# SEROLOGIC AFFINITY BETWEEN BLOOD HEMOCYTOBLASTS OF PATIENTS WITH ACUTE LEUKEMIA AND HUMAN FETAL THYMUS TISSUE

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A common antigen not found in other human organs is present in the hemocytoblasts of patients with acute leukemia, in the human fetal thymus, and in the spleen, lymph glands, and mucous membrane of the gastrointenstinal tractin adult humans. This antigen was not found in the leukocytes of healthy donors. It is postulated that this antigen may be associated with cells of thymic origin.

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It is now firmly established that thymectomy presents the development of some spontaneous or virus-, radiation-, and chemical-induced leukemias in mice [6, 7, 9-11]. Implantation of the thymus into thymectomized mice restored their ability to develop leukemias [9]. The thymus evidently contains cells which are most sensitive to the action of leukemogenic factors.

The object of this investigation was to analyze the antigenic structure of human leukemic tissues and of certain organs of the human fetus and adult.

## EXPERIMENTAL METHOD

Antisera were obtained by immunization of rabbits with saline extracts of human fetal thymus glands or of the spleens of persons dying from acute hemocytoblastosis. Normal laboratory methods [3, 4] were used to isolate leukocytes from the blood, to prepare antigens for immunization and for tests, to immunize rabbits, to exhaust antisera and isolate the total globulin fraction from them, and to determine protein in the antigens. The gel-precipitation reaction was carried out in a micromodification [1]. Immunoelectrophoresis [5] was also carried out in a micromodification at a potential gradient of 8.3 V/cm using a current of 20-40 mA.

## EXPERIMENTAL RESULTS

Sera of rabbits immunized with saline extracts of the spleens of persons dying from acute hemocytoblastosis were exhausted with a mixture of sera from healthy donors of blood groups O(I), A(II), and B(III) and with saline extracts of liver, kidneys, lungs, and spleen cadavers of persons dying accidentally. The exhausted antisera were concentrated 5-6 times by isolation of their total globulin fraction.

Antihemocytoblastosis sera obtained from 5 rabbits were used in the experiments. The antigens for the gel-precipitation reaction contained 5-7 mg protein/ml extract. During interaction between exhausted antihemocytoblastosis sera and antigens prepared from the spleen, liver, kidneys, or lung of a person dying from the leukemic form of acute hemocytoblastosis, one common precipitation line was formed, its ends in contact with the walls with normal liver, kidney, and lung antigen and with normal and leukemic human sera (Fig. 1, A and B). This line bends near the wells containing antigens prepared from normal human lymph glands and spleen (weakly positive reaction). The antigen corresponding to this line was conventionally described as hemocytoblast antigen (HA).

To identify it a test serum consisting of antihemocytoblastosis serum and saline extract of spleen from a person dying from the leukemic form of acute hemocytoblastosis was always used.

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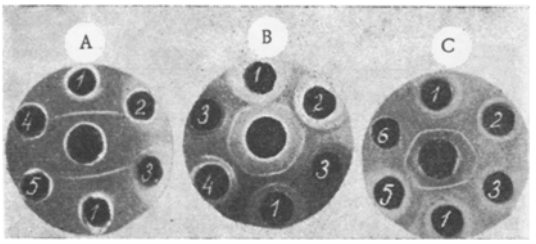


Fig. 1. Gel-precipitation reaction. A: 1) Extract of lung from a person dying from the leukemic form of acute hemocytoblastosis; 2) serum of a patient with acute hemocytoblastosis; 3) serum of a healthy donor; 4 and 5) extracts of normal human lung; central well contains exhausted antihemocytoblastosis serum. B: 1, 2, 3) and 4) extracts of organs from a person dying from the leukemic form of hemocytoblastosis; 1) extract of spleen; 2) extract of lung; 3) extract of liver; 4) extract of kidney; central well contains exhausted antihemocytoblastosis serum. C: 1) extract of human fetal thymus; 2 and 5) extracts of mucous membrane of adult human stomach; 3 and 6) extract of mucous membrane of adult human small intestine; central well contains antithymus serum exhausted with extracts of adult and fetal human tissues.

Immunoelectrophoretic experiments showed that HA is similar in its electrophoretic mobility to γ-globulins of human serum. It did not lose its activity during lyophilization and was precipitated by half-saturation with ammonium sulfate. Saline extracts of normal human organs were concentrated 3-5 times either by salting out or by lyophilization, and their content of HA was then studied. HA was detected in the concentrated extracts of normal spleen as a well-defined line; it could not be detected in concentrated extracts of the liver, kidneys, and lungs.

