APPEARANCE OF AN ENTODERMAL ANTIGEN IN THE ORAL MUCOSA OF DOGS WITH RECURRENT SPONTANEOUS STOMATITIS

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In dogs with stomatitis an additional component appears in the mucous membrane of the cheeks, tongue, and hard palate (ectodermal derivatives), which is not found in the homonymous areas of the normal oral mucosa. The component discovered was completely identical immunochemically with antigens of the mucous membrane of the soft palate, esophagus, stomach, and large and small intestines, which are entodermal derivatives. In other organs and tissues studied no entodermal antigen was found.

KEY WORDS: entodermal antigen; spontaneous stomatitis.

Recent immunological studies of chronic recurrent aphthous stomatitis observed in clinical practice [13] and in experiments on animals with recurrent spontaneous stomatitis [5, 6] have revealed antigens in the oral mucosa not normally present in it. The specificity of these antigens is disputed. Attempts to induce an immune response to injection of autologous tissue with the maximal content of this component were unsuccessful, possibly because natural immunological tolerance exists to the material studied and in normal animals antigens responsible for the maintenance of this tolerance exist.

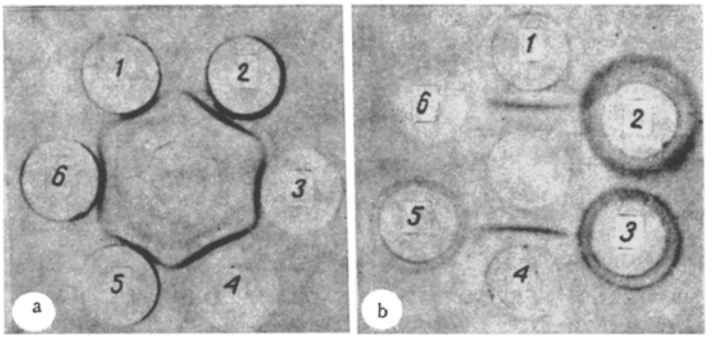

Fig. 1. Distribution of entodermal component in tissues of oral mucosa of dogs with spontaneous stomatitis. a: Central well contains specific rabbit antiserum reacting only with test antigen; 1) mucosa of cheek, pathological eruption in stage of development (antigen No. 35); 2) mucosa of cheek, pathological eruption in stage of development (antigen No. 25); 3) mucosa of cheek on side opposite to eruption; 4) mucosa of cheek around pathological eruption (no visible changes); 5) mucosa of cheek in period between relapses; 6) mucosa of cheek in period of active epithelization of pathological eruptions; b: Central well contains monospecific rabbit antiserum against pathological component; 1, 4) entodermal antigen; 2) staphylococcus; 3) streptococcal polysaccharide; 5) total microbial antigen; 6) physiological saline.

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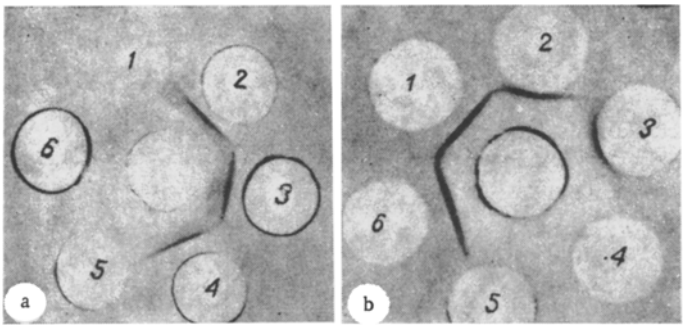

Fig. 2. Identity of antigens of soft palate of healthy dog with antigens of pathologically changed cheek tissue in spontaneous stomatitis. A: Central well contains specific rabbit antiserum reacting only with test component; 1) mucous membrane of hard palate; 2) mucous membrane of pathologically changed cheek tissue; 3) mucous membrane of healthy solft palate; 4) soft palate (spontaneous stomatitis); 5) mucous membrane of lateral surface of tongue; 6) mucous membrane of dorsum of tongue. B: Central well contains monospecific rabbit antiserum against pathologically changed cheek tissue; 1) mucous membrane of soft palate (spontaneous stomatitis); 2) mucous membrane of cheek (spontaneous stomatitis, pathological eruptions in stage of development); 3) mucous membrane of cheek (spontaneous stomatitis, pathological eruptions in stage of epithelization); 4) liver; 5) spleen; 6) mucous membrane of soft palate of healthy dog.

The object of this investigation was to study the distribution of this antigen in healthy dogs and in animals with spontaneous recurrent stomatitis.

