[11], the participation of AA metabolites in the pathogenesis of FMF becomes more evident still.

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DETECTION OF GLUCOCORTICOID-SENSITIVE DNA SEQUENCES IN AKR MOUSE THYMUS AND THEIR INACTIVATION IN THYMOMA

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Glucocorticoids are known to affect differentiation and functional activity of cell populations of the immune system. The discovery of glucocorticoid receptor complexes in nuclei of thymocytes and spleen cells [15] is evidence that glucocorticoids act on lymphoid cells by a mechanism of genetic induction: binding of glucocorticoid receptors complexes with hormone-sensitive regions of chromatin induces synthesis of specific mRNA [12]. It was shown previously that cortisol induction causes an increase in the content of moderately repeated sequences [2], including, it is considered, regulatory regions of the genes controlled by glucocorticoids [8], in the transcriptionally active DNA fraction (TA DNA) of rat liver. It was logical to suggest that the action of glucocorticoids in lymphoid tissue, just as in the liver, takes place through activation of regulatory regions in the genome. It also seemed probable that disturbances of mechanisms of regulation in transformed lymphocytes (for example, in lymphatic leukemia) may be the result of conformational changes in chromatin in the region of repeat sequences (RS) of DNA.

The aim of this investigation was to compare the distribution of glucocorticoid-controlled RS between functionally different DNA fractions in AKR mouse thymoma and also in the

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thymocytes of young AKR mice and (CBA × C57BL/6)F_1 mouse hybrids as the controls, which do not develop spontaneous leukemia. It was recognized that T leukemia developing spontaneously in AKR mice under the influence of a retrovirus induces several changes in chromosome structure and in oncogene activity, which are analogous to changes in leukemia cells induced by chemical carcinogens [9, 10]. This suggests that the AKR line is an adequate model with which to study processes lying at the basis of lymphoid cell transformation.

EXPERIMENTAL METHOD

AKR mice aged 3-4 and 10-11 months and (CBA × C57BL/6)F_1 hybrid mice aged 3-4 months were used.

Mouse thymus DNA fractions were isolated by a modified phenol-salt nondetergent method [1]. TA DNA (DNA I) was obtained by deproteinization of the tissue homogenate with a mixture of 1 M NaCl and 5% phenol, pH 6.4, in the ratio of 1:1 by volume. Transcriptionally inactive DNA (TI DNA, DNA II) was isolated by extraction of the interphase layer with a mixture of 0.14 M NaCl and 66% phenol, pH 8.5, in the rato of 1:1 by volume. The 3rd DNA fraction (DNA III), firmly bound with protein, was extracted from the spinal interphase layer by treatment with pronase. The DNA preparations were purified by the method in [13] and subjected to ultrasonic fragmentation on the UZDN-2T apparatus at 22 kHz and 18 μA for 1 min, with a total duration of sonication of 12 min. The sedimentation coefficient of the DNA fragments was 5-6S.

^{32}P-cDNA was synthesized on polyA$^+$-mRNA with the aid of reverse transcriptase from avian myeloblastosis virus by the method in [6], using ^{32}P-dGTP and unlabeled dNTP as precursors. The yield of ^{32}P-cDNA with specific activity of 10^6 cpm was 0.3-0.5 μg for 1 μg of polyA$^+$-mRNA. The cDNA varied in size from 4- to 9S, with a maximum in the 7S region. PolyA$^+$-mRNA were isolated from the liver of rats which were given hydrocortisone acetate in a dose of 50 mg/kg 4 h before killing. It was considered that the nucleotide sequences of many mouse genes have a high percentage of homology with rate genes [4]. Hybridization of ^{32}P-cDNA with a 10^5-fold excess of the test fraction of fragmented and denatured thymocyte DNA was carried out in capillary tubes, basically according to the method in [14]. The degree of hybridization was determined from the ratio of the quantity of ^{32}P-cDNA resistant to S_1 nuclease (from Sigma, USA) to the total quantity of ^{32}P-cDNA in the sample. The hybridization curves were compared with respect to Cot $\frac{1}{2}$ (Cot denotes the product of DNA concentration and hybridization time, in moles/liter/sec).

