LOCALIZATION OF ENTODERMAL ANTIGEN IN ORGANS AND TISSUES OF DOGS

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During an investigation of chronic recurrent aphthous stomatitis in dogs the writers found an additional antigen in extracts from the mucous membrane of the cheek, tongue, and hard palate, which was absent in corresponding extracts from healthy dogs. In the latter, this antigen was found in the mucous membrane of the soft palate and along the length of the distal segment of the gastrointestinal tract, and also in saliva from the submaxillary and sublingual salivary glands. The antigen was not found in the saliva from the parotid salivary glands. Because of this localization of the antigen we were studying, it was conventionally called entodermal antigen [4].

The aim of the present investigation was to study the intracellular localization of entodermal antigen in different parts of the alimentary tract.

EXPERIMENTAL METHOD

Material for investigation consisted of samples of mucous membrane from the mouth and gastrointestinal tract of six healthy dogs. Antiserum against entodermal antigen was obtained by immunizing rabbits with a saline extract of the mucous membrane of the soft palate, directly into the popliteal lymph nodes, together with an equal volume of Freund's adjuvant (from Difco, USA) [1]. An eluate of antibodies against entodermal antigen was obtained on Sepharose antigen immunosorbents [7]. Antigen for preparation of the immunosorbent was isolated from saliva from the submaxillary salivary gland of a dog by preparative electrophoresis in polyacrylamide gel [2]. The antibodies thus obtained gave one band in the precipitation test with entodermal antigen and were identical with previous monospecific antisera.

The indirect immunofluorescence method was used [3]. Donkey antiserum against rabbit globulins, labeled with fluorescein isothiocyanate (FITC), was obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

The localization of the antigen was studied on cryostat sections; blocks of tissue (2×3 mm) were fixed in 4% paraform in 0.1 M phosphate buffer (pH 7.3) for 4-6 h at 4°C, washed for 14-16 h in three changes of 0.1 M phosphate buffer (pH 7.3), and transferred into 10% glycerol in the same buffer. The tissues were embedded at 4°C. Before cutting, the blocks were frozen in petroleum ether cooled with liquid nitrogen.

The indirect immunofluorescence test was performed by the standard method [3]. Preparations were examined in the ML-2 luminescence microscope and photographed on RF-3 film.

An immunoperoxidase study of the localization of entodermal antigen was carried out by the unlabeled antibody method [10], using the PAP complex (peroxidase and rabbit antibodies against peroxidase), from Daco, Denmark.

The first stages of the immunoperoxidase test were similar to those of the immunofluorescence test. After application of donkey anti-rabbit serum and washing the sections, the PAP preparation was applied in a dilution of 1:20. The sections were incubated for 30 min, unbound PAP was washed off, and peroxidase was detected with diaminobenzidine by