EXPERIMENTAL METHOD

Mongrel dogs with recurrent spontaneous stomatitis [7] and healthy dogs were used as the test objects. Material for investigation included: pieces of oral mucosa with pathological eruptions, during the period of their development and epithelization and also in the latent period between recurrences, as well as adjacent areas of visibly unchanged oral mucosa, mucous membrane of the cheeks on the side opposite to the eruptions and of other organs (different levels of the esophagus, stomach, duodenum, large and small intestines, liver, spleen, kidneys, lungs, pancreas, trachea, and skin), milk and blood serum, and also the oral mucosa (hard and soft palate, lateral surface and dorsum of the tongue, inferior surface of the tongue, cheek, and retromolar space).

Saline extracts of the organs and tissues were prepared in the usual way with slight modifications [2]. Total microbial antigen was prepared from the oral cavity of healthy dogs in the Microbiological Laboratory of the Central Research Institute of Stomatology. Total protein in the extracts was determined by the biuret method [10]. Hyperimmune antisera were obtained by immunizing rabbits directly into the lymph nodes [3] with Freund's complete adjuvant (Difco, USA). Rabbit antisera were absorbed with glutaraldehyde sorbents and tissues of heterologous organs under the control of the gel-diffusion test [8, 12]. The sera were concentrated by isolation of the globulin fraction by the addition of an equal volume of saturated ammonium sulfate solution [1] followed by dialysis and also with the aid of Sephadex G-25 (coarse).

Semiquantitative titration analysis of antigens in gel [4] was used. Monospecific sera were tested in the gel-diffusion test with all saline extracts of the organs and tissues. Staining with azocarmine and Amido Black 10B were carried out after the material had been washed and dried. Altogether 120 saline extracts and more than 30 rabbit antisera against pathological and normal oral mucosa were tested.

EXPERIMENTAL RESULTS

The component studied was found in the tissue of the mucous membrane of the cheek in recurrent spontaneous stomatitis during the period of development and epithelization and also between eruptions, and also on the side opposite to the eruptions in the mucous membrane of the cheek; it was not identical with the microbial antigens of the oral cavity of adult dogs (Fig. 1).

TABLE 1. Distribution of Entodermal Antigen in Organs and Tissues of Dogs.

Organs and tissues of healthy and sick animals	Antigen found	Titer of antigen	Detection rate, %
Healthy dogs			
Mucous membrane			
of hard palate	–		
of lateral surface of tongue	–		
of dorsum of tongue	–		
of cheek	–		
of soft palate	+	1/128	100
Wall of esophagus at different levels	+	1/64	100
Wall of stomach	+	1/8	100
Wall of duodenum	+	1/32	100
Wall of small intestine	+	1/32	100
Wall of large intestine	+	1/64	100
Liver	–		
Spleen	–		
Kidneys	–		
Pancreas	–		
Trachea	–		
Lungs	–		
Skin	–		
Blood serum	–		
Milk	–		
Dogs with spontaneous stomatitis			
Mucous membrane			
of cheeks	+	1/28	100
of lateral surface of tongue	+	1/8-1/16	35
of hard palate	+	1/28 and over	10

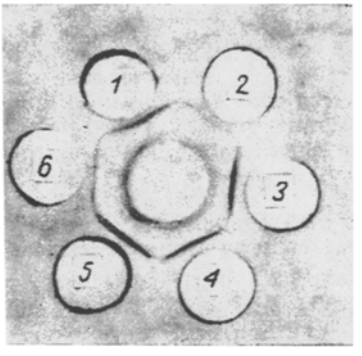

Fig. 3. Identity of test component of pathologically changed mucous membrane of cheek with antigens of gastrointestinal tract. Central well contains monospecific rabbit antiserum reacting only with pathologically changed mucous membrane of cheek in spontaneous stomatitis; 3) esophagus; 4) duodenum; 5) small intenstine; 6) large intestine.

In the oral cavity of healthy dogs it was discovered only in the mucous membrane of the soft palate, where it was detected in the greatest amount. This component was in fact immunologically completely identical with the antigen of the pathologically changed tissue of the cheek (Fig. 2A), as also was confirmed by experiments

in vivo in which rabbits were immunized with pathologically changed cheek tissue and, to stimulate the immune response, antigen of the mucous membrane of the palate was injected instead of homologous antigen.

The component being studied also was completely identical with antigens of the gastrointestinal tract but was not found in the liver, kidneys, spleen, and so on Figs. 2B and 3).

The distribution of the test component in the organs and tissues of healthy dogs and of dogs with spontaneous stomatitis is shown in Table 1. Clearly under normal conditions it was present not only in the mucous membrane of the soft palate, but also in tissues of organs of the gastrointestinal tract, and was present in the smallest amount in the stomach; in spontaneous stomatitis, on the other hand, it began to be detectable in parts of the oral mucosa where under normal conditions it is absent – in the mucous membrane of the cheeks, the lateral surface of the tongue, and the hard palate.