The HA content in organs of patients with leukemic (one case) and aleukemic (six cases) forms of hemocytoblastosis was studied with the aid of antihemocytoblastosis sera. In acute hemocytoblastosis with a leukemic blood picture HA was found in all the organs studied (spleen, liver, kidney, and lung), while in the aleukemic form it was found in all samples of the spleen, bone marrow, and lymph glands, but not in liver, kidneys, and, except in one specimen, the lung. It was postulated that HA is a constituent of the hemocytoblasts, and this explains its discovery in all organs of the patient with the leukemic form of hemocytoblastosis.

To test this hypothesis, the HA content was studied in the leukocytes of healthy donors and patients with various forms of leukemia. In patients with the leukemic form of acute hemocytoblastosis, HA was clearly determined in 5 of 7 samples of leukocytes consisting almost entirely of hemocytoblasts; with 2 samples the antihemocytoblastosis serum gave a weak positive reaction. In other forms of leukemia HA was found less regularly in the leukocytes. With 3 of the 4 samples of leukocytes from patients with chronic myeloid leukemia the reaction was weakly positive, and with 1 it is negative; of 6 samples of leukocytes from patients with chronic lymphatic leukemia, HA was detected in one and in the rest the reaction was negative.

HA was not found in any of the 6 samples of leukocytes from healthy donors that were studied. It likewise was not found in the sera of healthy donors and of patients with various types of leukemia. These results indicate that HA is in fact a component of the hemocytoblasts.

The HA content in the organs of human fetuses aged 18-40 weeks was studied by means of the antihemocytoblastosis sera. HA was clearly detected in the thymus of fetuses at or before term. The reaction of the antisera with spleen extracts from these fetuses was weakly positive. No HA was found in the liver or kidney of the human fetuses.

To continue the study of HA, antithymus sera were obtained and absorbed with the sera of healthy donors and with lyophilized powders of extracts of the liver, kidneys, and lungs of adults dying from injury and from human fetuses. Total globulin fractions were isolated from these antisera, and as a rule they reacted only with hemocytoblast antigen. Experiments with these antisera confirmed the results obtained in the experiments with antihemocytoblastosis sera. At the same time, HA was clearly detected in the mucous membrane of the adult human stomach and small intestine (Fig. 1C). Traces of it were possibly present also in the mucous membrane of the large intestine, because in some experiments the precipitation line corresponding to HA was bent toward the wells containing extracts of mucous membrane of the large intestine. No HA was found in the liver, kidneys, lungs, limb muscles, brain, adrenals, or pancreas of human adults and fetuses, or in the thyroid and pituitary of adults (the thyroid and pituitary of the fetuses were not investigated).

The experiments thus showed that serologic affinity exists between the blood hemocytoblasts of patients with acute leukemia, human fetal thymus tissue, and the peripheral lymphoid organs and mucous membrane of the gastrointestinal tract. Two hypotheses can be put forward to explain the nature of the affinity.

The first possible explanation is that HA is an antigen characteristic of young, mitotically active hematopoietic cells of the lymphoblast and hemocytoblast type. This antigen is present in much smaller amounts in more highly differentiated blood cells. It is detected most clearly in the thymus, because the number of lymphoblasts in the thymus is 4-8 times greater than in the peripheral lymphoid organs. The submucosa of the gastrointestinal tract contains collections of lymphoid tissue which are difficult to separate from the mucous membrane by dissection. Traces of this lymphoid tissue were perhaps present in the preparations of mucous membrane used for making the extract, and they may have been responsible for detection of HA in them.

Second, it may be postulated that hemocytoblasts in acute leukemia are formed as a result of transformation and malignant degeneration of cells of thymic origin, or thymocytes, which are evidently derived from the epithelium of the thymus [2]. The mucous membrane of the gastrointestinal tract and the epithelium of the thymus develop from the entoderm of the gut. Their common cytogenesis may determine the serologic affinity of leukemic hemocytoblasts, lymphoid cells of thymic origin, epithalial cells of the thymus, and the mucous membrane of the stomach and intestine.

Hemocytoblastoses are probably one of the forms of human leukemias in whose pathogenesis, just as in the case of certain mouse leukemias, an important role is played by the thymus, the source of cells most sensitive to the action of leukomogenic factors. Under normal circumstances these same cells may participate in the formation of the immunocompetent system of the body.

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