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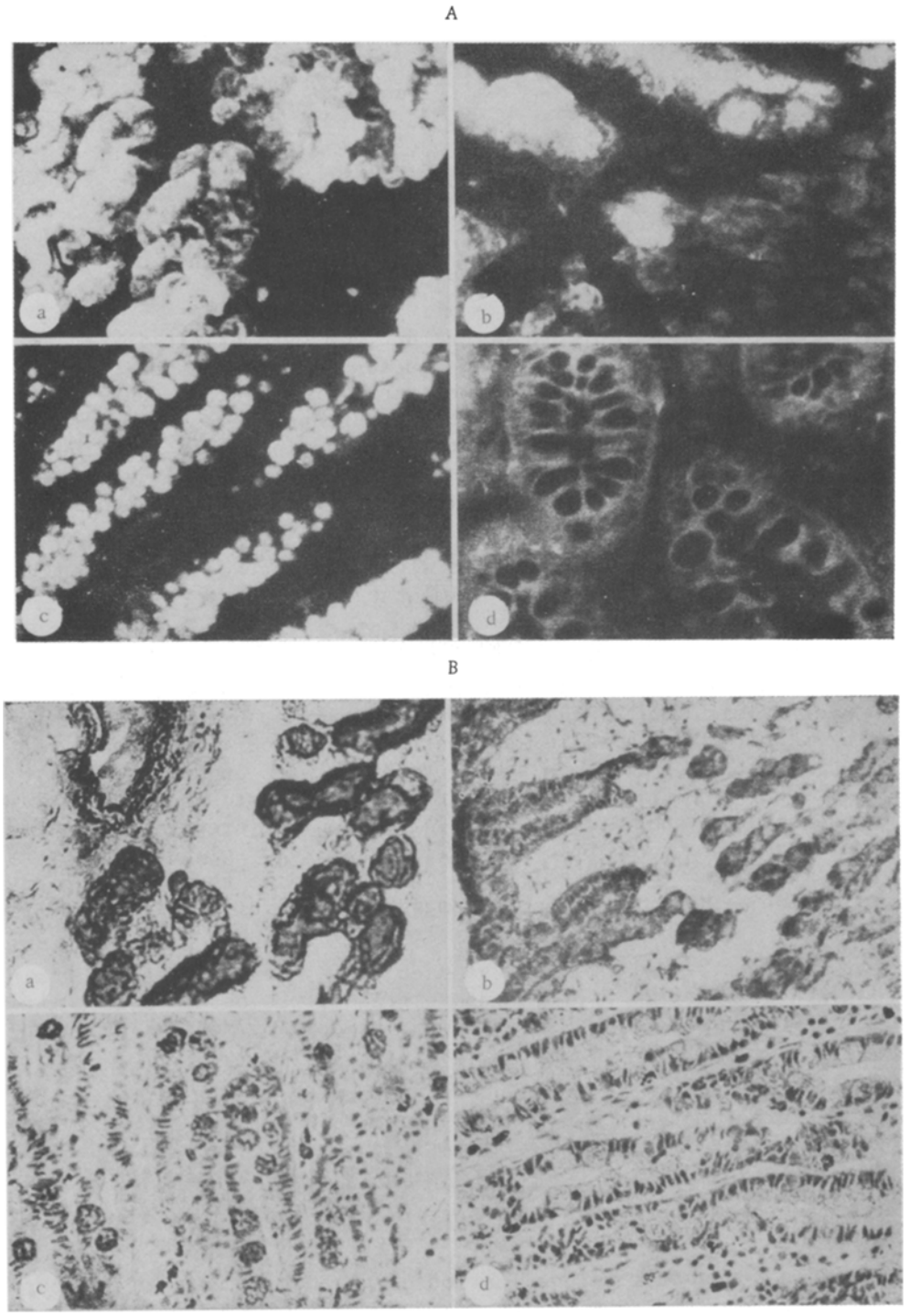

Fig. 1. Localization of entodermal antigen according to data obtained by immunofluorescence methods (A) and immunoperoxidase method (B). A: a) mucous glands in soft palate; b) fundal glands of stomach; c) mucous glands of large intestine; d) goblet cells in large intestine (neutralization). a, b, d) magnification 320×, c) 160×; B: a) mucous glands in soft palate; b) mucous glands of esophagus; c) goblet cells in small intestine; d) goblet cells in small intestine (neutralization); a-d) magnification 320×.

the method of Graham and Karnovsky [8]. In both cases, for control of the specificity of staining of the detected antigen, the test was carried out with antibodies neutralized by pure antigen.

EXPERIMENTAL RESULTS

At the beginning of the investigation an attempt was made to use paraffin sections. However, tissues from the oral cavity if embedded for a short time, while preserving the test antigen, were very poorly penetrated by the paraffin, so that it was impossible to obtain good paraffin sections. Later, therefore, we were forced to use cryostat tissue sections, fixed with paraform. To prevent the test material from thawing while being frozen to the stage, the fixed blocks were frozen on a base of mouse liver. In this way the tissue could be correctly oriented and sections in a definite plane obtained.

The results obtained by the immunofluorescence method agreed completely with those obtained by the immunoperoxidase technique, but the latter is more convenient for morphological analysis.

The results of the investigation showed that the entodermal antigen in the mucosa of the mouth and alimentary tract is located in mucus-forming structures which stood out in sharp contrast against the general background. Incubation of the sections with antibodies neutralized by pure antigen confirmed the specificity of detection of entodermal antigen in mucus-forming cells and glands.

In the large intestine the test antigen was revealed clearly and in sharp contrast only in the goblet cells of the crypts and villi. It evidently was located in the secretion produced by these cells (Fig. 1A). In the small intestine only goblet cells stained (Fig. 1B). In the gastric mucosa the antigen was found in the apical part of the fundal gland (Fig. 1A), and in the esophagus exclusively in glandular structures (Fig. 1B).

Fluorescence of the entodermal antigen in the mucosa of the soft palate was bright and in sharp contrast. All mucus-forming glands were uniformly fluorescent. No fluorescence was found in other structures. The glandular cells of the submaxillary salivary gland also gave bright fluorescence, evidence of the abundance of entodermal antigen contained in the secretion (Fig. 1A, B). In other parts of the mucosa of the oral cavity (mucous membrane of the cheek, tongue, and hard palate) no entodermal antigen could be detected, in full agreement with results obtained previously by the gel precipitation test.