The oral mucosa develops from two embryonic formations: the ectoderm and entoderm [11]. It has been suggested that the boundary between them in adults is at the junction between the hard and soft palate. This hypothesis was confirmed by the present investigation, for an antigen common with the antigen of the gastrointestinal tract was discovered in the mucous membrane of the soft palate but it was not found in the mucous membrane of any of the parts of the oral cavity of ectodermal origin that were studied.

The discovery of the test component in the mucous membrane of the cheek in recurrent spontaneous stomatitis at different stages of the pathological process and also in the period between relapses is, in the writers' view, a reflection of the special relations between tissues of different genesis that arise only in a pathological state of one of them.

The appearance of entodermal antigen in areas of mucous membrane affected by pathological changes may perhaps be attributable to derepression of the gene responsible for synthesis of entodermal protein in the genome of the ectodermal cells. This derepression may perhaps take place as a result of the action of the virus or other factor causing recurrent spontaneous stomatitis.

It is considered that the relationship thus discovered may in the future be used to study recurrent spontaneous stomatitis, a condition widely used at the present time as a model for the investigation of chronic recurrent aphthous stomatitis in man [9].

The phenomenon observed by the writers also deserves attention from the general biological point of view, for it characterizes the special relationship between derivatives of the ectoderm and entoderm in the oral cavity under normal and pathological conditions. The antigen isolated previously in spontaneous stomatitis is thus not immunologically specific. Normally it is absent from those parts of the oral mucosa that are of ectodermal origin. During the development of spontaneous recurrent stomatitis the entodermal antigen appears in ectodermal derivatives in the oral mucosa: constantly in the mucous membrane of the cheeks and much less frequently in the mucous membrane of the lateral surface of the tongue and of the hard palate.

In conclusion the writers are grateful to G. I. Abelev, Director of the Laboratory of Immunochemistry of Cancer, N. F. Gamaleya Institute of Epidemiology and Microbiology, to A. I. Gusev and A. K. Yazova, on the staff of that Laboratory, and also to V. Ya. Rogal'skii, Senior Scientific Assistant at the P. A. Gertsen Moscow Oncological Research Institute, for their help with many aspects of this research.

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ISOLATION OF A THERMOLABILE ENTEROTOXIN FROM Escherichia coli AND THE STUDY OF ITS BIOLOGICAL PROPERTIES

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An enterotoxin was isolated from strain Escherichia coli 015 by salt precipitation and gel chromatography. In the process of isolation and purification the toxic activity of the preparation increased: by 60 times according to the ligated segment of rabbit intestine method and 66-100 times according to the skin test. The plateau and second fraction obtained by gel chromatography were inactive according to the ligated segment of intestine method but possessed permeability factor (PF) activity in the skin test. Two hypotheses were put forward: The vascular permeability factor and the diarrheagenic factor are possibly two different substances (molecules) and the skin test is more sensitive as a method of determining toxicity than the ligated segment of rabbit intestine method.
KEY WORDS: enterotoxin of E. coli; vascular permeability factor; diarrheagenic factor.

Recent work has shown that certain species of escherichias which produce thermolabile and thermostable enterotoxins are the agents of acute cholera-like diseases in man and domestic animals [3]. In its biological properties, including immunological specificity, the thermolabile enterotoxin is similar to the cholerogen of the cholera vibrio and, in particular, it reacts with anticholera serum [4, 5].

The biological properties of the thermolabile colienterotoxin have been inadequately studied, since it has not been isolated in a purified form. The question of identity of the vascular permeability factor (PF activity) and the diarrheagenic factor, both of which are found in preparations of enterotoxin, still remains open.

The object of this investigation was to isolate and purify a thermolabile colienterotoxin and to study its diarrheagenic effect and PF activity.

EXPERIMENTAL METHOD

Strain E. coli O15, generously provided by Dr. Gorbach (USA), was used. The strain was grown on nutrient medium consisting of 2% casaminic acid (Difco), 0.6% yeast extract (Difco), and inorganic salts for 24 h at 37°C with aeration. The culture was then centrifuged (18,000g, 30 min, 4°C) and the residue (microbial cells) discarded.

The supernatant was filtered through millipore membranes with a pore diameter of 0.45 μ. The filtrate was lyophilized and designated the original preparation of enterotoxin. Part of the cultural filtrate was treated with ammonium sulfate (to 90% saturation) and the residue separated by centrifugation (6000 g, 30 min, 4°C), redissolved in distilled water and lyophilized. This preparation was described as the residue. Purification was then carried out by gel chromatography on a Sephadex G-150 column. The original preparation or residue was applied in an amount of 150 mg to the column (3 × 90 cm). Elution was carried out in distilled water (40-

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