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Research Article

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Experimental Study of Cellular – Antibody Response to Hyaluronidase

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Abstract Cellular and humoral immunity on antigene Hyal is studied at mice-hybrids. Hyal possessed low immunogenic properties.Use DS,PO as immunoadjuvant towards Hyal raised it immunogenic the activity expressed in strengthening humoral of the immune response on Hyal at mice.DS also was capable to activate specific cellular reactions DTH on Hyal.

Keywords Cellular humoral immunity, activity, cellular reactions.

Introduction

It is well-known that the enzyme hyaluronidase (Hyal.) is a lysosomal ferment dissolving the hyaluronic. Hyaluronic acid is included in the capillary wall structure.

Hyal., produced by parasites, indices pathological alternations in the host organism. Particular enzyme is a factor in the pathogenesis and aggressiveness of parasites, as it "facilitates" the penetration and distribution of parasites in the infected organism. Hyal. plays a crucial role in the pathogenesis of parasitic diseases. In the consideration of foregoing, it is topical to study antigenicity of Hyal. in specific cellular – immune responses.

The objective of the composition was pursuing the study of specific cellular – immune responses to Hyal. in the experiment on mice.

Research Materials and Methods

Experiments involved mice, that is, hybrids (CBAxC57B1/6) F1 with a mass of 18-24 g and Hyal. was applied as an antigen of the firm "ServaFinebiochem" Hyal. was dissolved in 0.9% solution of NaCl. Mice were immunized in the following way: Hyal., Hyal. in a complex with polyoxidonium, Hyal. in a complex of sulphate dextran ("Sigma Chem. Co"), with emulsion Hyal. in complete Freud's adjuvant ("Calbiochem") at the base of the tail, after a month repeated immunization. Secondary immunization was conducted in the same way as the primary one at the rate of 10 mcg Hyal./mouse. The dosage of sulphate dextran constituted 300 mcg, polyoxidonium is 200 mcg per mouse. The quantity of specifics to Hyal. antibodies of classes M and J, as well as their isotopic variant were determined in the serum of mice through the method of enzyme-immunoassay in the variant A Voller [1].

In order to research dynamics of anti-formation of enzyme-immunoassay all serums obtained after 1, 2, 3 and 4 weeks after first and the second repeated immune enzyme analysis.

The intensity of the response of hypersensitivity of the delayed-type was evaluated according to Magiel-Plotkovski M. S. et. al. [2] in size of induced Hyal. edema of pads of hind paws. To take into account the hyperactivity of delayed-type allowing microinjection of Hyal. in 20 mcg in 0.9% sterile solution NaCl in the pad of left hind paw experimental and controlling mice. In the first paws injected 20 mcg of NaCl. In 24 hours with the aid of micrometer measured the thickness of left and right paws. The difference between the average values and the thickness of left

and right paws taken for the size of the edema. Edema index (IE) calculated through the formula: IE = (VE ex. group – VE cont. group) where VE ex. group is the value of edema by experimental group, VE cont. group is the value of edema by controlling group [3].

Research Findings and Discussion

Conducted experiments reveal that Hyal. possessed very low antigenic specificity (enzyme-immunoassay titers in dynamics changed from 1:100 to 1:300). In particular group the value of edema of cellular responses of hyperactivity of delayed-type varied from 0.02 mm to 0.05 mm (Tables 1, 2), the maximum level of cellular response (T-effectors of hyperactivity of delayed-type) on Hyal. accounted for 4 weeks of secondary immune response) (Table 2).

Immunization of mice by the complex of the polyanionic nature Hyal.+SD resulted in the induction specific hyaluronic (antibody) response to Hyal. in ten times more intense than with the injection of only antigen Hyal. After momentary immunization the synthesis of antibodies was characterized by IEA – with titers of 1:900 (peak response for 2 weeks). Secondary produce of antibodies (principally of IgG type) increased in 90 times up to the values IEA – titers of 1:25000 (peak response was 1 week after reimmunization) Spectrum of isotopes of antibodies Hyal. was the following: IgG1 (IEA – titers up to 1:12000) > IgG2a (IEA – titers up to 1:5000) > IgG2b (IEA – titers up to 1:2000).

