REACTION OF MONKEYS TO INJECTIONS OF HUMAN LEUKEMIC BLOOD (PRELIMINARY COMMUNICATION)

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UDC 616-006.446-092.9-022.1

It has been found that leukemia can be produced in laboratory animals by injection of material from leukemic patients [1, 4, 5, 7, 8]. However, it is often difficult to interpret the results obtained, expecially in experiments on mice, because these animals are frequently carriers of various leukemogenic viruses. For this reason the authors selected monkeys—animals in which spontaneous leukemia is exceptionally rare—as experimental animals for studying the etiology of human leukemia. Furthermore, the closeness of monkeys to man in evolution and the similarity of their nosological disease profile suggest that experiments on these animals will be most likely to bear fruit. Admittedly, the few experiments so far carried out on monkeys with the object of reproducing leukemia [2, 3, 6] have not yielded definite results.

The object of the present investigation was to study the etiology of human leukemia by attempting to reproduce this disease in monkeys and to isolate the leukemogenic virus.

EXPERIMENTAL METHOD

Experiments were carried out on 144 monkeys of different ages (1.5-17 years) and different species (red and green guenons, macaques of different species, and baboons). In addition, material was injected into pregnant monkeys (5 animals), into the fetus followed by further injections into the young monkeys after birth. Materials for inoculation consisted of whole blood and blood filtered through a Seitz filter. Blood was taken from 11 untreated patients with various forms of leukemia (hemocytoblastosis, chronic lymphatic and myeloid leukemia) and injected into monkeys on three successive days parenterally in a volume of 3-5 ml, 15-60 min after being taken from the patient. In some cases the material was stored for 1-1.5 days at 4° before being injected.

As control material the monkeys were injected with citrated and defibrinated donors' blood and also with blood from patients with lymphosarcoma. The material from the leukemic patients and the inoculated monkeys was studied by histological, virological, and pathomorphological methods.

EXPERIMENTAL RESULTS

In the course of the experiment it was found that the sensitivity of the monkeys to the injected material depended on the species of the animal. The various species of macaques (rhesus, brown, and lapunder), which developed a disease in 100% of cases, were most sensitive. The latent period varied from 5 to 40 days. At the end of this time the animals became apathetic, lost their appetite, and developed a hemorrhagic diathesis. The characteristic signs in the sick animals were "headache," shivering and convulsions in the pre-agonal period. The disease was accomponied by elevation of the ESR from 7-10 to 75 mm in 1 h. Most monkeys developed a relative or absolute leukopenia during the first week of the disease, followed by leukocytosis in the second half of the disease. A distinguishing feature of the leukocytosis was that it was associated with an increase in the absolute lymphocyte count and with a shift to the left of the neutrophils (including a few myelecytes and juvenile cells). The blood formula showed monocytosis, lymphocytosis and, in some animals, an increased basophil count. In the bone marrow of the sick monkeys 1-1.5 weeks after the beginning of the disease an increase in the number of young forms of the neutrophil series and also in the number of reticular and undifferentiated cells was observed. The erythroid series was relatively

Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR; Central Institute of Hematology and Blood Transfusion (Presented by Active Member of the Academy of Medical Sciences of the USSR S. R. Mardashev). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 8, pp. 78-83, August, 1967. Original article submitted April 28, 1967.

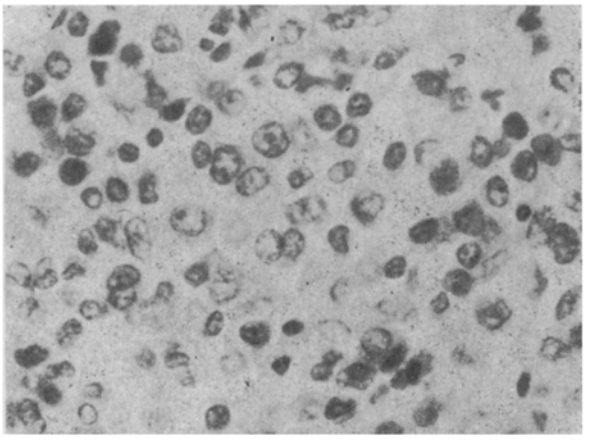

Fig. 1. Proliferation of reticular cells in the bone marrow of a monkey injected with the blood of a patient with chronic myeloid leukemia. Hematoxylin-eosin. 400 ×.

