ONCOLOGY

ACTION OF BLOOD PLASMA AND BONE MARROW EXTRACTS FROM LEUKEMIA PATIENTS ON CULTURES OF LEUCOCYTES FROM NORMAL BLOOD

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Considering leukemia as a disease not only of the hemopoietic system but also of the entire organism, involving disturbances of metabolism and neuroregulation, we thought it of interest to find out whether there are any substances present in the blood and organs of leukemia patients which affect the morphology of the leucocytes of healthy individuals.

Norris and Majnarich [2] have shown that addition of normal blood serum to bone marrow cultures accelerates, and of serum from leukemia patients inhibits, proliferation of the myeloid cells. Plum [3] concluded that addition of blood serum from persons suffering from myeloid leukemia or lymphogranuloma to bone marrow cultures suppressed erythropoiesis. Aleksandrowicz and Miklaszewska [1] found that the blood plasma of myeloid leukemia patients (in particular, acute myeloid leukemia), contains substances which promote breakdown of leucocytes of peripheral blood.

In our study of the effect of substances present in the tissues of leukemia patients on leucocytes present in bone marrow cultures, we endeavored to compare the biological properties of these leucocytes with those of similar cells cultured under normal conditions, and to determine the extent and nature of the differences.

We here present the results of a study of the action in vitro of blood plasma, and of bone marrow extracts from patients suffering from various forms of leukemia, on the leucocytes of normal blood.

For the study of the effect of leukemic blood plasma, we performed 11 series of experiments, of which 4 were with plasma from acute myeloid leukemia cases, 4 from chronic myeloid leukemia, and 3 from chronic lymphadenoma patients. Each series involved 50-80 experimental and control cultures.

The experiments were performed in Carrel dishes. The solid phase was prepared from 1:1 mixtures of leukemic blood plasma and rabbit plasma; for the controls plasma was taken from healthy individuals, instead of from leukemia patients. The liquid phase in all systems was Group IV human blood serum diluted with 2-3 volumes of Tyrode solution.

We used leucocytes taken from the buffy coat of the blood of a donor, having a normal differential blood cell count. Fragments of buffy coat about 1.5-2 sq mm in area were transferred to Carrel dishes for culturing, and the cultures were observed over a period of 10-15 days, examining them once daily. Whole preparations were made, as well as smears, by the method of Timofeevsky.

We used methanol or Carnoy's solution for fixation, and stained with azure II-eosin.

Addition of blood plasma from leukemic patients to the nutrient medium had a clearly adverse effect on leucocytes in vitro. Degenerative changes were evident (pyknotic changes in the nucleus, vacuolization, etc.), and these affected not only the polymorphonuclear cells, which are usually thus affected in cultures, but also many lymphocytes and cells derived from them. In general, the survival time of the cells was much shorter than in the

control cultures. Retarded development of the cells was observed in some of the experiments, and polymorphism of the cells was less pronounced than in control cultures.

The most marked and uniform changes were seen with plasma from cases of acute myeloid leukemia; plasma from chronic forms of the disease and from lymphadenoma patients gave only a weak effect.

We next studied the effect of extracts of bone marrow of leukemia patients (it is known that bone marrow is the most seriously affected tissue in this disease).

Sternal marrow was taken within 5-7 hours of death of persons suffering from leukemia, weighed, ground with powdered glass in a porcelain cup, and shaken with 5-10 volumes of Ringer's solution. The suspension was then incubated for 2-3 hours at 37°, and centrifuged. The supernatant was filtered through a Chamberland candle, and collected in a glass ampoule, which was sealed and stored in a refrigerator. In the experiments, one part of extract was added to 9-19 parts of nutrient solution. In all, 14 series of experiments were performed (80-110 cultures to each series), as follows: 6 series with extracts of bone marrow from cases of acute, 5 of chronic, myeloid leukemia, and 3 of lymphadenoma.

The survival time of cells in cultures containing extracts from acute leukemia cases was much shorter than in the control cultures. Degenerative changes (spherocytosis, vacuolization of the cytoplasm, pyknotic nuclei, stripped nuclei, granular karyolysis) supervened much earlier, and in a higher proportion of the cells than in the control cultures. In some cultures were were no surviving cells by the 5th-7th day, and in all the cultures by the 10th day. Most of the control cultures were in good condition on the 12th-15th day of culture.

We also found that maturation of lymphocytes was retarded, as compared with controls. Polymorphism, which is characteristic of lymphocytes in tissue culture, was not uniformly found.

Similar effects were found with extracts of bone marrow from cases of chronic leukemia, but to a lesser degree. The degenerative changes were of the same nature. Together with shortened survival times of the cells, we observed in two cases the appearance of immature cells of the erythrocyte line (of the type of proerythroblasts); such cells were never encountered in the control cultures. We also observed a certain degree of stimulation of granulopoiesis in these experiments. Thus, in one experiment we found 1.2% of eosinophile myelocytes (control 0.2%), and lymphomyeloblast type cells 1.8% (control 0.8%), in a 24-hour culture. In a 3-day culture there were myelocytes 0.8% (control 0.2%), and lymphomyeloblasts 1.4% (control 0.6%). Active development of lymphocytes and macrophages was also observed.

In all 3 series of experiments on the effect of extracts of bone marrow from chronic lymphatic leukemia, we found intensification of degenerative changes of the cells, as compared with those of control cultures, to a lesser degree than with material from acute forms. There was a certain stimulation of development of cells of the lymphocyte series, in the direction prolymphocyte-lymphomyeloblast. Thus in one 24-hour culture we counted prolymphocytes 12.4%, and lymphomyeloblasts 4.8%, as compared with 7.2 and 2.8%, respectively, in the controls. After 2 days there were prolymphocytes 12% and lymphoblasts 10%; controls 9 and 5%, respectively. In the same experiment development of lymphocytes in the direction polyblast-macrophage was retarded. Thus in a 24-hour culture we found 8.8% of polyblasts, as compared with 17% in the control. After 2 days the values were 21 and 36%, and after 3 days 24 and 42%, respectively.

The experimental cultures contained a smaller variety of cell forms than did the control cultures.

It may be concluded from the above results that the blood plasma, and in particular the bone marrow, of leukemia patients contain substances which adversely affect the viability of leucocytes from healthy individuals in tissue culture. These substances cause intensification of degenerative processes taking place in leucocytes outside the organism, and retard their development. Normal blood leucocytes, when cultured in the presence of these substances, acquire certain of the properties of the leucocytes of leukemia patients, i.e. they exhibit more or less pronounced pathological changes. The most pronounced effects are given by plasma and bone marrow from cases of acute myeloid leukemia.

LITERATURE CITED

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