LEUKEMOGENIC ACTIVITY OF SUPERNATANT FROM HUMAN LEUKEMIC TISSUE AFTER PASSAGE THROUGH TISSUE CULTURES

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In this investigation an attempt was made to detect a viral agent in human leukemic tissue cultivated in vitro on the basis of its leukemogenic activity in mice of lines with a low incidence of spontaneous leukemia.

EXPERIMENTAL METHOD

The spleen was taken 14 h after death from the cadaver of a 14-year-old boy dying from acute reticulosis (leukocyte count in the peripheral blood 92 000/mm^3; 91.5% of reticulum cells) and a saline extract was prepared from it in the proportion of 1 g tissue to 5 ml Hanks's solution (pH 7,0). The spleen tissue was first cut up with scissors into small pieces, after which the required volume of Hanks's solution was added and it was homogenized in a microblendor. The homogenate was kept for 5 h in a refrigerator at 4° and then centrifuged at 4000 rpm for 20 min. The residue was discarded and the supernatant centrifuged again at 120 000 g for 1 h. The supernatant was withdrawn and the residue resuspended in Hanks's solution with the aid of an electric homogenizer and washed by centrifugation at 120 000 g for 1 h. The supernatant was decanted and the residue resuspended in Hanks's solution in half the volume of the original extract taken for ultracentrifugation. The suspension was centrifuged at 5000 rpm for 10 min. The supernatant was withdrawn and penicillin and streptomycin added to it at the rate of 100 units of each antibiotic per ml fluid.

Two further supernatants were prepared by the same method from the spleen taken from the cadaver of a man aged 53 years dying from subacute (treated) reticulosis–hemocytoblastosis (leucocyte count in the peripheral blood 1700/mm^3: hemocytoblasts 31.5%, myelocytes 2.5%, juvenile 0.5%, stab cells 3%, polymorphs 8%, lymphocytes 54.5%, monocytes 0.5%), and from the enlarged lymph glands of a woman aged 61 years with a treated tumor-like form of chronic lymphatic leukemia (450 000 leukocytes/mm^3 blood; lymphocytes 92%). The organs used for preparation of the supernatants were taken 24 h after death.

The supernatants thus obtained from human leukemic tissue were used to infect a surviving culture of normal human spleen. The surviving culture was prepared from the spleen from a woman aged 33 years killed in an automobile accident, by the method described previously [1]. The surviving culture of normal human spleen was infected with the supernatant at the rate of 0.2–0.3 ml/ml medium. After 27 days the culture fluid of the surviving culture of normal human spleen was used to infect a monolayer of human embryonic kidney (HEK) in a dose of 0.2–0.3 ml of culture fluid/ml medium. Successive passages thereafter took place at intervals of 20–30 days (the 7th passage after 11 days). As a rule culture fluid was used for the passages, except for the 4th in which HEK cells were used instead. To obtain HEK, the embryonic kidneys were obtained from mothers with a negative family history. As the medium became acidified, alkali was added to it or it was changed.

Biological tests were carried out on CC57BR mice, into which the culture fluid of a 7-day surviving culture of normal human spleen infected with supernatant from human leukemic spleen was injected intraperitoneally or intracerebrally (the control animals were injected with the analogous uninfected culture). Other mice of the same line were injected intraperitoneally with material from a surviving culture of

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Results of Experiments in which CC57BR Mice were Injected with a Surviving Culture Infected with Supernatant from Human Leukemic Tissue and Effect of Passage through HEK

Diagnosis and material	No. of mice, dose, and mode of injection of preparation	No. of mice surviving until appearance of first case of leukemia (2 mos.)	Number of cases of leukemia developing
Reticulosis–hemocytoblastosis; 7-day surviving culture	10; 0.05 ml, intracerebrally	8	0
Chronic lymphatic leukemia; 7-day surviving culture	20; 0.5 ml, intraperitoneally	17	2
Uninfected 7-day surviving culture of normal human spleen (control)	10; 0.05 ml, intracerebrally	9	0
Acute reticulosis			
2nd passage through HEK	16; 0.5 ml, intraperitoneally	16	1
4th " " "	30; 0.5 ml, intraperitoneally	24	7*
13th " " "	8; 1 ml, intraperitoneally	3	3†
Uninfected HEK (control)	33; 0.5 ml, intraperitoneally	27	0

*Two cases of leukemia were confirmed by successful transplantation.
†One case of leukemia was confirmed by successful transplantation.

human spleen at the 2nd, 4th, and 13th passages through HEK infected with supernatant of human leukemic tissue. In control experiments the mice were injected intraperitoneally with an uninfected HEK culture.

