TISSUE-SPECIFIC ANTIGEN OF THE CHICK ADENOHYPOPHYSIS APPEARING IN THE EARLY STAGES OF HISTOTYPICAL DIFFERENTIATION

V. M. Barabanov and D. B. Nikolova-Kitova

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A water-soluble tissue-specific antigen with the electrophoretic mobility of α_1-globulin was found in the chicken adenohypophysis. It was shown by the indirect immunofluorescence method that the antigen arises during embryogenesis in the early stages of histotypical differentiation of the adenohypophysis. The first cells with specific fluorescence appear in 6-day embryos simultaneously in the cephalic and caudal lobes of the adenohypophysis. At the 8th-10th day of development bright mosaic fluorescence is observed in all cell bands of the adenohypophysis. The antigen detected can be used as a general marker antigen for differentiation of the chick embryonic adenohypophysis.

KEY WORDS: adenohypophysis of chick embryos; tissue-specific antigen; normal development.

The primordial and histotypical periods of differentiation are clearly distinguished in embryonic development of the adenohypophysis. In chick embryos the histotypical period of differentiation of the adenohypophysis begins on the 6th day of embryogenesis. An antigen immunochemically identical with the delta-crystallins of the lens [1] has been shown to appear in the anlage of the adenohypophysis (Rathke's pouch) of chick embryos [1]; in the histotypical period of development ACTH appears in the adenohypophysis of the 9-day embryo, and on the 13th day of development the specific tissue antigen of the caudal lobe of the adenohypophysis appears [2].

The object of the present investigation was to study tissue antigens characteristic of the initial stages of histotypical differentiation of the adenohypophysis in chick embryos.

EXPERIMENTAL METHOD

Aqueous extracts in a volatile buffer at neutral pH were obtained from the cephalic and caudal lobes of the adenohypophyses of hens of the Russian White breed. Lyophilized extract (40-60 mg) of the cephalic lobe was fractionated by electrophoresis in agar gel in 0.1 M Tris-EDTA-borate buffer system, pH 8.2-8.5. The anodal fraction of the extract, corresponding in electrophoretic mobility to human blood serum albumin was used as material for immunization. Two rabbits were immunized subcutaneously with a fraction of extract mixed with Freund's complete adjuvant in equal volume, and homogenized in agar. The rabbits were reimmunized three weeks and 1, 2.5, and 6 months later; antisera active in immunofluorescence tests were obtained after 2 to 4 reimmunizations.

The method of immunoelectrophoresis with determination of the relative electrophoretic mobility of the antigens [12] and the method of indirect immunofluorescence [4] were used.

TABLE 1. Relative Electrophoretic Mobility of Tissue-Specific A_2 Antigen of Chick Adenohypophysis

Lobes of adenohypophysis	Number of determinations	Electrophoretic mobility	
		$M \pm m$	P
Cephalic	12	91±0,5	<0,001
Caudal	9	78±0,4	

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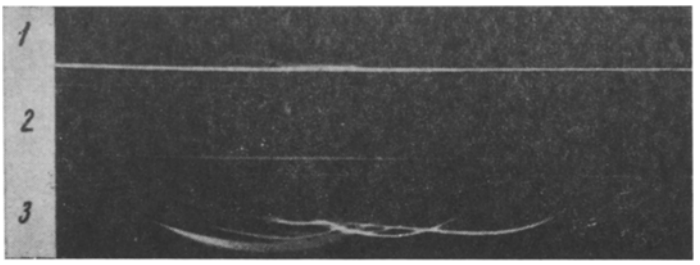


Fig. 1. Immunoelectrophoresis of antigen A_2 of the chick adenohypophysis. 1 and 2) Reaction of extract of cephalic and caudal lobes respectively of chick adenohypophysis with antiserum against anodal fraction of extract of cephalic lobe; 3) control reaction of normal human blood serum (NHS) with anti-NHS.

Adenohypophyses of 5-18-day chick embryos were fixed in Bouin's mixture [6], ethyl alcohol [9], and a 1% solution of acetic acid in ethyl alcohol [2], and embedded in paraffin wax; serial sagittal sections 5 μ thick were cut. The sections were incubated with rabbit antisera exhausted with lyophilized extract of chicken liver and blood serum, and at the next stage of the reaction, with a donkey luminescent antiserum against rabbit globulin (from the N. F. Gamaleya Institute of Epidemiology and Microbiology).

