INDUCTION OF TRANSMISSIBLE LEUKEMIA-LIKE SYNDROMES IN MICE BY INJECTION OF ANTIGENS AND IMMUNOSTIMULANTS

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UDC 616.155.392-039-092.9

Splenomegaly characterized by myeloid metaplasia was induced in BALB/c mice by injection of antigens and immunostimulants. By contrast with the leukemoid reaction, this syndrome can be transmitted by both plasma and spleen cells. Small virus-like particles (30-50 nm) were found in the plasma and RNA-containing type C viruses in the spleen cells.

KEY WORDS: *splenomegaly; myeloproliferation; retraviruses; small virus-like particles.*

The connection between the immune system and oncogenesis and ontogeny is diverse and has received little study. The possible role of viruses as functional factors in these processes has received even less study.

Repeated injection of different antigens [8] and also of antigens with different immunodepressants [6] and allogeneic lymphocytes [5] is known to cause reticular and lymphoid tumors in mice. Injection of adjuvant [7] or mineral oil [9] induces plasmacytomas.

In the chronic graft versus host reaction induced by injection of parental cells into F_1 hybrids, RNA-containing viruses of type C, which are oncogenic, are activated [2, 5]. Activation of viruses of the papova group has been described in recipients of kidneys [4] and in patients with the Wiskott–Aldrich syndrome [10] and with leukoencephalopathy [12]. Virus of the Mason–Pfizer type also has been activated in kidney recipients [11].

In this investigation induction of a leukemia-like syndrome, characterized by splenomegaly and myeloid metaplasia, was induced by injecting antigens and immunostimulants into mice. In this syndrome small virus-like particles are found in the plasma and RNA-containing type C viruses in the spleen cells.

EXPERIMENTAL METHOD

BALB/c mice (18-20) were obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR. Virus-free plasma from mice with Rauscher leukemia was fractionated on a column with Sephadex G-150 and the second peak fractions (LP1/2) collected. By mixing LP1/2 with bovine gamma-globulins (BGG) in the ratio of 1:10 and by "cross-linking" them with glutaraldehyde, an LP1/2–BGG complex was obtained. Normal plasma was fractionated (NP1/2) and "cross-linked" in the same way (NP1/2–BGG). Mice of the various groups were injected with: 1) 0.1 ml Freund's complete adjuvant (FCA, Difco) and 5.5 mg of LP1/2–BGG; 2) 0.1 ml FCA and 5.5 mg of NP1/2–BGG; 3) 0.1 ml FCA; 4) 1 mg dextran sulfate 5000 (DS, Fevvak) and 5.5 mg of LP1/2; 5) 1 mg DS and 5.5 mg NP1/2; 6) 1 mg DS and 11 mg ovalbumin; 7) 1 mg DS; 8) control.

Antigens were mixed with FCA and injected every 2 weeks into 10 points. When DS was used, the antigens were injected into 10 points 30 min after intraperitoneal injection of DS.

To sediment the virus-like particles, clarified plasma of mice with the leukemia-like syndrome was centrifuged in the SW39 rotor of a Spinco centrifuge for 180 min at 36,000 rpm.

Laboratory of Immunochemistry and Diagnosis of Tumors, N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 9, pp. 344-346, September, 1977. Original article submitted December 23, 1976.

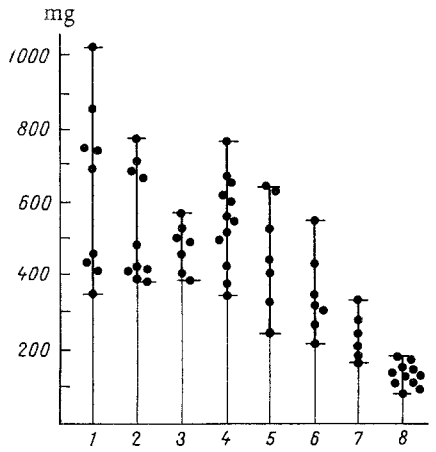

Fig. 1. Induction of leukemia-like syndrome in BALB/c mice. Abscissa, groups of animals; ordinate, weight of spleen (in mg).

