe^{2+}, Fe^{3+}, Co^{2+} and Cu^{2+} on coupled ion transport in human erythrocytes, we showed that the opposite effects of these preparations on Ca^{2+}-dependent K^+-channels result from their ability to modulate Ca^{2+} transport and participate in redox reactions [6]. However, the mechanisms of action of ADR complexes with transition metal ions on immunocompetent cells remain little studied.

The purpose of the present study was to examine the effect of ADR complexes with Fe^{3+}, Co^{2+} and Cu^{2+} on humoral and cell immune response in cultured human lymphocytes.

MATERIALS AND METHODS

Human tosillar lymphocytes were isolated by centrifugation in a Ficoll-Paque (Pharmacia) density gradient. Lymphocytes (3.5×10^6 cells/ml) were cultured in RPMI-1640 medium (Serva) supplemented with 15% fetal calf serum (Serva), 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol and 80 μg/ml gentamycin. The induction of lymphocyte humoral immune response was performed during 6 days [3]. Complexes of ADR with Fe^{3+}, Co^{2+} and Cu^{2+} were prepared at an ADR:metal 3:1 molar ratio [6] and added to the incubation medium in a final concentration of 2 μg/ml. For the induction of antigen-specific immune response, standard strains of *Salmonella typhimurium, Shigella flexneri, Staphylococcus aureus, Yersinia enterocoli-*

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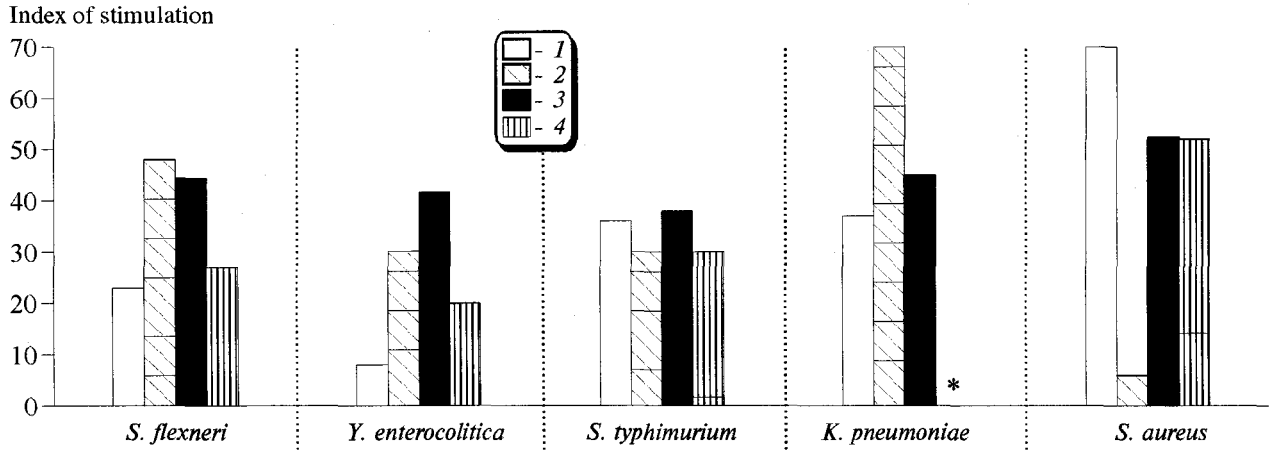

Fig. 1. Effect of adriamycin (ADR, *1*) and its complexes with Fe^{3+} (*2*), Cu^{2+} (*3*) and Co^{2+} (*4*) on the number of antibody-forming cells (AFC) in culture. *Not determined.

tica and *Klebsiella pneumoniae* were used as the antigens. To this end stock bacterial suspension with optical density of 0.6 arb. units at 600 nm was diluted 1:200. Individual antigen-forming cells (AFC) were identified in lymphocyte culture by a modified method of immunoenzyme assay on bacterial antigen-coated nitrocellulose filters [2]. Index of stimulation (IS) was calculated by the formula:

$$IS=1-\frac{\text{AFC (control)}}{\text{AFC (experiment)}}\times 100.$$

Cytotoxic activity of the lymphocytes was determined as described elsewhere [4] with our modifications. Native sheep erythrocytes (SE) and SE treated with specific antierythrocyte serum (SE-AB) were used as the target cells. After 72-h incubation in the presence of 25 U/ml recombinant interleukin-2 (Boehringer Mannheim), 5 μg/ml pokeweed mitogen, and ADR or its complexes, the lymphocytes were washed three times and incubated with equal volume of 4% SE or SE-AB suspension for 18 h. Hemolysis was assessed spectrophotometrically at 540 nm. Lymphocyte bactericidal activity against *Y. enterocolitica* strain 03 was estimated by the method elaborated in our laboratory. To this end, lymphocytes cultured for 72 h in the presence of pokeweed mitogen and ADR or its complexes were washed three times and incubated with bacteria for 24 h. Thereafter, the culture was seeded to meat-peptone agar containing sucrose, rhamnose, lactose, urea, mannitol, maltose, and phenylalanin. Biochemical activity of the standard bacterial strain was regarded as 100%.