EXPERIMENTAL RESULTS

It was shown previously that the DNA I fraction has increased sensitivity to the hydrolytic action of DNase I, it contains a high percentage of hybrid RNA, is rich in unique sequences, and belongs to the transcriptionally active regions of the genome [1-3]. The content of DNA I in rat liver does not exceed 20% of the total DNA. The DNA II fraction accounts for up to 70% of the total rat liver DNA, it is resistant to DNases, and it belongs to transcriptionally inactive regions of the genome. The DNA III fraction, firmly bound with proteins and, evidently, with membrane components, accounts for up to 5-10% of the total DNA [1-3].

The present investigation showed that the distribution of thymocyte DNA of hybrid mice among fractions was similar to that obtained previously for rat liver DNA fractions [1]. Meanwhile the content of DNA I in the thymoma and thymocytes of young AKR mice was sharply increased. For instance, whereas the DNA I content in thymocyte DNA of young hybrid mice was about 20%, in thymocytes of young AKR mice it was increased to 35%, and in the thymoma to 50-60%. The content of DNA III was increased two to threefold in the thymoma compared with that in the hybrids. These data suggest that conformational changes take place in the chromatin in the thymocytes of AKR mice still at an early age, and that they may perhaps determine a change (intensification) of transcription of certain DNA sequences participating in cell transformation.

It accordingly was considered important to discover whether these conformational changes in chromatin involve DNA sequences activated by an external regulator such as cortisol. For this purpose a comparative study was made of the kinetics of hybridization of TA DNA from thymoma, thymocytes of young AKR mice, and thymocytes of hybrid mice with ^{32}P-cDNA, synthesized on polyA$^+$-mRNA from cortisol-induced animals. Significant differences were found in the character of hybridization of the TA DNA studied (Fig. 1). For instance, Cot $\frac{1}{2}$ for thymoma DNA was 1318, whereas for thymocyte TA DNA of young AKR mice it was 32, and from hybrid mice

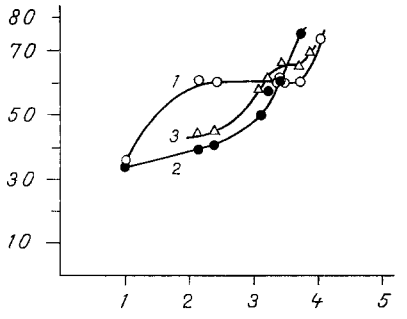

Fig. 1

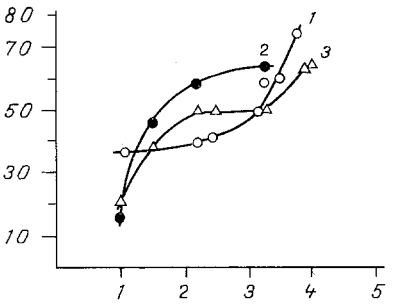

Fig. 2

Fig. 1. Hybridization of ^{32}P-cDNA synthesized on polyA$^+$-mRNA of cortisol-induced animals with TA DNA of thymoma (1), of thymocytes of young AKR mice (2), and of hybrid mice (3). Here and in Fig. 2: abscissa, log Cot; ordinate, hybridization (in %).

Fig. 2. Hybridization of functionally different thymoma DNA fractions from AKR mice with ^{32}P-cDNA, synthesized on polyA$^+$-mRNA of cortisol-induced animals. 1, 2, 3) DNA of fractions I, II, and III, respectively.

56. It follows from these data that the number of cortisol-induced sequences in thymocyte TA DNA from young AKR mice is 40 times greater than in thymoma TA DNA, and 23 times greater than in thymocyte TA DNA from hybrid mice. No significant differences were found in the number of unique sequences (Cot > 10^3) corresponding to cortisol-induced polyA$^+$-mRNA in thymocyte TA DNA from AKR mice and hybrid mice.

