SYSTEMIC BLOOD DISEASES IN CC57W MICE

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In the course of 1971-1976 outbreaks of systemic blood diseases were observed frequently at the breeding station for CC57W mice, with low predisposition to cancer, at the "Rappolovo" nursery.

The object of this investigation was to study the character and causes of the blood lesions in these animals.

EXPERIMENTAL METHOD

Altogether 137 mice of both sexes and of the CC57W strain bred at the Rappolovo nursery were used. The animals were divided into two groups: Group 1 consisted of 50 intact mice; the 87 mice of group 2 received subcutaneous injections of 0.05 ml sunflower oil once every 10 days throughout life. The reason for these experiments with injection of sunflower oil was that this substance is widely used as a solvent or emulsifier in oncologic experiments.

The animals were kept under observation until natural death. Some mice with marked clinical and hematologic manifestations of leukemia were killed. Periodically hematologic investigations were carried out on samples of animals from both groups (determination of the hemogram: ESR, hemoglobin, cell count, color index, platelet count). All mice which died or were killed in a terminal state were investigated with fixation of the organs in neutral formalin followed by preparation of histological sections. If a systemic blood disease was suspected in mice, films of bone marrow were prepared to determine the myelogram, and affected organs were removed for study in the electron microscope.

Pieces of organs for electron microscopy were fixed in 2-2.5% glutaraldehyde solution in phosphate buffer (pH 7.4) at 0°C, then postfixed with 1% osmium tetroxide solution, stained with uranyl acetate, dehydrated with alcohol, and embedded in Epon. Sections were cut on the LKB 880LA Ultrotome, stained with lead citrate by Reynolds' method, and examined in the JEM-7A electron microscope under an accelerating voltage of 80 kV. Blood and bone marrow cells were stained by Pappenheim's method.

In some cases a cell homogenate from leukemic organs, extracted under sterile conditions, was injected subcutaneously into 2-month-old mice of the same strain. One of the transplanted strains was studied in 13 generations. Altogether 95 animals receiving leukemic material were examined.

EXPERIMENTAL RESULTS

According to data in the literature, leukemia was found in 0.4-0.8% of cases in CC57W mice in 1944-1969 [3, 4]. The results of observations made in 1971-1976, given in Table 1, showed that the character of the lesions to the blood system and also the frequency of diseases of this type in mice receiving sunflower oil did not differ significantly from those in intact animals. Among the diseases of the blood system discovered, the great majority were generalized forms of reticulosarcomatosis. This disease was manifested as a lesion of

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TABLE 1. Frequency of Onset and Characteristics of Tumors in CC57W Mice in 1971-1976

Indices and mouse tumors studied	Group of animals	
	1	2
Total number of mice	50	87
Time of appearance of first tumor, days	308	189
Number of mice surviving until appearance of first tumor	27	52
Number of mice with neoplasms	10 (37%)	24 (46,1%)
Systemic blood diseases:		
lymphatic leukemia		3 (5,8%)
generalized reticulosarcomatosis	9 (33,3%)	13 (25%)
lymphoblastoma		1 (1,9%)
thymoma		1 (1,9%)
Total	9 (33,3%)	18 (34,6%)
Adenomas of the lungs	—	4
Other tumors	2*	2

*Including one folliculoma of the ovary combined with generalized reticulosarcomatosis.

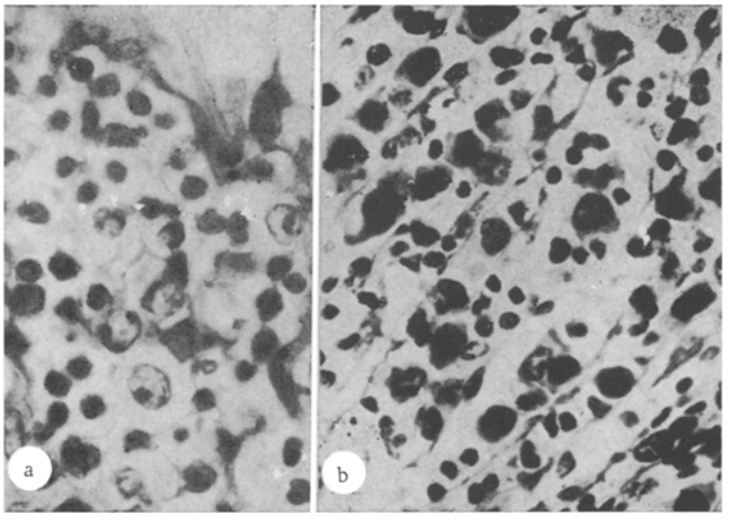

Fig. 1. Areas of peripheral zone of mesenteric lymph nodes of CC57W mice. a) Lymph node of healthy mouse aged 320 days; stromal cells and lymphocytes, 1200 ×; b) generalized reticulosarcomatosis (intact mouse, 336th day of observation in animal house of Institute); reticuloid tumor tissue, replacing normal lymph node cells, 900 ×.

