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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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TABLE 1. Skin tests with Various Antigens on Guinea Pigs with Allergy of Delayed and Immediate Types to Brucellas

Type of allergy	Number of animals	Antigen			
		sonicated	P fraction	PS fraction	RNA fraction
Immediate skin tests (active cutaneous anaphylaxis, mm)					
HIT	14	14,8±0,93	10,7±0,87	0	0
HDT	14	9,9±0,63	11,7±1,52	0	m/t
Control	5	0	0	0	0
Delayed skin tests, ml					
HIT	14	0	0	0	0
HDT	14	11,3±1,94	9,2±1,42	0	m/t
Control	5	0	0	0	0

Legend. 0) Reaction negative; n/t) not tested.

TABLE 2. PCA Test with Different Brucella Antigens (latent period for each serum 4 and 24 h)

Type of allergy in donors of serum	Reacting antigen				
	corpuscular (500 million cells)	sonicated (5 mg)	P fraction (5 mg)	PS fraction (5 mg)	RNA fraction (5 mg)
HIT	0/7	65/70	3/4	0/4	0/4
HDT	0/10	0/24	0/7	0/8	0/2
Control	0/2	0/4	0/4	0/4	0/4

Legend. Numerator shows number of sera giving positive results, denominator number of sera tested.

TABLE 3. AS Index to Injection of Various Microbial Preparations into Guinea Pigs with HIT to Brucellas

Type of allergy	Reacting antigen			
	corpuscular (500 million cells)	sonicated (1-3 mg)	P fraction (1-3 mg)	PS fraction (5-10 mg)
HIT	0 (22)	4 (8)	4 (6)	0 (6)
Control	0 (16)	0 (4)	0 (4)	0(4)

Legend. Here and in Table 4, number of animals shown in parentheses.

was separated by centrifugation for 45 min at 18,000 rpm. The P fraction was purified by treatment with isopropyl alcohol and by dialysis. The purity of the preparation was confirmed by spectrophotometric and chemical methods of investigation. The fourth preparation – the polysaccharide (PS) fraction – was isolated after precipitation of the proteins and DNA by treatment with ethyl alcohol in the cold. Purification was by alkaline hydrolysis and dialysis. Chemical reactions confirmed the purity of the preparation. The fifth (RNA) fraction was isolated after removal of the P and PS fractions by a method developed by one of the writers, consisting

TABLE 4. Schultz–Dale Reactions with Various Antigens in Animals with HDT and HIT to Brucella Antigens (in % of contraction to histamine)

Type of allergy	Antigen				
	corpuscular (24-30 mg/ml)	sonicated (0.3 mg/ml)	P fraction (0.3 mg ml)	PS fraction (0.3 mg/ml	RNA fraction (0.3 mg/ ml)
HIT	0 (23)	107,3±10,85) (10)	46±6,13 (10)	0 (10)	0 (10)
HDT	0 (35)	0 (24)	0 (5)	0 (5)	0 (5)
Control	0 (33)	0 (20)	0 (6)	0 (12)	0 (5)

of evaporation of the extracts at 40°C in vacuum followed by dialysis; the purity of the preparation was confirmed by spectrophotometric and chemical methods of investigation.

The above-mentioned preparations were used in the following allergic reactions of delayed and immediate types: 1) an allergic skin test with differential reading of the immediate (by the active cutaneous anaphylaxis test with dye [6]), and delayed (by the greatest diameter of the focus of infiltration and of hyperemia) reactions; 2) the passive cutaneous anaphylaxis reaction (PCA) by Ovary's method [6]; 3) anaphylactic shock (AS) by intravenous injection of antigens, the degree of which was assessed by means of the AS index [7]; 4) the Schultz–Dale reaction by the usual method, with isotonic recording of contraction of isolated segments of the small intestine in the presence of the antigens or the physiological stimulus – histamine. The strength of anaphylactic contraction of the muscles was expressed as a percentage of the contraction in response to a standard (10^{-6}) concentration of histamine.

As a preliminary step before all the tests listed above, working doses of each antigen completely inactive for the unsensitized animal were determined for healthy animals. For instance, in the allergic skin tests doses of 100 μg, as protein or weight of dry substance (PS and RNA) in 0.1 ml were used; healthy animals did not react to intravenous injection of 5 mg or more of soluble (sonicated) antigen, of the P and RNA fractions, of the PS fraction up to 10 mg, and of microbial cells of corpuscular antigen up to 30×10^9 (see [2] and our own data). Sonicated antigen, P and RNA fractions in doses of up to 0.3 mg/ml nutrient solution, PS fraction up to 1 mg/ml, and corpuscular antigen up to 30 mg/ml in the Schultz–Dale reaction did not affect the smooth-muscle organs of normal guinea pigs.

Experiments were carried out on 244 noninbred guinea pigs of both sexes weighing 250-350 g: 49 of the animals were in a state of HDT, 89 in a state of HIT to brucella antigens, and 106 were intact animals used for selecting working doses of antigens and for the PCA test.

EXPERIMENTAL RESULTS

Soluble antigens were studied in allergic skin tests (Table 1). As regards the corpuscular antigen, a suspension of killed brucellas is known to give rise to positive delayed reactions in patients with brucellosis, not disappearing for several days.

Analysis of the data in Table 1 shows that the PS and RNA fractions of brucellas caused neither early nor late skin reactions. A negative result was obtained in both types of allergy.

Immediate and delayed reactions were produced only by whole sonicated antigen and the P fraction. Comparison of the intensity of the skin reactions to these antigens showed them to be about equal. The differences were not statistically significant, except in the case of immediate reactions in a state of HIT ($P < 0.001$). In this case the stronger reaction to whole sonicated antigen could evidently be attributed to sensitization of the animals with precisely this antigen, which is more complex in composition than the purified protein.

In the PCA (Table 2) with homocytotropic skin-sensitizing antibodies (in HIT) only the whole sonicated antigen and the P fraction also reacted. Corpuscular antigen and the PS and RNA fractions of the microorganisms gave negative results with the same sera. The absence of reaction to corpuscular antigens will be noted. According to existing data [4], during anaphylactic sensitization with egg albumin this antigen produces systemic and local passive anaphylaxis only in the soluble form, and not in the corpuscular form (bound with the erythrocyte membrane).

Results in full agreement with those of the PCA were obtained by the study of the ability of the various microbial antigens to induce AS, which was carried out on animals with HIT (Table 3).

The Schultz–Dale reaction (Table 4) as might be expected, was observed only in HIT to the microbial antigen. Under these circumstances contraction was induced by whole sonicated antigen and the P fraction. Just as was observed with respect to immediate skin reactions, the sonicated antigen homologous with the sensitizing antigen caused a significantly stronger specific contraction of the muscles.

Hence, both delayed and immediate allergic reactions in microbial sensitization were produced only by preparations containing protein, and not by purified PS and RNA fractions of the microbial cell. Corpuscular antigen, in the form of a suspension of killed microorganisms (brucellas) did not induce anaphylactic reactions in HIT to brucellas.

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