It can be concluded from these results that in the modification [5] of Jerne's method arbitrary quantities of tissue sample and of isotonic fluid were used, and this led to the appearance of an unverifiable quantity of inorganic ions; "immune" plaques are formed when this modification is used under conditions of high osmotic pressure; investigation of such plaques in the phase-contrast microscope showed the absence of hemolysed erythrocytes, possible evidence of the absence of autoantibodies; the author of the modification in [6] showed that when hypertonic solutions are used false plaques are obtained, i.e., plaques of nonimmune nature, In my own opinion, the description is evidence that false plaques are plaques formed under conditions of increased osmotic pressure and they differ from "immune" plaques in their mechanism of formation.

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EXPRESSION OF VIRUS-SPECIFIC RNA IN CELLS OF MICE INFECTED WITH MAZURENKO AND RAUSCHER VIRUSES

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KEY WORDS: leukemia; oncornaviruses; RNA; expression.

In 1957 a virus which subsequently proved to be highly leukemogenic for mice not only of the CC57Br line, but also for CC57W, C57BL, and C3H mice and also for noninbred rats, was isolated by N. P. Mazurenko from organs of CC57Br mice developing leukemia after receiving an injection of vaccinia virus.

Mazurenko virus has the morphology of particles of type C oncornaviruses [6]. The viriens are composed of 60-70S RNA and RNA-dependent DNA-polymerase, and the density of the virus particles in a sucrose gradient is 1.16 g/ml [3]. In its immunologic properties the virus belongs to the Friend, Moloney, Rauscher (FMR) group of viruses [2].

Despite the fact that Mazurenko virus was discovered more than 20 years ago, its molecular-biological properties have so far received little study. Yet some of the properties of this virus, distinguishing it from the mouse leukemia viruses known today, namely its extremely high oncogenicity, and the method of activation of the virus – make the study of its molecular-biological properties and their comparison with those of known mouse leukemia viruses most interesting.

The object of this investigation was to study expression of RNA of Mazurenko and Rauscher (RLV) viruses in tissues of various organs of CC57Br and BALB/c mice infected with these viruses.

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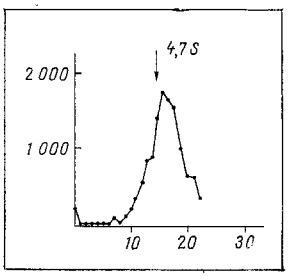

Fig. 1. Distribution of ^{3}H-DNA transcript of RLV in a sucrose gradient. ^{3}H-DNA-transcript of RLV was centrifuged in an alkaline sucrose gradient (5-20%) at 34,000 rpm for 16 h (VAC 602 centrifuge, 3 × 5 rotor). The sucrose solutions included 0.3 M NaOH, 0.7 M NaCl, and 0.01 M EDTA. Arrow indicates marker, for which bovine serum albumin with sedimentation coefficient 4.7S was used. The marker was centrifuged in a parallel gradient. Ordinate, cpm; abscissa, nos. of fractions.

EXPERIMENTAL METHOD

1. Sources of material. Mice of the BALB/c line were infected with RLV (which was generously provided by E. N. Rozinova, Oncologic Scientific Center, Academy of Medical Sciences of the USSR), by intraperitoneal injection of 0.5 ml of a 50% suspension of minced spleens of mice of the same line with signs of leukemia induced by RLV.

CC57Br mice were infected with Mazurenko virus, generously provided by Mazurenko himself, by intraperitoneal injection of 0.3 ml of a 25% suspension of minced organs of mice of the same line with leukemia induced by Mazurenko virus. The development of leukemia in the mice was observed on the 7th-9th day.

2. Isolation of RNA. Cytoplasmic RNA was isolated from the mouse organs by the standard phenol method at pH 6.0, with water-saturated phenol [1] and additional treatment with pronase (from Calbiochem), which was subjected to autodigestion at 37°C for 2 h. To remove low-molecular-weight RNA from the preparations, chromatography on a column with cellulose LK (from Chemapol) was used [12].