To suppress possible coexistent bacterial infection the animals received antibiotics (monomycin, penicillin, colimycin) in a daily dose of 2000 units per mouse.

The diagnosis of leukemia was made on the basis of the macroscopic picture and a cytological study of the liver, spleen, bone marrow, and blood films and the leukocyte count of the sacrificed mice. To confirm the diagnosis of leukemia, material was transplanted into other mice.

The morphologic study revealed no differences between the experimental and control tests in relation to HEK cultures at the 2nd, 3rd, 4th, 6th, 7th, 8th, 9th, 10th, 12th, 13th, and 14th passages.

Examination of the control and experimental cultures at the 5th passage revealed foci of monolayer arrangement of the cells, forming branching shapes consisting of long cells, flattened from side to side, on the delicate monolayer. Foci of growth of this type were more rarely seen in the controls.

Not until the 11th passage on the HEK monolayer were sharp differences observed between the experimental and control cultures, expressed by different types of cell growth. In the control culture, for instance, most cells were long and split lengthwise, with marked opalescence and with a strong growth potential, whereas in the experimental culture the cells were much smaller, round in shape, and less opalescent. Judging from the state of the monolayer in the experimental culture, growth of the cells was clearly depressed.

The 15th passage was carried out on a monolayer of kidney from an adult monkey Macaca rhesus; this passage of the experimental culture differed very considerably from the control.

In the monolayer of the experimental culture rapid disintegration of the main body of cells was observed. Single cells with obvious signs of degeneration remained on the glass, whereas in the control culture a well developed monolayer of cells with high growth potential was found.

It is possible that in this case depression of growth in the experimental cell culture was due to a transplanted agent manifesting its action only episodically.

Injection of the 7-day surviving culture infected with supernatant from the spleen of a patient dying from reticulosis–hemocytoblastosis intracerebrally into CC57BR mice did not cause the development of leukemia in the animals.

The 7-day surviving culture infected with supernatant of the spleen from a patient dying from chronic lymphatic leukemia likewise was relatively inactive (two cases of leukemia in 17 mice; see table).

Highest leukemogenic activity was shown by HEK cultures infected with a 5-day surviving culture of normal human spleen to which had been added the supernatant from the spleen of a patient dying from acute leukemia (reticulosis). As the table shows, the second passage through HEK was relatively inactive, but the 4th and 13th passages showed well-marked leukemogenic activity. In three cases the leukemia was successfully transplanted into other mice of the same line, but a strain capable of prolonged transplantation could not be obtained. No cases of leukemia occurred in the control experiments.

Morphologically the leukemias which developed were hemocytoblastoses and myeloses with a typical picture: gross enlargement of the spleen with multiple white nodules; enlargement of the peripheral lymph glands, thymus, and liver; changes in color of the organs.

Microscopic study of impressions of the organs showed as a rule marked rejuvenation of cells of the myeloid series and absence of erythropoiesis in the bone marrow, with foci of extramedullary hemopoiesis in the liver and the spleen.

The leukocyte count of the experimental mice a few days before death usually increased to 30,000-80,000; blast cells appeared in the peripheral blood films.

When the mechanism of origin of leukemia in the experimental mice is analyzed, two most likely possibilities must be borne in mind: adaptation of the viral agent present in human leukemic tissue to mice and activation of a latent leukemogenic virus of mice by this agent. Which of these mechanisms was responsible for the appearance of leukemia in these experiments is not clear, and it cannot therefore be concluded categorically that the agent isolated from human leukemic tissue is an etiological factor in human leukemia.

LITERATURE CITED

1. V. M. Bergol'ts and V. V. Dement'eva, Byull. éksp. Biol., No. 10, 92 (1965).