EFFECT OF COMBINED INJECTION OF HYDROCORTISONE AND EDTA ON THE NUMBER OF ANTIBODY-FORMING CELLS IN THE SPLEEN OF MICE IMMUNIZED WITH SHEEP'S ERYTHROCYTES

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Experiments on (CBA × C57BL)F_1 mice showed that injection of hydrocortisone into the animals in a dose of 1 mg per mouse 24 h after immunization with sheep's erythrocytes, and against the background of repeated injections of EDTA, leads to a reduction in the relative number of plaque-forming cells by more than two-thirds in the spleen of the mice compared with the effect of the two agents separately, and by more than five-sixths compared with the control. It is suggested that this may be the result of the more intensive incorporation of hydrocortisone associated with the prolonged hypocalcemic action of the complexone, EDTA.

KEY WORDS: antibody-forming cells; hydrocortisone; ethylenediaminetetraacetic acid (EDTA).

Ca^{2+} cations play a key role in the regulation of many functions in the body. The mechanism of their action lies at the level of control of processes mediated by cyclic nucleotides [3, 8-10], the packing of the protein globules of membranes [2], and the polarization and stabilization of the latter [1]. For this reason, the permeability of membranes [10], the activity of receptor sites, and the ability of cells to receive a given mitogenic signal [12] are under the control of Ca^{2+}.

The possibility cannot be ruled out that this mechanism of action also lies at the basis of the effect of Ca^{2+} on development of the immune response in the body, as is confirmed by the results of numerous investigations in vitro into the inhibitory action of complexones ethylenediaminetetraacetic acid (EDTA) and ethyleneglycolbis(aminoethyl)-N,N,N',N'-tetraacetic acid on proliferation of stimulated lymphocytes [11, 12] and on the processes of antibody synthesis by spleen cells in response to sheep's erythrocytes [6].

Glucocorticoid hormones are also known to be effective inhibitors of many responses of cellular and humoral immunity [4]. These hormones have been found to be in the group of agents which induce the stage of differentiation in cells toward the Ca^{2+}-dependent pathway, mediated by cyclic 3',5'-AMP [7].

On the basis of these data the writers suggested that under hypocalcemic conditions the action of an immunodepressant such as hydrocortisone may be considerably intensified because of its increased incorporation into the cell and the increased transcription activity initiated by it [7].

Accordingly in the investigation described below the combined action of hydrocortisone and EDTA was studied on the accumulation of antibody-forming cells (AFC) in the spleen of mice immunized with sheep's erythrocytes (SE).

EXPERIMENTAL METHOD

Female (CBA × C57BL)F_1 mice obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, were used.

The mice were given subcutaneous injections of $1.61 \cdot 10^{-5}$ M EDTA per mouse daily for 4 days. On the 2nd day of EDTA administration the animals were immunized with a 5% suspension of SE. Hydrocortisone (Richter) was injected intraperitoneally in a dose of 1 mg per mouse on the day of immunization or 24 h after immunization. On the fourth day after immunization the animals were killed and the number of AFC counted

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TABLE 1. Effect of Combined Injection of EDTA and Hydrocortisone on Accumulation of AFC in Spleen of Mice Immunized with Sheep's Erythrocytes

Group No.	Experimental conditions	Number of experiment	Number of animals	Number of cells in spleen	Number of AFC per million spleen cells		Number of AFC in spleen	
					$M \pm m$	P	$M \pm m$	P
1	Control	3	21	152±13	302,3±22	—	46 532±5430	—
2	EDTA, 6.44×10^{-5} M	3	21	172±14	144,7±26	<0,001	24 617±4560	<0,001
3	Hydrocortisone, 1 mg per mouse, injected on day of immunization	3	16	124±14	232,9±44	>0,05	28 072±5780	<0,05
4	EDTA, 6.44×10^{-5} M + hydrocortisone, 1 mg per mouse, injected on day of immunization	3	16	127±15	229,0±39	<0,05	29 279±4140	<0,05
5	Hydrocortisone, 1 mg per mouse, injected 24 h after immunization	3	15	85±8	148,4±22	<0,001	11 375±2350	<0,001
6	EDTA, 6.44×10^{-5} M + hydrocortisone, 1 mg per mouse, injected 24 h after immunization	3	14	78±7	40,0±8	<0,001	3673±986	<0,001

in their spleen by a modified method of local zones of hemolysis in semiliquid medium [5]. Intact animals immunized with SE served as the control. The significance of the results was assessed by Student's criterion.