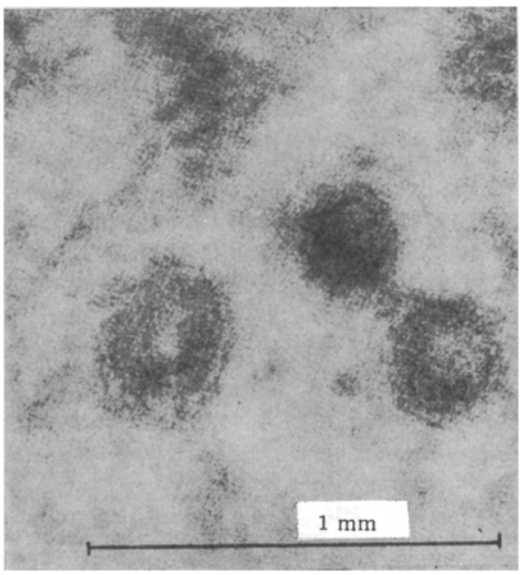

Fig. 2. Virus-like particles isolated from plasma of mice with induced leukemia-like syndrome.

The residue was injected intraperitoneally into mice of one group and the supernatant into mice of another group.

Organs for histological examination were fixed with formalin or Carnoy's solution and paraffin sections were stained by Giemsa's method or with hematoxylin–eosin. Films and squash preparations were stained with azure–eosin or by Pappenheim's method. Shabadash's reaction was carried out for glycogen, the Graham–Knoll reaction for peroxidase, and Lison's reaction for lipids with Sudan B.

Blood was taken from the retroorbital sinus and cells were counted in a Goryaev's chamber.

An ultraprecipitate was separated for electron microscopy from the plasma of mice with the leukemia-like syndrome in a sucrose gradient and fractions with density of 1.12–1.16 g/cm^2 were sedimented. Treatment with OsO_4 and uranyl acetate and embedded in Epon-812 were carried out actually in the centrifuge tubes. Sections were cut on the LKB III Ultrotome, stained with uranyl acetate and lead, and examined in the Tesla 513 and HS-11 microscopes.

EXPERIMENTAL RESULTS

After the fifth injection a well-marked splenomegaly was observed in most of the mice in groups 1-6 (Fig. 1). The spleens showed marked myeloid metaplasia, with an increase in the number of megakaryocytes and erythroblasts. Myeloid cells of all stages of maturity lay along the trabeculae. The total number of perosidase-positive cells was 20-50% (normally 11-13%).

On the 11th-16th day after injection of cells or of plasma obtained from mice with the leukemia-like syndrome and filtered through a Millipore filter (0.45 μ) beforehand into adult mice, the animals developed an identical syndrome. The structure of the spleens, the weight of which reached 500-1500 mg, was obliterated and was characterized by numerous blast cells.

The number of peroxidase-positive and Sudanophilic cells was increased, whereas the number of PAS-positive cells was normal. Besides myeloid cells in all stages of maturity, foci of erythropoiesis, an enormous number of megakaryocytes, and activated follicles were observed.

Perivascular reactive foci of infiltration of myeloid cells, lymphocytes, and histiocytes were present in the liver and other organs, and the circulation was disturbed.

On the 14th day after infection the blood showed anemia (a reduction to 4 million cells/mm^3 from a normal level of 10 million) and leukopenia (down to 1700 cells/mm^3 from a normal figure of 8500), but by the 6th week the blood picture was restored to normal, and thereafter slight leukocytosis was observed.

The syndrome observed followed a chronic course and the mice survived at least 3 months with splenomegaly. The problem of whether this syndrome can change into true myeloid leukemia requires further investigation. However, in some cases 4 months after infection death of the mice was observed with leukocytosis (60,000 leukocytes/mm^3, mainly myeloid and blast cells) and with leukemic infiltration of all organs by myeloid cells.

Injection of the ultraprecipitate obtained from the plasma of mice with the leukemia-like syndrome led to splenomegaly on the 14th day, but the supernatant was inactive, indicating the corpuscular nature of the agent. When this precipitate was fractionated in a sucrose gradient, a maximum of absorbance was obtained at 280 nm, corresponding to a density of 1.14 g/cm^3, and absent when the analogous precipitate from normal plasma was fractionated.

The fractions of this peak were biologically active and in the electron microscope they were found to contain virus-like particles 30-50 nm in diameter (Fig. 2) with two types of electron-dense nucleoids: ring-shaped and circular, filling the whole space as far as the membrane. They were similar to the virus-like particles found in women with breast cancer [3] and to the minioncornaviruses of types C' and A' described by Bykovskii [1]. However, there is no evidence that they belong to the group of retraviruses, for preliminary tests for revertase and gs-1 (group-specific antigen of mouse leukemia viruses) were negative. No small particles could be found in sections through the spleen, but on the other hand, type C RNA-containing viruses were observed there.

The possible interaction between these two types of particles is of particular interest.

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