NUCLEAR RNA POLYMERASE ACTIVITY OF L_{929} CELLS PRODUCING INTERFERON IN THE PRESENCE OF ADULT AND NEONATAL MOUSE SERA

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KEY WORDS: RNA polymerase activity; interferon; neonatal and adult sera.

The time course of interferon formation and of nuclear RNA polymerase activity was studied in L_{929} cells producing interferon in the presence of neonatal and adult mouse sera. Nuclear RNA polymerase activity was found to be identical in both cases. The results indicate that the low level of interferon formation by L_{929} cells in the presence of neonatal mouse serum is not connected with disturbances of transcription of messenger RNA for interferon.

The writers showed previously that peritoneal exudate cells of both adult and neonatal mice equally produced much larger quantities of interferon in the presence of serum from old animals than from neonatal animals [1-3]. This sharp decrease in interferon formation under the influence of serum factors from newborn mice could be due to changes taking place at the transcription level, i.e., at the level of synthesis of messenger RNA for interferon. To test this hypothesis, nuclear RNA polymerase activity was studied in L_{929} cells producing interferon in the presence of neonatal and adult mouse sera.

EXPERIMENTAL METHOD

L_{929} cells were grown in roller flasks at a speed of 10 rev/h. After monolayer formation the cells were treated with allantoic fluid from chick embryos infected with vaccine strain H of Newcastle disease virus. The virus was introduced in a dose of 100 CPD_{50} per cell. Control cultures were grown without addition of the virus. The cultures were incubated with virus for 1 h at 37°C, after which they were washed with medium without serum to remove the virus, and Eagle's medium with a double set of amino acids and vitamins and 2% adult or neonatal mouse serum, inactivated beforehand at 56°C for 30 min, were added. The quantity of interferon in the culture fluid was determined 2, 4, 6, 8, 10, and 12 h after induction. Samples of interferon were titrated on L_{929} cells by a micromethod against 100 CPD_{50} of vesicular stomatitis virus (Indiana strain). In each measurement system a mouse reference preparation (Mouse G 002-904-5, USA) was used to calibrate the interferon titers. The results were expressed in international units (i.u.).

Nuclei from the culture of L_{929} cells were isolated by a modified method [4]. The cells were harvested in Earle's solution and sedimented by centrifugation. All subsequent operations were carried out in the cold (4°C). The cell residue was suspended in buffer A (0.25 M sucrose; 0.005 M Tris-HCl, pH 7.5, 0.005 M $MgCl_2$, for concentration 10^7/ml) and after swelling of the cells the suspension was homogenized for 10 min in a tightly ground Dounce homogenizer (glass/glass, 20 tractions). The homogenate was centrifuged for 7 min at 480g. The residue was suspended in buffer B (0.25 M sucrose, 0.005 M Tris-HCl, pH 7.5, 0.005 M $MgCl_2$, 0.007 M 2-mercaptoethanol), homogenized again (10 tractions), and centrifuged. The residue of nuclei was suspended by gentle homogenization in incubation buffer C (0.25 M sucrose, 0.05 M Tris-HCl, pH 7.5, 0.04 M KCl, 0.02 M ammonium sulfate, 0.004 M $MgCl_2$, 0.001 M $MnCl_2$, 0.007 M 2-mercaptoethanol, and 100 μg/ml tRNA). The final number of nuclei was $1\text{-}2 \cdot 10^7$/ml. To determine RNA polymerase activity 0.05 ml of a mixture of ribonucleoside triphosphates was added to each 0.2 ml of suspension of nuclei in buffer C up to a final concentration of 0.4 mM of each of the three unlabeled ribonucleoside triphosphates and 0.05 mM of the fourth [^{3}H]cytidine triphosphate (^{3}H-CTP) (2 μCi per sample). The reaction was carried out at 25°C with constant

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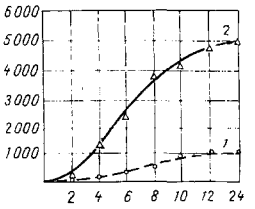

Fig. 1. Kinetics of interferon production by L_{929} cells in presence of neonatal (1) and adult (2) mouse sera. Abscissa, time of observation (in h); ordinate, interferon titer (in i.u./ml).