RESULTS

During 72-h incubation, ADR and its complexes with Fe^{3+}, Co^{2+} and Cu^{2+} exhibited no pronounced cytotoxicity against unstimulated and mitogen-stimulated lymphocytes isolated from peripheral blood and tonsills (lymphocyte survival rate in the presence of ADR or its complexes was 73-86 *vs.* 79-90% in the control). The stimulating effect of the drugs depended on the antigen and metal ion incorporated into the anthracyclic structure of the ADR molecule (Fig. 1). The highest stimulatory effect of ADR on antibody production was observed in the experiments with *S. aureus*. At the same time, the number of AFC formed in response to *Y. enterocolitica* in the presence of ADR practically did not differ from the control. ADR stimulated antibody formation in response to *K. pneumoniae, S. typhimurium* and *S. flexneri*. Unlike ADR, its complex with Fe^{3+} increased the number of AFC induced by *Y. enterocolitica* and produced an about 2-fold increase in the number of AFC stimulated with *K. pneumonia* and *S. flexneri* in comparison with ADR alone. At the same time, no significant changes in the number of AFC in response to *S. aureus* in the presence of ADR-Fe^{3+} complex were observed. The most stable increase in the number of AFC in the presence

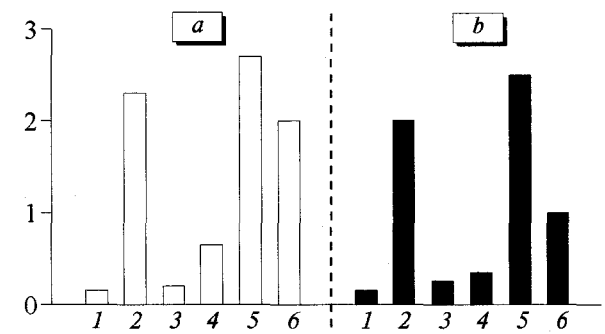

Fig. 2. Effect of pokeweed mitogen (*1*), interleukin-2 (*2*), adriamycin (*3*) and its complexes with Cu^{2+} (*4*), Fe^{3+} (*5*) and Co^{2+} (*6*) on lymphocyte cytotoxicity. Sheep erythrocytes untreated (*a*) and treated with antierythrocyte antiserum (*b*) were used as target cells. Ordinate: optical density at 540 nm (arb. units).

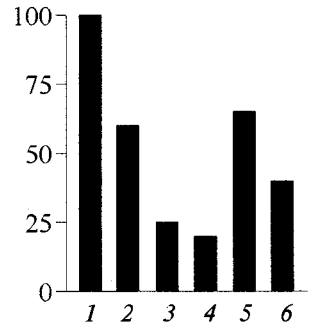

Fig. 3. Effect of standard strain (*1*), pokeweed mitogen (*2*), adriamycin (*3*) and its complexes with Cu^{2+} (*4*), Fe^{3+} (*5*) and Co^{2+} (*6*) on bactericidal activity of cultured lymphocytes. Ordinate: biochemical activity of *Y. enterocolitica*, %.

of all antigens was caused by ADR-Cu^{2+} (Fig. 1). The effect of ADR-Co^{2+} complex on antibody formation was close to that of ADR under the conditions of stimulation with *S. flexneri* and *Y. enterocolitica* (Fig. 1).

When evaluating the effect of test drugs on cell immune response in lymphocyte culture, we found that the stimulatory effect of ADR-Fe^{3+} and ADR-Co^{2+} on lymphocyte cytotoxicity against SE was close to that of interleukin-2, while ADR-Cu^{2+} complex produced only a weak stimulatory effect (Fig. 2, *a*). At the same time, pure ADR produced no significant stimulatory action. Antibody-dependent lymphocyte cytotoxicity against SE-AB as target cells was enhanced in the presence of interleukin-2 and ADR-Fe^{3+}, while pure ADR and ADR-Cu^{2+} had no effect and ADR-Co^{2+} only slightly stimulated antibody-dependent cytotoxicity (Fig. 2, *b*). Lymphocyte cytotoxicity against *Y. enterocolitica* was maximum after treatment with ADR and ADR-Cu^{2+}, while ADR-Fe^{3+}-treated lymphocytes possessed low cytotoxicity (Fig. 3).

Thus, molecular modifications of ADR, in particular complexation of anthracycle molecule with various metal ions changes immunomodulatory properties of ADR, i. e. potentiates or reduces the effect of ADR on effector cells of humoral and cell immunity.

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