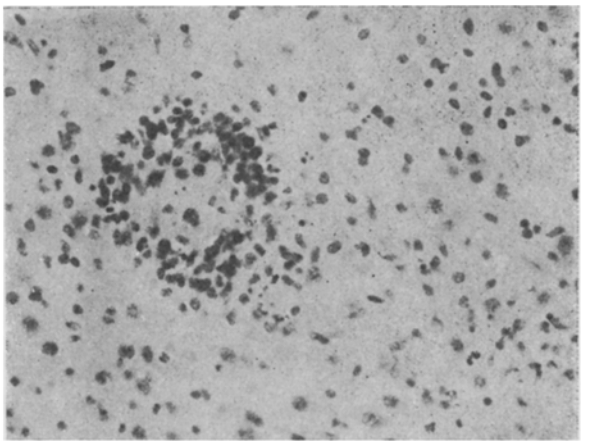

Fig. 2. Proliferation of cells in the wall of a blood vessel of the brain in an infected rhesus monkey. Hematoxylin-eosin. 250 ×.

depressed. Towards the end of the disease erythroblasts and normoblasts, sometimes in the state of division, appeared in the peripheral blood. The hemoglobin concentration and the erythrocyte count were lowered but not significantly.

The duration of the disease in the monkeys varied from 5 to 12 days, and it terminated as a rule in death of the animal (of the 41 macaques infected only one survived).

At necropsy on the monkeys which died, consistent findings included a hemorrhagic diathesis, enlargement of the spleen and lymph glands, slight parenchymatous degeneration of the liver, myocardium, and kidneys, and the development of cerebral edema and hydrocephalus. Histological examination of the bone marrow (Fig. 1) and lymph glands of the dead monkeys revealed proliferation of the reticular and undifferentiated myeloid cells, more marked in the animals sick for a long period of time. Proliferation of reticular and yound myeloid cells was also observed in the meninges and the brain tissues along the course of the blood vessels (Fig. 2). Similar proliferation could be seen along the course of the vessels in the lungs (Fig. 3) and, to a lesser degree, in the liver and spleen (Fig. 4). The enlargement of the spleen was not due to an increase in the number of cells, but to accumulation of protein in the septa between the sinuses. The splenic pulp was aplastic.

The course of the disease in the guenons and baboons was milder and fewer of them died. In the process of the disease the hematological changes in these animals were less marked then in the macaques, and the first leukopenic phase of the disease was often absent. The animals which died were mainly unacclimatized, and most of them had concomitant diseases (dysentery and pneumonia). In the uncomplicated cases the pathomorphological manifestations of the disease were the same as in the macaques, but the signs of proliferation in the reticular and myeloid tissues were less conspicuous.

No appreciable difference was found in the clinical and pathomorphological manifestations of the disease in the monkeys receiving blood from patients with different forms of leukemia.

To determine the possibility of reinoculation of the infecting agent, material from monkeys dying in the agonal period (blood and extracts from organs) were injected parenterally into other monkeys. The inoculations were carried out with crude materials and with material filtered through Seitz filters. The results of these experiments showed that the animals regularly developed the disease after inoculation with both the filtered and the unfiltered material. In these experiments the latent period was shorter then after primary inoculation and the disease in every case terminated in death of the animal. The clinical and pathomorphological pictures in the sick animals used for reinoculation was the same as in those used for primary inoculation. So far the filtered material has induced the disease in four passages through monkeys. In the animals used for these passages, the signs of proliferation of the reticular cells, expecially in the brain, the lungs, and the bone marrow, were more marked than in the monkeys receiving the primary inoculation from which the material used in the subsequent passages was obtained.

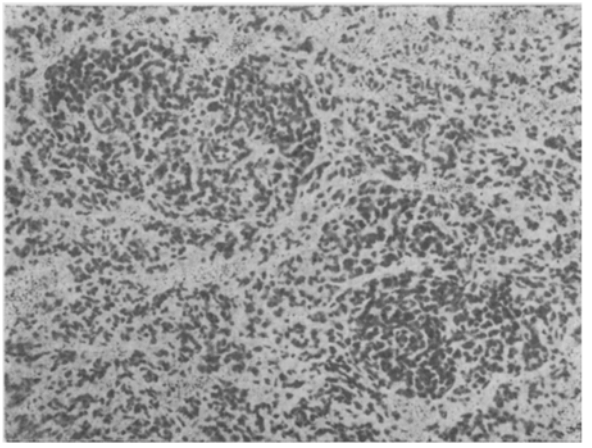

Fig. 3. Nodules of perivascular proliferation in the lung. Hematoxylin-eosin. 80 ×.