EXPERIMENTAL RESULTS

Injection of either of the test substances – EDTA or hydrocortisone – into the animals was found to cause a virtually equal reduction in the number of AFC (by about half compared with the control).

After combined injection of EDTA and hydrocortisone a sharp decrease in the relative (by more than 85%) and absolute (by more than 90%) number of AFC was observed only if the hydrocortisone was given 24 h after immunization. If injection of hydrocortisone on the day of immunization was combined with injection of EDTA, the changes in the number of AFC did not differ significantly from those observed after injection of EDTA or hydrocortisone alone, and this was most clearly reflected in figures for the absolute number of AFC in the spleen.

These results indicate that the hypocalcemic action of EDTA on the membranes differs in intact and antigen-stimulated lymphocytes, and that Ca^{2+} cations evidently influence the activity of the corticosteroid receptors. It can evidently be postulated that the cumulative immunodepressive effect obtained is the result of the greater mobility of Ca^{2+} on the excited membranes of the antigen-stimulated lymphocytes compared with their mobility on intact lymphocytes. The possibility cannot be ruled out that the complex-forming ability of EDTA in this case may be manifested to a much greater degree. The more intensive processes of polarization of the membranes [1] and of the conformational changes in their protein globules [1] thus produced create the conditions for maximal incorporation of hydrocortisone into the cell.

Allowance must also be made for data in the literature according to which B-lymphocytes in the spleen are more sensitive to the action of hydrocortisone [4], further confirmation that the action of hydrocortisone depends on the time of its injection.

Absence of the cumulative effect when hydrocortisone was injected on the day of immunization could perhaps be the cause of the lower susceptibility of the macrophages to the action of EDTA, considering that they are more resistant at the same time to hydrocortisone.

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PHYSICOCHEMICAL PROPERTIES OF MICROBIAL ANTIGENS AND IMMUNOALLERGIC TESTS

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Experiments were carried out on guinea pigs with the delayed or immediate type of allergic sensitization. Different antigens, obtained from Brucella abortus strain 19-VA were used for the sensitizing and reacting injections. The reacting properties of the corpuscular and soluble (sonicated) antigens and of purified protein (P) and polysaccharide (PS) fractions and RNA were compared in skin tests of immediate and delayed types, passive cutaneous anaphylaxis (PCA), acute anaphylactic shock (AS) and the Schultz-Dale reaction on the isolated intestine. Delayed and immediate and allergic reactions were produced by whole soluble antigen and the P fraction. Immediate reactions to purified P fraction were weaker than to whole soluble antigen, by which the animals were sensitized. The PS and RNA fractions were inactive in allergic reactions.

KEY WORDS: Microbial antigens; increased sensitivity of delayed and immediate types; allergic reactions.

Data in the literature are still incomplete on the nature of the substances causing generalized reactions of immediate and delayed types and also allergic reactions of the skin, smooth muscles, and other tissues and cells of the sensitized organism. Data on microbial polysaccharides with definite ability to stimulate immune antibody formation are contradictory. Interest has recently been shown in the diverse biological properties of cell RNA. Information on the allergodiagnostic properties of microbial RNA is scanty. When testing pure synthetic RNA Boxel et al. [5] obtained no allergic reactions.

The object of this investigation was to study the ability of various native and fractionated purified microbial antigens to cause allergic reactions of delayed and immediate types in animals with delayed (HDT) or immediate (HIT) types of hypersensitivity to the homologous microorganism.

EXPERIMENTAL METHOD

Cells of the vaccine strain of Brucella abortus strain 19-VA, with low virulence and high allergenic properties, were used as the test object. HDT was induced by a single subcutaneous injection of 2×10^9 cells of a living culture of the microorganism and HIT by two subcutaneous injections, at an interval of 2-3 days, each containing 5-8 mg (as protein) of the soluble (sonicated) brucella antigen with an equal volume (0.5 ml) of incomplete adjuvant [3].

The following preparations obtained from strain Br. abortus 19-VA were used in the tests: corpuscular – a suspension of brucellas of different concentrations, inactivated by heating to 60°C for 1 h; 2) soluble (sonicated) antigen, obtained by treatment with ultrasound for 2 h on the UZDN-1 apparatus. The supernatant after centrifugation at 8000-10,000 rpm for 20 min was used as the antigen. The antigen contained chiefly the cytoplasmic components of the cell. To obtain the other two preparations the microbial suspension was treated with ultrasound but not centrifuged. They consisted of purified fractions. The protein (P) fraction was prepared by weak-alkaline hydrolysis followed by precipitation with 1 N acetic acid at pH 6.5-5.5. The P residue

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