the peripheral and visceral lymph nodes and spleen, in which massive foci of proliferation of reticular cells, often completely replacing the parenchyma of the organ, were discovered (Fig. 1). Areas of infiltration from reticular cells, often very large, were found in the liver, kidneys, and lungs. The bone marrow, however, was by no means always involved in the process and the myelogram was not significantly different from the normal state found in clinically healthy mice of the same strain and of the same age, and in agreement with data in the literature [5-8]. As regards the peripheral blood, characteristic changes usually were not observed for a long time. Not until 1-2 weeks before the animal's death did the number of reticular cells and, sometimes, of monocytes begin to rise. In the terminal period (1-2 days before death) the number of reticular cells and monocyte-histiocytes in the blood could amount to 20-40% when the total number of nucleated cells was 6000-20,000/mm^3 blood. In some cases, however, characteristic changes were not observed in the peripheral blood despite the presence of lesions of the lymph nodes, spleen, liver, and other internal organs.

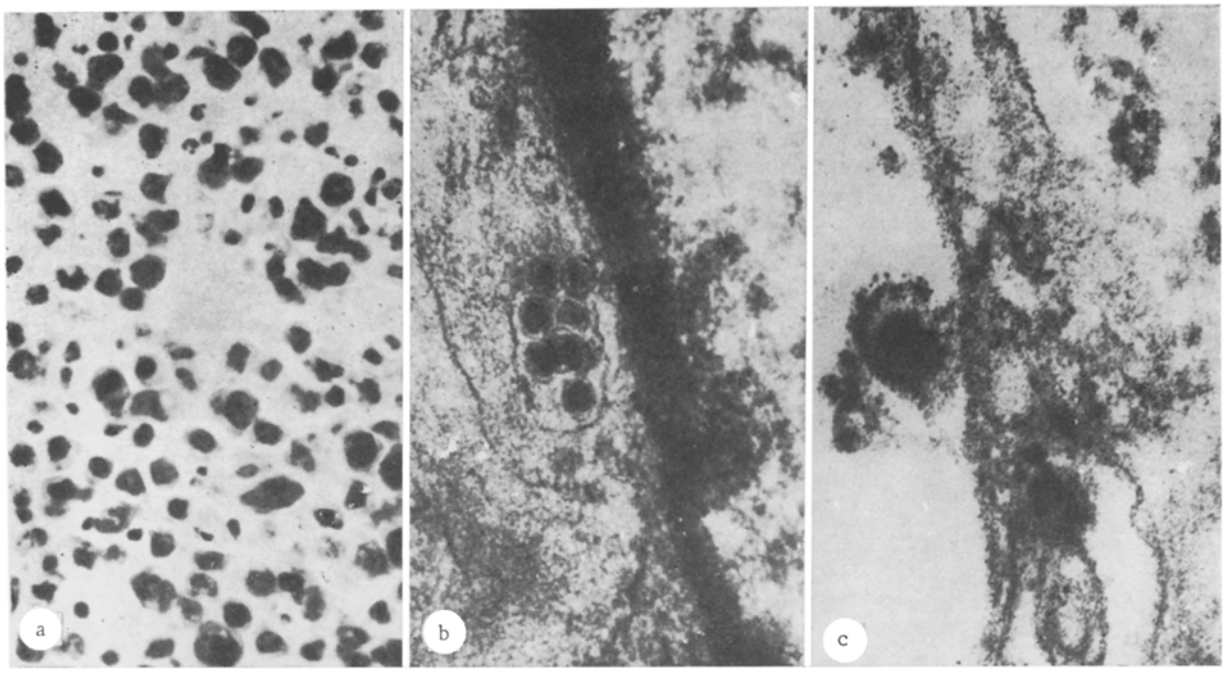

Fig. 2. Virus particles in cells of mouse lymph nodes. Generalized reticulosarcomatosis. a) Budding of virus on membrane of endoplasmic reticulum; b) virus particles in cytoplasm of cell; c) group of viruses in vacuole near nucleus; electron micrographs. Magnification: a) 112,000, b) 126,000, c) 52,000 ×.