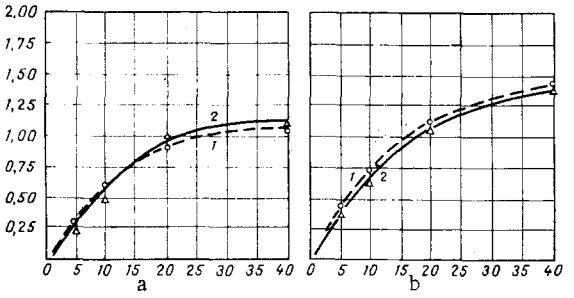

Fig. 2. Kinetics of nuclear RNA polymerase activity of L_{929} cells at different stages after interferon induction with neonatal (1) and adult (2) mouse serum: a) 1 h, b) 7.5 h after induction. Abscissa, time of observation (in min); ordinate, radioactivity, in cpm/min$\cdot 10^3$ per nucleus.

stirring. Aliquots of 0.25 ml were transferred to cold test tubes and an equal volume of cold 10% TCA containing 2% sodium pyrophosphate was added. The precipitates were transferred to G and F glass filters (Whatman, England) and washed with 20 ml of 5% TCA and 5 ml of 70% ethanol. The filters were dried and their radioactivity determined in toluene scintillator on a liquid scintillation counter (Packard, USA). The radioactivity was expressed in cpm per nucleus.

EXPERIMENTAL RESULTS

Curves of the kinetics of interferon production by L_{929} cells in the presence of neonatal and adult mouse sera are shown in Fig. 1. It can be seen that the cells produced much more interferon in the presence of adult mouse serum than of neonatal mouse serum, in agreement with the writers' previous experiments on other cells [1-3].

Because of the character of the curves thus obtained, the following time points after induction were chosen for analysis of nuclear RNA polymerase activity of the L_{929} cells: 1 h and 7.5 h. In the course of an hour after induction, no interferon was observed to appear in the culture fluid. The level of nuclear RNA polymerase activity in this case could reflect the initial state of the RNA-synthesizing apparatus of the cell. After 7.5 h, as Fig. 1 shows, interferon synthesis by the cells was increasing (the middle of the ascending curves in Fig. 1). This stage of interferon production by L_{929} cells must correspond to activation of the nuclear RNA polymerase. Kinetic curves of RNA synthesis by endogenous RNA polymerase of isolated cell nuclei at different times after induction are illustrated in Fig. 2a, b. Endogenous RNA synthesis under these conditions continued until 40 min as a linear function of the number of nuclei in the sample, for it was DNA-dependent (completely inhibited by actinomycin D in a concentration of 10 μg/ml), and it did not take place in the absence of the three unlabeled ribonucleoside triphosphates. Under the conditions chosen for the RNA polymerase reaction, up to 70% of nuclear RNA polymerase activity was accounted for by RNA polymerase II [4], which is known to be responsible for messenger RNA synthesis. The curves given in Fig. 2 therefore largely reflect the level of synthesis of messenger RNAs, including that for interferon. Comparison of the kinetic curves in Fig. 2 shows that the increase in interferon production from the first hour until 7.5 h after induction was accompanied by some increase in

nuclear RNA polymerase activity. However, during the first hour (Fig. 2a), before any interferon had been synthesized, and 7.5 h after induction (Fig. 2b), when active interferon synthesis was in progress, the course of the curves and the level of RNA synthesis were the same for nuclei isolated from L_{929} cells producing interferon in the presence of adult and neonatal mouse serum. In other words, the nuclear RNA polymerase activity of cells synthesizing interferon in the presence of adult and neonatal mouse sera was identical.

These results are evidence, in our opinion, that the low level of interferon formation in the presence of neonatal blood serum is not due to disturbances of transcription of RNA for interferon. Results obtained previously indicate the role of post-translation mechanisms in the inhibition of interferon formation by serum factors. It has been shown, for instance, that inhibition of cathepsin D in the presence of adult mouse serum and its activation and release in the presence of neonatal serum determined the level and composition of the molecular populations of interferons [2]. It may be that the mechanism of serum regulation of interferon production consists of the proteolytic degradation of interferon in the presence of neonatal serum.

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DETERMINATION OF MYELIN MARKER ENZYMES IN THE BLOOD SERUM OF PATIENTS WITH PERIPHERAL NERVOUS DISEASES

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Insufficient attention has been paid to the study of the enzyme composition of myelin. The presence of at least two enzymes — leucine aminopeptidase (LAP) and 2',3'-cyclic nucleotide-3-phosphohydrolase (CNP), nowadays considered to be myelin markers, has been reliably demonstrated [1]. LAP activity has been found in various tissues of the body. As regards CNP, its activity in the myelin sheath is known to be some 10 times higher than in other tissues [5]. It has been shown that this enzyme, which carries out hydrolysis of 2',3'-cyclic nucleotides to the 2'-derivatives, exhibits maximal activity during ontogeny in the period of myelination of the nervous system.

In demyelinating diseases and, in particular, in multiple sclerosis, CNP activity falls in the substance of the sclerotic plaques, possibly due to passage of the enzyme into the blood. No CNP can be found in healthy human blood [2].

The object of this investigation was to study activity of myelin marker enzymes in the blood serum of patients with diseases of the peripheral nervous system and in animals with experimental injury to nerve trunks.

EXPERIMENTAL METHOD

LAP activity was determined by the classical method using ready-made kits supplied by Fermognost (East Germany).

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