Table 1: Intensity of hyperactivity response of delayed-type, evaluated by the Value of edema and Edema index. In the parenthesis is indicated confidential interval under p=**0.05**

Ser. No.	Term	Primary immune response	
	Groups	1 week	
		Value of edema (mm)	Edema index
1.	10 mcgHyal.	0.04 (0.02-0.06)	1.0
2.	10 mcgHyal., 300 mcgDS	0.08 (0.03-0.13)	3.0
3.	10 mcgHyal., 200 mcgPO	0.03 (0.02-0.04)	0.5
4.	10 mcgHyal. InCFA	0.17 (0.07-0.27)	7.5

Table 2: Intensity of hyperactivity response of delayed-type, evaluated by the Value of edema and Edema index. In the parenthesis is indicated confidential interval under p=0.05

Ser. No.	Term	Secondary immune response			
	Groups	2 week		4 week	
		Value of edema (mm)	Edema index	Value of edema (mm)	Edema index
1.	10 mcgHyal.	0.02 (0.01-0.03)	1.0	0.05 (0.02-0.08)	1.6
2.	10 mcgHyal., 300 mcgDS	0.09 (0.05-0.013)	8.0	0.07 (0.04-0.10)	1.3
3.	10 mcgHyal., 200 mcgPO	0.03 (0.02-0.05)	2.0	0.02 (0.01-0.03)	-0.3
4.	10 mcgHyal. InCFA	0.16 (0.07-0.27)	15.0	0.09 (0.05-0.13)	2.0

Polyanion Hyal.+SD reinforced cellular responses specific in relation to Hyal. value of edema from 0.07 mm to 0.09 mm (Table 1, 2). The maximum level cellular response of hypersensitivity of delayed-type accounted for 2 weeks of secondary immune response (effectors of hypersensitivity of delayed-type) when edema index constituted 8.0 (Table 2).

Joint injection of mice Hyal. with a covalent complex polycationic nature with polyoxidonium (Hyal. + PO) stimulated predominant secondary synthesis of antibodies, that is, from 5 to 20 times (IEA – titers up to 1:2000-1:6000) with the maximum of response in a week. In such a case dominated antibodies of isotope IgG1 (IEA – titers up to 1:2000), fixed the antibodies of isotope IgG2b (IEA – titers up to 1:100), and the antibodies of other isotopes were recorded at all (background level).

In addition, with the aid of polyoxidonium was a failure to induce a specific response of hypersensitivity of delayed-type to the present antigen, that is, the value of edema changed from 0.02 mm to 0.03 mm, and peak of cellular immunity from the edema index, that is, 2.0 accounted for two weeks. The secondary synthesis of specific antibodies was much intensive compared to the primary. In this case, recorded 200 times increase of specific synthesis of IgG in comparison with the response to the antigen Hyal. (IEA – titers is 1:25000-1:52000) its maximum level IgG reached in four weeks of secondary response. Under the CFA (complete Freund's adjuvant) produced antibodies of all isotopes antibodies to Hyal., where their concentrations slightly exceeded the analogous values of group Hyal.+SD [4].

Immunization of mice Hyal.in CFA also contributed to strengthening of cellular immune responses expressed in the strengthening of formation T-effectors of hypersensitivity of delayed-type in the regional lymphoid tissue. In response to the injection of Hyal., in the pad of hind paw evolved response of hypersensitivity of delayed-type with value of edema 0.09-0.17 mm, that was in 2-8 times more in comparison with cellular response in mice, immunized only with Hyal. in this case in the present group of edema index achieved its maximum values and changed within the limits 7.5-15.0 (Table).

Obtained experimental findings testify that polyanion SD really performs a significant immunoadjuvant effect on the immune system of mice in relation to the antigen Hyal. and most importantly, Hyal. is a very weak antigen for mice hybrids of abovementioned line: even double-immunization of mice Hyal.+SD ca be at the latter induce intensive cellular – antibody responses in comparison with the response only to the antigen Hyal. (without adjuvant).

Polycationpolyoxidonium in the composition of the complex with Hyal. also was capable to increase specific antibody response to Hyal., but the levels which generated under this antibodies IgG class significantly inferior to the following values of other groups that is, Hyal.+SD and Hyal.+CFA [3].

Overall, with the aid of polyions it is possible to induce cellular-antibody immune responses to Hyal. SD as distinct from polyoxidonium has proven to be more effective immunoadjuvant to Hyal. according to its immunogenic properties SD approached the CFA. These attachments can be quite utilized to creation of effective protection against parasites, in particular when constructing artificial antigens (vaccines) on the basis of antigen Hyal. andpolyions possessing high immunogenic activity and at the same time having no side effects.

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