To discover what caused the sharp decrease in cortisol-activated RS in thymoma TA DNA, ^{32}P-cDNA was hybridized with all three fractions (I, II, and III) of thymoma DNA. The results showed (Fig. 2) that the sharp decrease in the number of cortisol-activated RS in thymoma TA DNA takes place mainly on account of transfer of these sequencies into DNA II. Whereas Cot $\frac{1}{2}$ for thymoma TA DNA was 1318, for TI DNA Cot $\frac{1}{2}$ was 50, and for DNA III it was 178. In other words, the number of cortisol-activated RS in thymoma TI DNA was 26 times greater than their number in TA DNA and more tham 7 times greater than their number in DNA III.

It can be concluded from these results that conformational changes in chromatin in regions containing cortisol-activated RS of DNA have already taken place in thymocytes of AKR mice in the period preceding their transformation. This may perhaps be one cause of the increased vulnerability of these RS in DNA to external influences (in this case, proviral DNA). This results in their inactivation in the thymoma and transition into a transcriptionally inactive state. It can only be conjectured at present that this escape from cortisol control may relate primarily to regulation of the differentiation of thymocytes and expression of the antigenic markers on their surface, for we know that cortisol participates in this process during induction by thymic factors [7] and that malignant transformation of lymphocytes and, in particular, of AKR thymocytes leads to reduction of expression of products of the H-2^k genes [11], to loss of some antigenic markers, and to the appearance of cells not belonging to the T series [5]. To a certain degree this may be due to the fact that regulatory regions in RS of thymocyte DNA lose their ability to be induced (controlled) by cortisol.

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EFFECT OF FUSARIC ACID ON PHOSPHOINOSITIDE METABOLISM OF ERYTHROCYTE MEMBRANES OF SPONTANEOUSLY HYPERTENSIVE RATS

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A factor which accompanies hypertensive states is high activity of the sympathicoadrenal system, as is shown by the fall of arterial pressure (BP) of hypertensive rats in response to injections of antisympathetic drugs or to immunologic "sympathectomy" [3]. One substance which lowers the level of mediators of the sympathicoadrenal system is fusaric acid (FA, 5-butylpicolinic acid), which was discovered by screening products of fungal metabolism [4]. FA inhibits activity of the enzyme dopamine-β-hydroxylase, which catalyzes the conversion of dopamine into noradrenalin. It lowers the concentrations of noradrenalin and adrenalin in the body tissues and depresses BP both in chronic experiments and after injection of a single dose [5, 8]. Spontaneously hypertensive rats (SHR), whose pathology is evidently determined by a genetic defect of permeability of the tissue plasma membranes for ions [7], are used as a model of human essential hypertension.

Di- and triphosphoinositides (DPI and TPI, respectively: phosphatidyl-myo-inositol-4-phosphate and phosphatidyl-myo-inositol-4,5-diphosphate) participate in regulation of plasma membrane permeability for ions and in the mechanism of activation of cells under the influence of Ca^{++}-mobilizing hormones on plasma membrane receptors [1, 2].

The aim of this investigation was to study the effect of FA on metabolism of TPI, DPI, and monophosphoinositides (MPI, phosphatidyl-myo-inositol) of erythrocyte membranes of SHR of different ages.

EXPERIMENTAL METHOD

Male SHR aged 2 and 4 months were used: These animals are characterized by labile (BP 160/95 mm Hg) and stable (205/130 mm Hg) forms of hypertension respectively. The control group for comparison consisted of normotensive Wistar rats (NWR) of the same age and sex (BP 125/70 mm Hg). FA was injected subcutaneously (50 mg/kg) 150 min before subcutaneous injection of ^{32}P-orthophosphate (^{32}P-OP) without a carrier (74 MBq/kg body weight). The rats were decapitated 90 min after injection of ^{32}P-OP, and 5 min later the native erythro-

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