3. Synthesis and purification of ^{3}H-DNA-transcript. RLV obtained from the National Cancer Institute of the USA under the terms of the Soviet–American collaboration in the field of oncology, was used. The virus was purified by means of two zonal gradients.

The ^{3}H-DNA-transcript of RLV was synthesized in a modified reaction mixture described previously for the synthesis of ^{3}H-DNA-transcripts of mouse retraviruses [10]. The reaction mixture included the following components in final concentrations of: 0.05 M Tris-HCl buffer, pH 7.8; 0.06 M KCl; 0.02 M dithiothreitol; 0.00125 M $MnCl_2$; 0.3% Triton X-100; 1 mg/ml actinomycin D; 0.2 μCi/ml ^{3}H-TTP with specific activity 46 Ci/mmole (from Radiochemical Centre, Amersham, England), and unlabeled triphosphates dATP, dGTP, and dCTP, each in a concentration of 0.2 mg/ml. The reaction mixture was incubated at 37°C for 18 h and the DNA-transcript was extracted by the dodecylsulfate-phenol pronase method. The specific activity of the labeled DNA-transcript was 1.4×10^6 cpm/μg. The ^{3}H-DNA-transcript was heated to 96°C for 10 min and self-annealed at 37°C for 20 h, after which it was fractionated on hydroxyapatite to remove the double-helical product. Single-helical ^{3}H-DNA-transcript of RLV, eluted with 0.14 M phosphate buffer, pH 7.0, and 60°C, was used for hybridization.

4. RNA-DNA-hybridization. The reaction mixture for hybridization included: 25 μl RNA in 5 × SSC buffer (1 × SSC buffer: 0.15 M NaCl; 0.015 M Na citrate, pH 7.4), 15 μl ^{3}H-DNA-transcript of RLV (2000 cpm per sample); 0.05 μl 50% Na dodecylsulfate; 10 μl 100% formamide (from Merck). The total volume of the mixture was 50 μl. The RNA concentration in the sample varied from 0.6 μg/ml to 5 mg/ml. The hybridization mixture was incubated at 65°C. Values of C_rt (concentration of nucleotides in moles/sec/liter) was calculated by the standard equation [7].

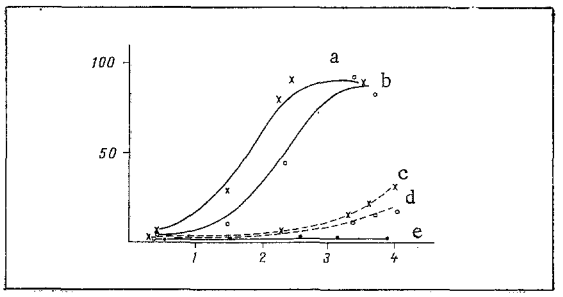

Fig. 2. Hybridization of ^{3}H-DNA-transcript of RLV with RNA from BALB/c mice, healthy and infected with RLV: a) spleen of infected mice; b) liver of infected mice; c) spleen of healthy mice; d) liver of healthy mice; e) yeast tRNA. Here and in Fig. 3: ordinate, resistance to nuclease S_1, %; abscissa, log C_rt.

5. Analysis of the hybrids. The RNA-DNA hybrids were analyzed by means of nuclease S_1, which specifically hydrolyzes single-stranded regions of nucleic acids. The enzyme was isolated from α-amylase (from Sigma) by the method in [14]. After incubation the samples with hybridization mixture were divided in halves and half of each sample was treated with nuclease S_1 by the method described previously [14].

EXPERIMENTAL RESULTS

1. Analysis of the ^{3}H-DNA-transcript of RLV in a Sucrose Gradient. On centrifugation of the ^{3}H-DNA-transcript of RLV in an alkaline 5-20% sucrose gradient the material was distributed heterogeneously with a principal peak at 5-7S (Fig. 1). Values obtained were characteristic for ^{3}H-DNA-transcripts of oncornaviruses synthesized in the endogenous RNA-dependent DNA polymerase reaction [11].

