ANTIGENIC COMPOSITION OF INTRACELLULAR FRACTIONS OF THE DENTAL PULP

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The pulp of the incisors of rats contains two organ-specific antigens (in the nuclear and cytoplasmic fractions), nonspecific tissue antigens, with the antigens of the liver, spleen, and lymph glands, and serum proteins.

Analysis of the distribution of antigens in intracellular fractions of tissues, their similarity and difference, and their participation in the formation of immunologic reactions is of great importance to the problem of homotransplantation and to discovery of the pathogenetic mechanisms of allergic and autoimmune processes [2, 5, 7, 8, 10].

Indirect evidence has been obtained for the antigenic composition of saline extracts of dental pulp [3, 4]. The effects of antipulp cytotoxic serum on structural components of the pulp, mineral metabolism in the hard tissues of the teeth in rats, and on the course of inflammatory processes, and so on, have been demonstrated. In the complement fixation reaction, antiserum against the pulp gave cross reactions with lymphoid tissue, splenic tissue, and serum proteins, indicating the complex antigen composition of preparations used for immunization.

In the present investigation the antigenic properties of fractionated intracellular structures of the dental pulp were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 110 Wistar rats weighing 90-120 g and 15 rabbits. After exsanguination of the rats the pulp of the upper and lower incisors was removed and washed in the cold to remove traces of blood with physiological saline by repeated centrifugation. The tissue was suspended, chopped with scissors, and ground with glass (1:1) in a cold mortar with the addition of 5 volumes of 0.14M NaCl solution. The homogenate was repeatedly frozen and thawed (8 times) and then centrifuged at 500, 1000, and 2000 rpm, for 15 min at each speed. The supernatant was preserved with merthiolate (1:5000), and after determination of its protein content it was used for immunization of rabbits together with Freund's complete adjuvant by the following scheme: triple primary immunization in the course of 3 weeks, a gap of 4 weeks, reimmunization, followed by exsanguination 6-7 days later. The total content of antigen protein was 67 mg per rabbit. In the course of immunization of the animals the total blood proteins were determined refractometrically, the protein fractions by electrophoresis on paper, and the antibodies in the blood by the precipitation reaction in agar and the complement fixation reaction.

Intracellular fractions were isolated by Schneider's method [6, 11]. A tissue homogenate in 0.25M sucrose solution (pH 7.4) was prepared in a mortar with a teflon pestle. The nuclear fraction was sedimented by centrifugation at 600 g for 10 min, the mitochondria at 8500 g for 20 min, the microsomes at 45,000 g for 100 min; the supernatant contained the hyaloplasm fraction. The fractions of nuclei, mitochondria, and microsomes were resuspended in 0.25M sucrose and resedimented by centrifugation under the same conditions, dialyzed against 0.14 M NaCl (pH 7.0), and concentrated by evaporation from a cellophane bag. All operations were carried out at 0-4°. After estimation of their protein content by Lowry's method [9] and equalization of their concentrations, the resulting fractions were used for analysis of the antigens by double immunodiffusion in agar in the micromodification of Ouchterlony's method [1]. Subsequently the agar plates were washed in physiological saline to remove unprecipitated proteins and stained with amido black. Densitometry was then carried out with the MF-2 microphotometer.

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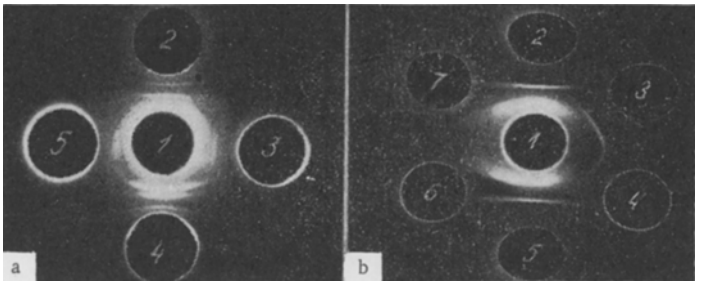

Fig. 1. Precipitation reaction in agar between pulp antiserum and different antigens. a: 1) Antiserum against pulp homogenate, 2, 4) pulp homogenate, 3) hyaloplasm fraction of pulp, 5) nuclear fraction of pulp; b: 1) antiserum against pulp after exhaustion with serum proteins, 2,5) pulp homogenate, 3) nuclear fraction of pulp, 4) hyaloplasm fraction, 6, 7) rat serum.

EXPERIMENTAL RESULTS

In the course of immunization, complement-fixing antibodies against rat pulp antigen were found in the serum in a titer of 1:160 by the end of primary immunization (21st day) and in a titer of 1:320 after reimmunization (50th day). Precipitating antibodies were detected at these same times in titers of 1:8 and 1:32 respectively.

One of the three precipitation lines (Fig. 1) formed between pulp homogenate and the corresponding antiserum was also found regularly in the nuclei, microsomes, and hyaloplasm. In the course of immunization this line appeared before the others and was due to serum albumin. The second precipitation line, intermediate in position between the wells, was also formed in the hyaloplasm fraction. The presence of this antigen in other intracellular fractions or in the blood serum could not be demonstrated. The third precipitation line between pulp homogenate and the corresponding antiserum, located nearer to the well containing antigen, was due to the presence of one of the serum proteins (not albumin).

The study of the intracellular fractions by means of antiserum exhausted by serum proteins revealed the presence of specific pulp antigen in the nuclear fraction and also the presence of serum protein in the mitochondria. The position of these precipitation lines in the pulp homogenate could not be identified (nonequivalent proportions).

The organ-specific properties of the pulp antigens were studied in cross reactions with intracellular fractions of rat liver, spleen, and lymph glands. Antigen of the pulp hyaloplasm showed marked organ-specificity: in cross reactions with antigens of other tissues, their difference was clearly revealed. The same was true of specific pulp antigen discovered in the nuclear fraction: antigens of the pulp nuclei showed no similarity with liver, spleen, or lymph gland proteins.

Evidence both of antigenic similarity and difference was found between the mitochondrial and microsomal fractions of the pulp and other tissues, but they were due mainly to the presence or absence of serum proteins.

These results shed light on the complex antigenic composition of dental pulp homogenate and its intracellular fractions. The presence of at least two organ-specific antigens – in the nuclei and hyaloplasm – was established. A further study of the nature of the specific antigens of the dental pulp and their immunochemical identification are needed.

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