ON THE POSSIBILITY OF ELEVATING THE BIOLOGICAL ACTIVITY OF EXTRACTS FROM HUMAN LEUKEMIC TISSUE

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A number of investigations [1-5 et al.] have served to establish that acellular extracts of human leukemic tissue, when injected into mice of low-leukemia lines, cause the appearance of leukemia in them, which is rarely encountered in the corresponding control experiments. However, as a rule, the percent that develop leukemia in these experiments were lower than that which is observed from inducing leukemia in mice by the use of the mouse leukemia viruses.

The goal of this investigation was to elucidate the possibility of elevating the leukemogenic activity in extracts of human leukemic tissue. With this purpose, it was decided to try two routes for injecting the mice with the extracts (directly into the brain or directly into the thymus gland), and also different variations for preparing the human leukemic tissue extracts under study. We did not attempt to clarify the specific activity of human leukemic extracts as compared to normals, inasmuch as that question has already been studied by other investigators [1-5].

EXPERIMENTAL METHOD

The tissue being studied was taken from patient G., 34 years old, who died of acute hemocytoblastosis. The last blood analysis showed: 3,200,000 erythrocytes, 153,000 leukocytes, 98 hemocytoblasts, 12,800 thrombocytes. Homogenates were made of tissue from the spleen or brain in physiological saline, using a dilution of 1:5 and keeping the material refrigerated, and was then centrifuged at 3,000 rpm for 10-15 minutes.

The supernatant fluid was used for injecting the mice. A portion of the crude spleen or brain extract was heated at 56° for 30 minutes. We postulated that heating of the extract exerts an inactivating effect on the inhibitor of the leukemia virus. The heated extract was added to the untreated one in the ratio of 1:1. The extracts were injected into the mice via two routes: 1) into the left hemisphere of the brain in a dose of 0.03-0.04 ml, using mice of the low-leukemia line, C3HA, at the age of 4 weeks, and 2) directly into the tissue of the thymus gland in a dose of 0.1 ml, using white mice of mixed breeding at the age of 20-22 days. Preliminary model experiments involving injection of a stain into the thymus gland showed that in mice, 20-22 days of age, the thymus gland is sufficiently well defined, and thus it is possible to inject the extract directly into the tissue of the gland. In certain series of the experiments, hyaluronidase was added to the extracts in a concentration of 5 mg per ml of extract.

All the experimental animals were divided into 6 groups, each containing 15 mice. The mice of the first group were injected in the thymus gland with the untreated spleen extract and untreated brain extract in a ratio of 1:1, with the addition of hyaluronidase; mice of the second group got untreated spleen extract and untreated brain extract; mice of the third group received untreated brain and "inactivated" spleen; mice of the fourth group got untreated brain and "inactivated" brain; mice of the fifth group were injected in the tissue of the brain with untreated spleen extract and "inactivated" brain extract; mice of the sixth group got untreated spleen extract and "inactivated" spleen extract.

A diagnosis of leukemia was made on the basis of morphological study on the mice that died or were sacrificed. Smear-impressions were made from the bone marrow, spleen and liver of all the mice, and these were stained by the method of Pappengeim. In a portion of the cases, subcutaneous transfers of the leukemia were made to mice of the same breed, in order to confirm the diagnosis of true leukemia.

EXPERIMENTAL RESULTS

The largest number of leukemias were observed in the experiment in which the mice were injected in the thymus gland with untreated spleen extract, untreated brain extract and hyaluronidase. The first case of leukemia in this experiment appeared at 2 months after its initiation. Out of 10 mice that survived the interval of the appearance of the first leukemia, leukemia developed in 7 of them. Two cases of leukemia out of the 7 were confirmed by successful subcutaneous transfer, with the formation of nodules at the site of transfer; out of these leukemias we obtained transplantable strains, which, up to February of 1961, have passed through more than 15 generations. The average latent period for development of leukemia has been 3 months.

In a similar experiment, with injection into the thymus gland of untreated spleen and brain extracts, but without hyaluronidase, the extracts were also active, but to a lesser degree. The average latent period for the appearance of leukemia in this experiment was 5 months.

Out of 9 mice that survived for 2 months after the beginning of the experiment, leukemia developed in three. One case of leukemia was confirmed by transfer.

In the experiment in which the mice were injected in the thymus gland with untreated brain extract and "inactivated" spleen extract, we diagnosed leukemia in two animals out of 10 that survived for 2 months after initiation of the experiment, with an average latent period for development of leukemia of $4\frac{1}{2}$ months.

Injection of mice in the thymus gland with untreated and "inactivated" brain extracts did not produce a substantial leukemogenic effect. Out of 10 mice that survived the interval for the appearance of the first leukemia, leukemia was diagnosed in only one animal (the latent period was approximately 4 months).

In the experiment in which the mice were injected in the brain with untreated spleen extract and "inactivated" brain extract, not one of the 12 mice that survived for 2 months from the beginning of the experiment developed leukemia. Also, no case of leukemia was observed in any of the 14 mice which were injected in the brain with untreated and "inactivated" spleen extracts, and which survived for 2 months after the beginning of the experiment. The mice from these experiments were sacrificed 7 months after initiation of the experiment.

Judging from the results of the experiments presented above, induced leukemias begin to appear within 2 months from the beginning of the experiment, attaining a maximum by 4 months after injection of the extracts.

According to the morphological picture, all the leukemias that arose belonged to the myeloid type, characterized by total myeloid metaplasia of the bone marrow, with suppression of erythropoiesis and myeloid infiltration of the liver and spleen.

In previous investigations [1] it was established that, out of a number of investigated tissues which were taken from patients who died of acute leukemias (spleen, liver, lymph nodes, bone marrow, tonsil, blood), the greatest activity is found in the spleen or bone marrow extracts, and the least activity in the blood. The results of the experiments performed show that extracts of spleen and brain, taken separately, are less active than their mixture.

As for heating the extract in order to suppress the hypothesized leukemia virus inhibitor, on the basis of our data there is no foundation for assuming that this inhibitor can be inactivated by heating at 56°. Apparently, the very important problem of inactivating the virus inhibitor must be studied, using other means for its removal (treatment with organic solvents, etc.).

The results of these experiments show that maximum leukemogenic activity is found in the mixture of centrifugates of untreated leukemic spleen and untreated leukemic brain, with the addition of hyaluronidase (5 mg ml), when the preparations are injected directly into the tissue of the thymus gland of 3 week old mice.

In our opinion, the results obtained carry definite interest, since they again confirm that it is possible to obtain leukemias in mice by using extracts of human leukemic tissue. They also have methodological importance for experimental works in which it is necessary to obtain leukemias very quickly and in large number.

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