The complete agreement between the results obtained by immunofluorescence and immunoperoxidase methods (the method using PAP is one or two orders of magnitude more sensitive than the indirect immunofluorescence method), and the absence of reactions when antibodies neutralized by the pure antigen were used, are evidence of the specificity of localization of the entodermal antigen. The clear and sharply contrasting localization of the test antigen in all parts of the alimentary tract suggests that the test antigen is a component of the mucus secreted by glands of the alimentary tract of entodermal origin.

The absence of entodermal antigen in other parts of the mucosa of the oral cavity except the soft palate is noteworthy. According to the most widely held views on embryogenesis of the oral cavity, the boundary between ectoderm and entoderm is the boundary between the hard and the soft palate [5], in agreement with the results now obtained. Considering that until now the boundary between ectoderm and entoderm in the oral cavity has not been finally established [6], in our opinion the test antigen can serve as a marker for demarcation between derivatives of these two embryonic layers in the oral cavity. It is an interesting fact that although in the normal mucosa of the cheek very small mucus-forming glands are present [9], the antigen we have been studying, which is secreted by mucus-forming glands, is absent from that structure. The absence of entodermal antigen in the small mucus-forming glands of the anterior portion of the oral mucosa, the entodermal origin of which has been proved, a fact which may be used in the future in order to explain the possible mechanisms of antigenic conversions in the oral mucosa, deserves further study.

In conclusion, the writers are grateful to G. I. Abelev, Director of the Laboratory of Immunochemistry and Diagnosis of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and to N. V. Éngel'gardt and A. S. Gleiberman, on the staff of that laboratory, for their help with many aspects of this investigation.

LITERATURE CITED

1. A. I. Gusev and A. K. Yazova, Byull. Éksp. Biol. Med., No. 4, 120 (1970).
2. A. I. Gusev and A. K. Yazova, Biokhimiya, No. 1, 172 (1970).
3. L. A. Zil'ber (ed.), Immunochemical Analysis [in Russian], Moscow (1968).
4. V. N. Isaev and T. P. Séédre, Byull Éksp. Biol. Med., No. 10, 1234 (1976).
5. L. I. Falin, Histology and Embryology of the Oral Cavity and Teeth [in Russian], Moscow (1963).
6. Z. S. Khlystovaya (ed.), Morphology of the Epithelium of the Anterior Part of the Digestive and Respiratory Systems [in Russian], Moscow (1971).
7. P. Cuatrecasas, J. Biol. Chem., 12, 245 (1970).
8. R. C. Graham, Jr., and M. G. Karnovsky, J. Histochem. Cytochem., 14, 291 (1966).
9. H. Sicher, in: Orban's Oral Histology and Embryology, St. Louis (1961), p. 416.
10. L. Sternberger et al., J. Histochem. Cytochem., 18, 315 (1970).

NUCLEAR MEMBRANE INCLUSIONS IN CORTICAL NEURONS OF THE PROGENY OF RATS SENSITIZED WITH BRAIN ANTIGEN

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KEY WORDS: neurosensitization; progeny; cerebral cortex; nuclear membrane inclusions.

Considerable attention has recently been paid to the role of neuroimmune factors in the pathogenesis of nervous and mental diseases. Modelling of neuroimmune processes may provide the key to the understanding of the fine mechanisms of disturbances of the structure and function of the CNS.

Hardly any references can be found in the literature to the study of the effect of preliminary sensitization of female animals with brain antigens on the development of the brain structures of their progeny. The writer's previous investigations of the CNS of 30-day-old rats whose mothers had been treated in this way [2] revealed a decrease in size of the pyramidal cells in layer V of the sensomotor cortex and a reduced tigroid content of their cytoplasm compared with the control. Electron-microscopic investigation revealed membrane inclusions in the nuclei of the neurons and slight changes in the cytoplasm.

The aim of the present investigation was to study subcellular structural manifestations of the pathology of postnatal development of cortical neurons of an animal whose intrauterine development took place from the beginning under conditions of neuroimmune conflict. An attempt was made to study the dynamics of the ultrastructural changes discovered, by investigating the brain at different stages of postnatal life — from 2 to 60 days.

EXPERIMENTAL METHOD

A 20% solution of cerebral cortical isoantigen was injected intraperitoneally into sexually mature noninbred rats in a dose of 0.3 mg/200 g body weight three times on alternate days. On the 21st day after the first injection the rats were mated with healthy males. The presence of antibodies in the blood was established by the complement fixation test in the cold. The young were born on the 21st-22nd days after mating. The progeny of intact females served as the control. Altogether there were three series of experiments in which 120 experimental and 50 control young rats, obtained from 17 sensitized and nine control mothers respectively, were used. Pieces of sensomotor cortex taken for electron-microscopic investigation were fixed with glutaraldehyde and osmium tetroxide in phosphate

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