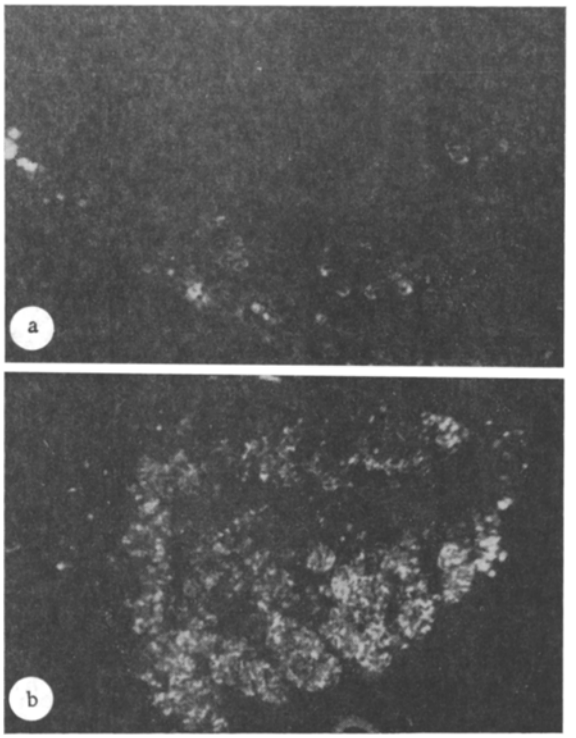
EXPERIMENTAL RESULTS

A tissue antigen contained in the cephalic and caudal lobes was found in the adenohypophysis of chickens by means of active sera obtained against the anodal fraction of cephalic lobe extract. This antigen was described as the second adenohypophysial antigen (A_2) to distinguish it from antigen A_1, described previously, which is characteristic of the caudal lobe of the chick adenohypophysis [2].

In immunoelectrophoresis with exhausted antisera the precipitation arc of antigen A_2 was masked by a denser arc of serum albumin, and it was detected when the antisera were exhausted with chicken blood serum or extract of chicken liver. Extract of the cephalic lobe of the adenohypophysis, in a concentration of lyophilized material of 40-60 mg/ml, formed a long distinct precipitation arc in the anodal zone on immunoelectrophoresis. The mean relative electrophoretic mobility of the A_2 antigen of the cephalic lobe was 91 (Table 1, Fig. 1). In reactions with extract of the caudal lobe of the chick adenohypophysis in the same concentration of lyophilized material a short diffuse or weak and thin precipitation arc was formed along the electrophoretic axis at the level of the cathodal half of the arc of A_2 antigen from the cephalic lobe. The differences in the behavior of A_2 antigen in reactions with extracts from different parts of the chick adenohypophysis and also the lower electrophoretic mobility of antigen from the caudal lobe (Table 1) are evidence of the quantitative predominance of A_2 antigen in the cephalic lobe and the greater degree of differentiation of the cephalic lobe in relation to this antigen compared with the caudal lobe.

Comparison of the action of the three fixatives, namely Bouin's mixture, ethyl alcohol, and a 1% solution of acetic acid in ethyl alcohol, showed the brightest specific fluorescence of the sections of the adenohypophysis fixed in Bouin's mixture, and this fixative was used in all subsequent work.

In the anlage of the adenohypophysis (Rathke's pouch) in 5-day chick embryos A_2 antigen was not present, according to the results of the immunofluorescence test. With the beginning of formation of epithelial cell bands in the adenohypophysis of the 6-day embryos, cells with weak specific fluorescence indicating the beginning of synthesis of A_2 antigen appeared. In the 6-day embryo they were located in the anterior superior band of the cephalic lobe and in the inferior bands of the caudal lobe of the adenohypophysis (Fig. 2a). During the next two days of development the number of cells with specific fluorescence and its intensity increased considerably. In the 8-day embryos, mosaic fluorescence was particularly bright in the lower part of the caudal lobe, and the region of specific fluorescence of the cells spread over the whole cephalic lobe, including the zone of the inferior bands (Fig. 2b).



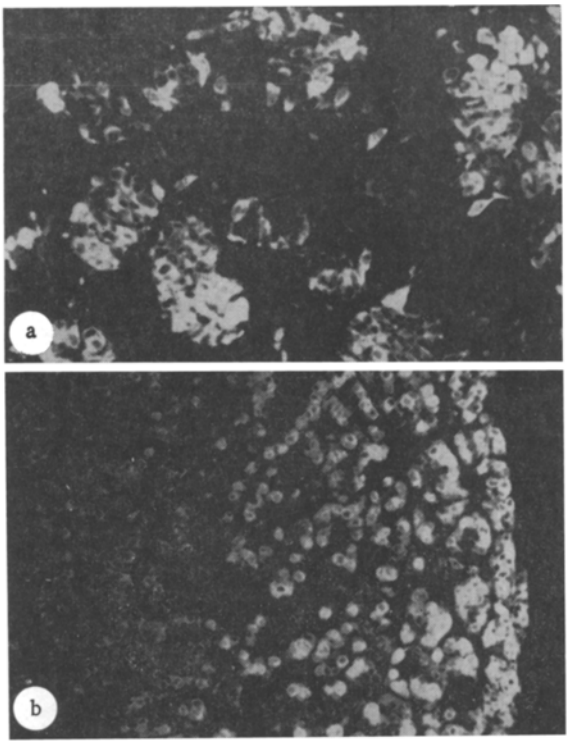


Fig. 2

Fig. 3

Fig. 2. Appearance of A_2 antigen in adenohypophysis of 6-8-day chick embryo. a) Caudal lobe of adenohypophysis of 6-day embryo (60 ×); b) adenohypophysis of 8-day embryo (30 ×). Indirect immunofluorescence.