2. Hybridization of the ^{3}H-DNA-Transcript of RLV with RNA from Spleen and Liver Cells of BALB/c Mice Infected with RLV and Healthy Mice. Cellular cytoplasmic RNA was hybridized with the ^{3}H-DNA-transcript of RLV in an excess of RNA. Different concentrations of RNA from the same organ were used for hybridization. Hybridization was assessed by resistance of ^{3}H-DNA-transcript of RLV to nuclease S_1. The hybridization curves given in this paper show dependence of resistance of the labeled DNA-transcript to nuclease S_1 on log C_rt.

The results of hybridization of the ^{3}H-DNA-transcript of RLV with cytoplasmic RNA from spleen and liver cells of BALB/c mice infected with RLV are shown in Fig. 2. The curve of hybridization with liver RNA came out on a plateau at a higher value of C_rt (10^3) than the curve of hybridization with spleen RNA, which came out on a plateau at $C_rt = 2 \times 10^2$, indicating the presence of a larger quantity of virus-specific RNA in spleen cells of BALB/c mice infected with RLV than in the liver cells of those mice. The level of hybridization of the ^{3}H-DNA-transcript of RLV obtained with RNA from the liver and spleen of BALB/c mice infected with RLV was identical and reached 90% (Fig. 2), evidence of the virtually complete transcription of the virus genome in these cells.

The level of hybridization of the ^{3}H-DNA-transcript of RLV with RNA from the spleen and liver of healthy BALB/c mice reached 35 and 20%, respectively, at $C_rt = 10^4$, and the hybridization curves did not come out on a plateau even at relatively high values of C_rt (Fig. 2). These curves differed in shape from the hybridization curves of the ^{3}H-DNA-transcript of RLV with RNA from the organs of mice infected with RLV: Whereas the first curves were concave the second were convex (Fig. 2). The results are evidence of expression of some virus sequences, which was much lower than the expression of virus-specific RNA in muscle cells infected with RLV, in good agreement with observations by other workers published previously [9].

The ^{3}H-DNA-transcript of RLV obtained for practical purposes did not hybridize with yeast tRNA (Fig. 2).

3. Hybridization of the ^{3}H-DNA-Transcript of RLV with RNA from the Organs of CC57Br Mice, Healthy and Infected with Mazurenko Virus. Because of various difficulties – the lack of an adequate cell system for reproduction of Mazurenko virus, the impossibility of synthesizing a ^{3}H-DNA-transcript of the virus isolated

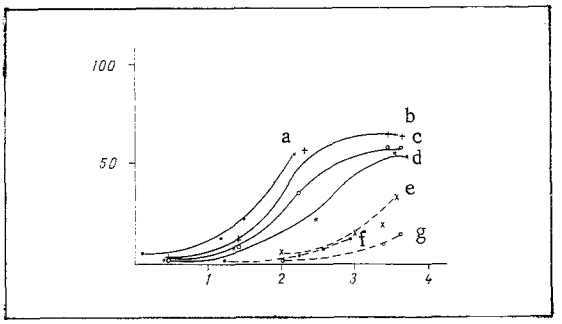

Fig. 3. Hybridization of ^{3}H-DNA-transcript of RLV with RNA from organs of CC57Br mice, healthy and infected with Mazurenko virus: a) thymus of infected mice; b) lymph nodes of infected mice; c) spleen of infected mice; d) liver of infected mice; e) thymus of healthy mice; f) liver of healthy mice; g) spleen of healthy mice.

from mouse plasma because of the presence of nucleases in such virus preparations, degrading DNA-transcript, it was impossible to obtain a ^{3}H-DNA-transcript of the Mazurenko virus. Only a DNA-transcript of RLV was therefore used. This experimental approach was used by certain workers to analyze the expression of virus-specific information in other experimental models of mouse virus leukemias [8].

