PHYSICOCHEMICAL AND IMMUNOLOGIC PROPERTIES OF TISSUE DNA FROM MICE WITH ONCORNAVIRUS-INDUCED LEUKEMIA

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Tumor transformation is known to lead to the appearance of tumor antigens in cancer cells, whatever the cause of the malignant degeneration. The process of malignant transformation is accompanied by profound disturbances of nuclear metabolism, which may lead to structural changes in DNA molecules.

According to data in the Soviet and Western literature, changes in the content of certain nucleotide blocks and methylated bases, and also the presence of nucleotide sequences not found in DNA of normal tissues, have been found in DNA from leukemic tissue [1, 2, 4, 7, 10].

Previous publications gave data on the immunologic specificity of DNA preparations from human and bovine leukemic tissues although the etiology of these diseases is not known [5, 9]. Accordingly it is interesting to study the immunologic specificity of DNA preparations from leukemic tissues of animals with virus-induced leukemia.

The object of the present investigation was to compare the physicochemical and immunologic properties of DNA preparations isolated from the tissues of mice with leukemia induced by Friend's and Rauscher's oncornaviruses.

EXPERIMENTAL METHOD

DNA preparations were isolated from the spleens of leukemic and normal mice by the method described in [3, 5], and subsequently repeatedly deproteinized with a mixture of chloroform and butyl alcohol. To free the DNA preparation as far as possible from protein and RNA impurities, the preparations were subjected to additional treatment with pronase and RNase. Altogether 3 DNA preparations were obtained from leukemic tissue and three from tissues of healthy mice. As physicochemical tests for comparative analysis, indices of nativeness were used, namely ε (p) DNA and its hyperchromism. The degree of polymerization of the DNA molecule was judged from the molecular weight measured by a viscosimetric method in a low-gradient viscometer at 25°C. All preparations of DNA used were highly polymerized (mol. wt. from 9,000,000 to 12,000,000) and in the native state: ε(p) from 5600 to 6400, hyperchromic effect 34-38%.

Comparative analysis of the physicochemical properties of DNA preparations from leukemic and normal tissues did not differ significantly with regard to parameters of nativeness of the DNA molecule or molecular weight.

For comparative immunologic analysis of DNA preparations from leukemic and normal mouse tissues, the quantitative complement fixation test (CFT) at 50% hemolysis was used, followed by exhaustion of the antisera by corresponding DNA preparations. Anti-DNA sera were obtained by prolonged and repeated immunization of rabbits with native DNA preparations [6].

Native and denatured DNA in a concentration of 0.25 mg/ml were used as test antigen.

EXPERIMENTAL RESULTS

The results are given in Table 1.

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TABLE 1. Interaction between Antisera against DNA from Normal and Leukemic Tissues and DNA Preparations in CFT at 50% Hemolysis

Antisera	Number of serum	Native DNA		Denatured DNA	
		normal	leukemia	normal	leukemia
Against DNA from leukemic tissues	14	1 : 18±2,5 $n=14$	1 : 65±8,5 $n=14$	1 : 116±8,7 $n=14$	1 : 560±35,8 $n=14$
		$P<0,001$		$P<0,001$	
Against DNA from normal tissues	12	1 : 97±18,6 $n=12$	1 : 34±5,8 $n=12$	1 : 480±53,1 $n=12$	1 : 164±22,7 $n=12$
		$P<0,001$		$P<0,001$	

The serologic tests showed that immunization of rabbits with native polymerized DNA preparations from normal and leukemic mouse tissues leads to induction of complement-fixing antibodies against DNA. To establish specificity of the antibodies for the DNA molecule, thermal denaturation was used [8] and the DNA preparations were treated with DNase.

As a result of denaturation a marked increase was observed in the serologic activity of the DNA preparations, whereas treating them with DNase led to disappearance of serologic activity. These results indicate that the antisera contained specific antibodies against the DNA molecule.