Fig. 3. Localization of antigen A_2 in adenohypophysis of 11-day and 18-day chick embryos. a) Caudal lobe of adenohypophysis of 11-day embryo (100 ×); b) cephalic lobe of adenohypophysis of 18-day embryo (60 ×). Indirect immunofluorescence.

Fluorescent cells could also be detected in the upper part of the adenohypophysis, mainly at the base of the cell band, whereas the apical cells of the bands did not yet contain A_2 antigen. A connection between the appearance of A_2 antigen and the specific differentiation of the adenohypophysis also was demonstrated by the fact that specific fluorescence was absent or only solitary fluorescent cells were found in the lining of the primary canal of the adenohypophysis in 8-day embryos. In 10-15-day embryos bright mosaic fluorescence was found in all bands of the adenohypophysis, and also in the tuberal lobe. Many cells in the lining of the primary canal did not exhibit specific fluorescence (Fig. 3a). The distribution of A_2 antigen in the adenohypophysis of the 18-day embryos was similar in character, but fluorescent cells were brightest at the periphery of the adenohypophysis in both the cephalic and the caudal lobe (Fig. 3b).

It can be concluded from these results that the chick adenohypophysis contains a tissue antigen whose appearance in embryogenesis is characteristic of the initial stages of histotypical differentiation of the adenohypophysis. Cytological differentiation of the adenohypophysis in birds in embryonic development is often regarded as a vectorized process starting in the cephalic lobe and gradually spreading in the caudal direction [3, 8, 10, 11], or a process flowing independently and divergently in the cephalic and caudal lobes [7]. Meanwhile the results obtained in this investigation show that tissue-specific A_2 antigen appears in the adenohypophysis of 6-day chick embryos in the cephalic and caudal lobes simultaneously, and the spread of the zones of localization of this antigen in the early stages of histotypical differentiation of the adenohypophysis takes place synchronously in both of its lobes. Accordingly, A_2 antigen can be used as a common marker antigen of the differentiation of the chick embryonic adenohypophysis in experimental research.

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EFFECT OF PRODUCTS OF DESTRUCTION OF TISSUE MACROPHAGES ON HEMATOPOIETIC STEM CELLS

L. I. Privalova, B. G. Yushkov, and B. A. Katsnel'son

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Products of destruction of mouse peritoneal macrophages (MDP), obtained aseptically by freezing and thawing the cells three times, when injected intraperitoneally into syngeneic mice cause an increase in the number of splenic colony-forming units (CFU_S) in the hematopoietic tissue of the bone marrow and spleen, revealed by Till and McCulloch's method. This increase is a true increase, for it was shown that the fraction of transplanted stem cells adsorbed by recipient's spleen in donor mice receiving MDP was relatively smaller than in the control. Besides an increase in the total number of splenic colonies a decrease was found in the number of erythroid colonies relative to the number of colonies of granulocytic and monocytic type. One possible mechanism of the effect of MDP on the number of CFU_S may be modification of the hematopoiesis-inducing microenvironment, as is indicated by the increase in the number of colonies in mice into which normal hematopoietic tissue was transplanted after preliminary repeated injection of MDP. Other possible mechanisms of the observed effects also are examined, allowing for the fact that no direct effect of MDP on the stem cell could be found in experiments with preincubation of the bone marrow tissue with MDP before its injection into lethally irradiated mice.

KEY WORDS: products of destruction of macrophages; hematopoietic stem cells.

The cellular phase of self-cleansing of the lungs from inhaled particles is a self-regulated process, controlled by the quantity of macrophage destruction products (MDP) formed [4, 5]. Besides causing local activation and attracting phagocytic cells and mobilizing them from the depots, MDP also promote granulocytopoiesis and monocytopoiesis [3], functions which can be regarded as a manifestation of the general principles of regulation of hematopoiesis, in which an essential role is ascribed to destruction products of blood cells [2, 7]. However, little is known of the action of such products on the initial stages of hematopoiesis. Accordingly, special attention must be paid to the study of the effect of MDP on hematopoietic stem cells.

Department of Work Hygiene, Institute of Work Hygiene and Occupational Diseases, and Department of Pathological Physiology, Sverdlovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 4, pp. 345-347, April, 1979. Original article submitted April 20, 1978.

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