The lymphatic leukemias also assumed a chronic, aleukemic or subleukemic form (about 40,000 leukocytes/mm³ blood, predominantly lymphocytes — up to 84% in the blood and up to 61% in the bone marrow).

On electron microscopic examination type C virus particles were found intracellularly or extracellularly in the reticular cells of organs affected with generalized reticulosarcomatosis, and in particular, budding viruses were seen on the cell membrane (Fig. 2). These observations indicate the very probable virus nature of generalized reticulosarcomatosis of mice.

Virus particles were found both in the original sick animals and also in mice into which a cell homogenate from the leukemic organs was transplanted. A study of the strain thus obtained showed that the morphology of the tumor cells remained unchanged. The disease resembled generalized reticulosarcomatosis in character. No tumors developed at the site of injection of the homogenate in the first 10 generations. The incubation period shortened in the course of the transplantations from 3 months to 4 weeks in the course of the first three generations. The take rate was 100%.

The clinical and morbid anatomical picture of the reticulosarcomatosis found in the animals resembles the virus disease described by Bergol'ts [2] in CC57BR mice — very close relatives of the animals studied in the present investigation. In mice infected with other known leukemic viruses the morphology of the process is somewhat different. Friend virus, for instance, usually causes not only reticuloblastosis, but also erythroblastosis, sometimes with hypervolemic polycythemia, whereas Stansly virus causes a disease resembling lymphogranulomatosis [1, 2]. Nothing of this nature was observed in the cases now described. To determine the precise nature and characteristics of the etiologic factor causing reticulosarcomatosis in CC57W mice, further investigations, especially immunologic, are necessary. Attention must also be directed to the known periodicity of the reticuloblastosis we have described. Whereas in 1971-1976 a very high incidence of the disease among mice was recorded, during 1977-1978 the number of spontaneous cases of this type in CC57W mice was much smaller.

This investigation thus has shown that in the course of 1971-1976 systemic blood diseases of the generalized reticulosarcomatosis (up to 33%) and lymphatic leukemia (up to 6%) types were observed in a high percentage of cases in mice of the CC57W strain, with low pre-

disposition to cancer. Type C particles were found in the cells of affected organs. These diseases affect mainly the lymphoid tissue of animals and they run a chronic course, in aleukemic and subleukemic forms.

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PRODUCTION OF A FACTOR INHIBITING MACROPHAGE MIGRATION AND GROWTH OF MELANOMA B16 UNDER THE INFLUENCE OF BCG

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Growth of malignant tumors has now been shown to be accompanied by immunologic changes and a disturbance of individual stages of immunopoiesis [5, 15]. BCG vaccine is widely used in clinical and experimental medicine as a nonspecific immunologic stimulator [8, 9, 12]. BCG is known to activate macrophage function and to stimulate the production of mediators of cellular immunity by lymphocytes [10]. Meanwhile the character of the effect of BCG on growth of melanoma B16 has not yet been explained. Contradictory data are to be found in the literature on this subject [4, 7, 11, 14].

The object of this investigation was to study the action of BCG on growth of melanoma B16 and on the production of macrophage migration inhibiting factor (MMIF) in C57BL/6 mice.

EXPERIMENTAL METHOD

Cells of a melanoma B16 were transplanted subcutaneously into C67BL/6 mice in a dose of 2×10^6. Two series of experiments were then undertaken.

In series I BCG was injected subcutaneously or intraperitoneally into the animals of one group in a dose of 1 mg simultaneously with melanoma cells. The mice of the other group received BCG subcutaneously in the same dose (on the side opposite to that of injection of the tumor) or directly into the tumor 14 days after transplantation of the melanoma cells. Growth of the tumor was determined 12 days after BCG immunization by counting the number of cells, and production of MMIF by the spleen cells was estimated in the direct capillary tube test [2] with modifications [1]. Phytohemagglutinin (PHA) and tuberculin (TB), in nontoxic doses for cells (2 and 100 μg/ml respectively) were used as antigen. The quantitative index of MMIF production was the migration index (MI), determined by the equation:

$$MI = \frac{\text{area of migration with antigen}}{\text{area of migration without antigen}} \cdot 100\%$$

In the experiments of series II, simultaneously with injection of the melanoma cells, 2×10^7 spleen cells were transplanted intravenously from syngeneic donors. The donors of

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