Analysis of the results in Table 1 shows that preparations of native leukemic mouse DNA exhibit immunologic specificity. Denatured DNA had higher serologic activity than native. Denaturation of leukemic DNA did not affect its immunologic specificity. The results in Table 1 show also that leukemic DNA, regardless of its conformational state, reacts less actively with antisera against normal DNA than DNA from normal tissues. Accordingly, the antisera against both types of DNA were exhausted with DNA preparations. Denatured DNA from normal and leukemic tissues, adsorbed on membrane filters, and also on formalinized sheep's erythrocytes, was used for exhaustion.

On the one hand, antigenic determinants not found in homologous normal DNA were found in leukemic DNA, and on the other hand, determinants present in DNA from normal tissue were lost.

The results thus showed the presence of immunologic specificity in DNA preparations isolated from the spleens of mice with leukemias induced by Friend's and Rauscher's oncornaviruses.

It must be emphasized that immunologic specificity was established not only in native (double helical) preparations of leukemic DNA, but also in DNA preparations with modified secondary structure. The specificity of leukemic DNA is evidently determined by changes in its primary structure, for this specificity is preserved in denatured DNA from leukemic tissues.

It follows from the results that immunologic differences are found in the DNA molecule in malignant neoplasms by comparison with DNA of normal tissues. Disturbances arising in the structure of DNA of tissues which have undergone malignant transformation lead to complex changes in the composition of the antigenic determinants; both the appearance of new and the disappearance of antigenic determinants present under normal conditions are observed in this case.

The appearance of new antigenic determinants, determining the specificity of leukemic DNA, can evidently be explained either by activation or mutation of genes or by the introduction of new genetic information as a result of integration of the virus genome with the cell DNA.

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ANALOGS OF HUMAN EMBRYONIC PREALBUMIN-2 IN ANIMALS

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The presence of immunochemical analogs of human embryonic proteins in animals is of great importance because their discovery has opened the way to the experimental study of these proteins.

The object of this investigation was to make an immunochemical study of analogs of embryonic prealbumin-2 (EPA-2) in animals. EPA-2 is a protein found in the blood serum and amniotic fluid of human fetuses. This protein has not been found in the blood serum of blood donors or pregnant women [1].

EXPERIMENTAL METHOD

Antisera against EPA-2 were obtained in rabbits, using the glycoprotein fraction of human amniotic fluid for immunization [1]. Immunochemical identification of EPA-2 analogs was carried out with the aid of a standard test system for this protein by the method of Khramkova and Abelev [5].

The cellular localization of EPA-2 in tissues of calf fetuses and adult animals was studied by the indirect immunofluorescence analysis method. Tissue sections 4-8 μ thick were fixed with alcohol and acetic acid [7] and embedded in paraffin wax [8]. The sections were labeled by means of antibodies against EPA-2 obtained with the aid of an immunosorbent. EPA-2 immobilized on ACA-34 ultrogel by means of glutaraldehyde [6] was used as the immunosorbent. The technique of immunofluorescence analysis was described by the writers previously [2]. To study analogs, amniotic fluid, tissue, and blood serum from fetuses in the first half of embryonic development, and tissue and blood serum from adult animals were chosen. The tissue extracts were prepared in Tris-glycine buffer, pH 8.3, with the addition of detergents [4].

EXPERIMENTAL RESULTS

Immunochemical analysis of EPA-2 showed the presence of analogs of this protein in the blood serum and amniotic fluid of calf, pig, and sheep fetuses (Table 1). EPA-2 was not found in the blood serum of adult animals. Analogs of EPA-2 in animals gave a reaction of complete immunochemical identity with human EPA-2 (Fig. 1) and they also had similar physicochemical properties and electrophoretic mobility to those of prealbumin (Fig. 2).

The results of the study of EPA-2 in biological fluids, tissue extracts, and tissue sections from various organs of calf fetuses and cows are given in Table 2. The highest content of EPA-2 in animals, just as in man, was found to be in the amniotic fluid of the fetuses. The EPA-2 level in tissue extracts of fetal organs varied from 0.5 μg/ml in extracts of the spleen, liver, lung, and brain to 16-32 μg/ml in extracts of skin and of the umbilical cord.

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