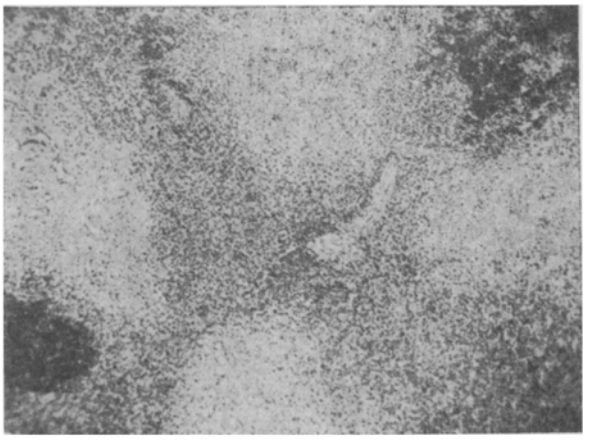

Fig. 4. Proliferation of reticular cells in the spleen. Hematoxylin-eosin. 80 ×.

In the animals of the control group (12 rhesus monkeys), whether receiving human donors' blood or receiving extracts of the organs of healthy monkeys, and also (in two cases) the blood of a patient with lymphosarcoma, no hematological, clinical, or pathomorphological abnormalities were found.

Immunologic investigations of the serum taken from the monkeys at various times after inoculation revealed a factor causing hemagglutination of group 0(I) erythrocytes of human blood; the titer of this factor rose in the course of the disease. The titers of the hemagglutinins at the height of the disease varied in the different monkeys from 1:64-1:128 to 1:512-1:2048. An increase in the titer of hemagglutinins was regularly observed in all the monkeys developing the disease, whether those inoculated with the original material or after passage. The hemagglutination reaction was negative with the serum of the control animals. The results of the test with trypsin showed that the appearance of hemagglutins was not caused by antibodies. Antibodies inhibiting hemagglutination caused by the serum of the sick monkeys appeared in the serum of the convalescent monkeys. Human sera from three patients with acute hemocytoblastosis taken during a remission led to inhibition of hemagglutination of material from six sick monkeys of the 12 animals investigated. The serum of healthy human subjects and monkeys did not produce inhibition of hemagglutination. In parallel experiments to the animal inoculations, blood from human leukemic patients and material from the infected monkeys were tested in cultures of various human and monkey tissues. The cytopathogenic agents were isolated and the transforming action of these materials on the cell cultures was determined. Primary kidney cultures from human and monkey embryos, and also transplantable HeLa, BHK, and other cells, were used in the experiments. In some cases the kidneys, thymus, and spleen of the sacrificed infected monkeys were cultivated.

The preliminary results of these experiments revealed no case of a cytopathogenic effect after treatment of these cell cultures with blood from leukemic patients. Meanwhile, during cultivation of kidney cells obtained from 11 infected monkeys, in three cases signs of transformation of the cells were seen. The most characteristic results were obtained in a culture of the kidney of a green guenon, injected with filtered blood from a patient with chronic myeloid leukemia. From the first days of cultivation signs of transformation were observed in this culture, in the form of intensive acidification of the medium, rapid proliferation of the cells, and the appearance of colonies of atypical cells. Cytopgenetic analysis demonstrated changes in the karyotype of the cultures: on the ninth day of growth 48.5% of the cells were aneuploid, whereas in the kidney of the normal green guenon, cultivated for the same time and in the same medium, the proportion of aneuploid cells was 18.5%.

Analysis of these results shows that a filter-passing agent pathogenic for monkeys circulates in the blood of patients with various forms of leukemia. After monkeys were injected with the blood of leukemic patients (both untreated and filtered through a Seitz filter), they developed an acute disease, often fatal, characterized by the appearance of a hemorrhagic diaphesis, the development of proliferative lesions in the hemopoietic system, and the formation of foic of proliferation of reticular and young myeloid cells in the internal organ. The various species of macaques were found to be most sensitive to this agent. Green and red guenons were less susceptible, and baboons were almost completely resistant.

The filter-passing agent regularly caused hemagglutination of human erythrocytes blood group 0(I). Regardless of the severity of the disease, antibodies inhibiting hemagglutination appeared in the blood serum of the surviving animals. A similar reaction was observed with the blood serum of human leukemic patients taken during a remission.

A well marked morphological and karyological transformation was observed in cultures of the kidneys of certain monkeys killed at the height of the disease.

It may therefore be postulated that this filter-passing agent was a hemagglutinating virus, regularly circulating in the blood of patients with various forms of leukemia. The marked affinity of the agent for the reticular and hemopoientic tissues of the inoculated to the development of leukemia.

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