USE OF SURVIVING SPLEEN TISSUE CULTURES TO ELICIT THE LEUKEMOGENIC AGENT IN HUMAN LEUKEMIC TISSUE

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A biological test on animals is a necessary stage in eliciting the active agent in human leukemic tissue. However, this method has a number of essential shortcomings associated mainly with the prolonged latent period of development of leukemias, the presence of latent leukemia viruses in experimental animals, and the difficulty of a regular reproduction of obtained results. At the same time the absence of any other quicker of more reliable tests for the presence of virus forces us to use the biological experiment on animals (with consideration, of course, of all its limitations) when studying the viral etiology of human leukemia. The present report gives an account of a new method of eliciting the leukemogenic agent (isolated from human leukemic tissue) which has a definite advantage over the previously proposed methods [1, 2].

EXPERIMENTAL

For the biological experiments we used the culture liquid of the surviving tissue of the normal spleen of a 58-year-old male who died from rheumatic heart disease. After removing the capsules the spleen was minced into pieces 1-2 mm in size. The minced pulp of the spleen was washed 5-10 times with No. 199 medium with 5% bovine serum and with antibiotics until complete removal of peripheral blood. Into each flask we placed up to 10 pieces of spleen and added 5-7 ml of No. 199 medium with 5% bovine serum. Then we added to the flasks 0.2 ml of a 10% suspension of spleen and bone marrow from a 52-year-old woman who died from acute leukemia, which was prepared on hydrolyzate lactalbumin with 2% bovine serum. The flasks were incubated at 37°C. Upon inspection of the flasks we observed oxidation of the medium, which indicated satisfactory survival of the spleen tissue. The surviving spleen cultures were bacteriologically sterile.

At different time intervals (4, 7, 22, and 29 days) after infection of the surviving spleen tissue, the culture fluid was injected in a dose of 0.1-0.2 ml directly into the spleen of 2 to 3-month-old mice of the low-leukemic strains CC57BR, $C_3H(f)$* and BALB/C. The mice that died or were killed were subjected to a pathomorphological investigation. Replicas of the organs (liver, spleen, bone marrow) were stained by Pappenheim's method.

RESULTS

The results of the experiments are shown in Table 1.

As we see from Table 1, the greatest leukemogenic activity is manifested by cultures taken 4-7 days after their infection by leukemic material (especially on the 7th day; it is interesting that the virus of poliomyelitis is also active after 8 days incubation in the surviving tissue culture). The biological activity of the culture drops later.

The leukemias that developed belong morphologically to granulocytic leukemias with a typical macro- and micropicture (see Figure 1).

*Leukemias in the mice of these strains occur spontaneously in less than 1% of the cases.

TABLE 1. Biological Activity of Fluid of a Surviving Culture of Normal Human Spleen Taken at Various Periods After Infection by a Suspension of Human Leukemic Spleen and Bone Marrow Injected into Mouse Spleen

Number of days after infection of surviving culture	Strain of mice	Number of experimental mice	Number of mice that survived the period of development of the first lukemia (10 months)	Number of leukemias that occurred
4	CC57 BR	20	8	2
	$C_3H(f)$	18	13	2
7	CC57 BR	20	16	7
11	CC57 BR	20	10	0
15	CC57 BR	20	11	2
22	CC57 BR	14	5	1
29	BALB/C	10	4	0
29 (Heated culture)	BALB/C	10	7	0

Fig. 1. Mouse with leukemia induced by the injection of a surviving culture of spleen infected with human leukemic material.

An attempt was made to preserve the leukemogenic agent by transplanting the induced leukemias to other mice and by passage on fresh surviving cultures of human and mouse spleen. Since the biological test on animals is virtually the only test for the presence of the leukemogenic agent in the given medium, we will be able to summarize the results of these experiments much later (owing to the very long latent period of action of leukemia viruses).

The data obtained in the present work do not, of course, answer the problem of the mechanism of development of leukemias in mice—whether they occur as a consquence of adaptation of the human leukemia virus to mice or as a result of activation of the latent mouse leukemia virus (the effect of the bovine serum is improbable for many reasons). The answer to this problem can be given only after obtaining a strain of leukemogenic virus capable of prolonged passage. The results of these experiments show that human leukemic tissue contains an agent which is retained (or multiplies) in a culture of the surviving tissue of normal human spleen and induces leukemias in a high percent of mice of the low-leukemic line CC57BR. This method of eliciting the leukemogenic activity of the agent in human leukemic tissue can be used in investigations of the viral etiology of human leukemias.

LITERATURE CITED

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