The hybridization experiments gave the following results: The ^{3}H-DNA-transcript of RLV hybridized to the extent of 53% with RNA from the thymus glands of CC57Br mice infected with Mazurenko virus at $C_rt = 2 \times 10^2$ but, unfortunately, the hybridization curve with this RNA could not be brought out on a plateau because the necessary quantity of material was not available (Fig. 3). The level of hybridization with RNA from the lymph nodes of these same mice reached 60%, with RNA from the spleens 56%, and with RNA from the liver 52%; the hybridization curves came out on a plateau at $C_rt = 10^3$, 2.4×10^3, and 3.6×10^3, respectively (Fig. 3). The character of the hybridization curves of the ^{3}H-DNA-transcript of RLV with RNA from the thymus glands, lymph nodes, spleens, and liver of CC57Br mice infected with Mazurenko virus, and also the C_rt values at which the curves came out on a plateau (Fig. 3) are evidence that cells of different organs, just as in the case of Rauscher leukemia, contain different quantities of virus-specific RNA. The smallest quantity of virus-specific RNA was found in the liver, approximately twice as much in the spleens, seven times more in the lymph nodes, and more still in the thymus.

The results of hybridization with RNA from organs of uninfected CC57Br mice agreed with the results of hybridization with RNA from the organs of uninfected BALB/c mice (see above).

Several conclusions concerning the problem of expression of RNA specific for leukemia viruses in different tissues of infected mice can be drawn from these results.

In the first place, differences can be clearly traced in the quantities of virus-specific RNA in the different organs. For instance, for Rauscher virus, the largest quantity of RNA was found in the spleens, and for Mazurenko virus, in the thymus and lymph nodes. Since, as we know, the spleen of BALB/c mice is the target organ for the action of Rauscher virus [4], it can be suggested that thymus of CC57Br mice also is the target organ for Mazurenko virus; this is in agreement with results obtained previously in a study of the biological properties of this virus [4, 5].

Comparative quantitative analysis of virus-specific RNA in the spleen and liver of the two lines of mice (Figs. 2 and 3) suggests that, first, in leukemia induced by RLV these organs contain 2-4 times more virus RNA than in leukemia caused by Mazurenko virus and, second, certain sequences specific for Rauscher virus are absent in the organs of mice infected with Mazurenko virus (the difference in hybridization levels reaches 40%). What may the nature of the common sequences expressed in the two forms of leukemia be? The possibility cannot be ruled out that Mazurenko virus, like Rauscher and Friend viruses, is a heterogeneous population of viruses, containing defective virus which gives rise to the characteristic pathology in mice, and a "helper" virus [11, 13, 15]. The "helper" virus can reproduce with equal efficiency in different organs with the ^{3}H-DNA-transcript of RLV. However, the nature and amount of information specific for Mazurenko virus

alone, and the degree to which this information is linked with the biological features of this virus, still remain unexplained.

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ACTIVATION OF MACROPHAGES BY BLASTOLYSIN

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KEY WORDS: blastolysin; macrophages; cytotoxicity; phagocytosis; adjuvant.

In recent years adjuvants, particularly BCG, Corynebacterium parvum, and lipopolysaccharides, have begun to be used for the treatment of certain tumors. It is suggested that macrophages, activated by these adjuvants, either become capable of causing lysis of tumor cells or they begin to secrete mediators which stimulate proliferation of precursors of cytotoxic T-cells [2, 3].

It was shown previously that blastolysin, the main components of which are glycopeptide fragments of the cell wall of Lactobacillus bulgaricus, exhibits antitumor activity against various transplantable and spontaneous tumors of animals [1].

The object of this investigation was to determine whether blastolysin possesses adjuvant activity and whether it can activate cells of the mononuclear phagocytic system and, in particular, macrophages. For this purpose the effect of blastolysin on antibody production, on the ability of macrophages to phagocytose various antigens, and on its ability to exert a cytotoxic action on syngeneic target cells with disturbed growth parameters was studied.

EXPERIMENTAL METHOD

Experiments were carried out on BALB/c and C57BL/6 mice.

Blastolysin was used in different doses: 1 mg per mouse (a dose causing up to 76% inhibition of growth of sarcoma S-180 and completely curing 30-40% of mice), and 4 and 20 mg per mouse. Sheep's red blood cells (SRBC) in a dose of 5×10^6 were used as the antigen.

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