CHARACTERISTICS OF PROTEINS OF TYPE A INTRACYTOPLASMIC PARTICLES PRODUCED BY HUMAN CELLS

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The study of type A cytoplasmic particles (ACP), associated with mouse mammary gland tumors, has shown that they contain a protein with molecular weight of 73,000, a precursor of proteins of the gag gene of mouse mammary gland tumor virus (MMTV) [12, 14, 15]. The RNA found in the composition of the ACP, moreover, has a high degree of homology with the MMTV genome [9], and the reverse transcriptase is indistinguishable in several characteristics from the enzyme of B-type virions [8]. On the basis of experimental data it has been suggested that ACP are immature precursors of extracellular oncogenic viruses. ACP also are found in cells of primates producing D-type oncogenic viruses [1, 3, 6, 11]. No proteins with common antigenic determinants with structural proteins of D-type oncogenic viruses have been discovered by immunodiffusion or by radioimmunologic investigation in ACP from human cells [4]. Differences also have been found in the properties of reverse transcriptase from ACP and the corresponding enzyme from D-type virions [4, 5]. These results evidently point to the independent origin of ACP and D-type oncogenic viruses.

To test this hypothesis, a comparative analysis was undertaken of proteins of ACP and D-type virions from HEP-2 (VIlB) cells.

EXPERIMENTAL METHOD

Preparations of Mason–Pfizer virus (MPMV) and also antisera against individual proteins of MMTV, MPMV, and Rauscher leukemia virus (RLV) were obtained from the National Cancer Institute of the USA under the auspices of the Soviet–American agreement in the field of oncology.

Hep-2 cells, washed in Hanks' solution, were suspended in NTE-buffer (0.1 M NaCl, 0.01 M Tris-HCl, pH 7.4, 0.001 M EDTA) with 0.25 M sucrose and 0.5% Triton X-100, incubated at 0°C for 30 min, and then centrifuged at 6000g for 15 min. The supernatant was applied to a stepwise gradient consisting of 5 ml sucrose in NTE-buffer with a density of 1.31 g/ml; 10 ml sucrose with a density of 1.218 g/ml, and 5 ml with a density of 1.18 g/ml, and then centrifuged at 70,000g for 3 h. Material from the zone with a density of 1.22–1.31 g/ml was diluted twofold with NTE-buffer and applied to a linear sucrose density gradient (1.21–1.31 g/ml), and then centrifuged at 70,000g for 16 h. All manipulations were carried out at 4°C.

Rabbit antisera against purified ACP were obtained by the method described previously [2].

Proteins were iodinated in the presence of chloramine T [7] and studied by radioimmunoprecipitation. Antisera were added in a dose of 1–2 µl to 50–100 ng ^{125}I-labeled proteins ($4–6 \cdot 10^5$ cpm·µg^{-1}) in 0.2 ml PIA-buffer [0.25 M NaCl, 0.01 M Tris-HCl, pH 7.4, 0.5% Triton X-100, 0.1% Na dodecylsulfate (DDS), 1 mM PMSF, 0.2% bovine serum albumin (BSA)] and the mixture was incubated at 22°C. The mixture was then treated with 10–20 µl protein-A-sepharose (with an initial concentration of 30 mg/ml) and incubated for 30 min at 4°C. The residues were washed several times and dissolved in 40 µl of disintegrating buffer (0.0625 M Tris-HCl, pH 6.8, 2% DDS, 5% 2-mercaptoethanol, and 10% glycerol). The samples were incubated for 2

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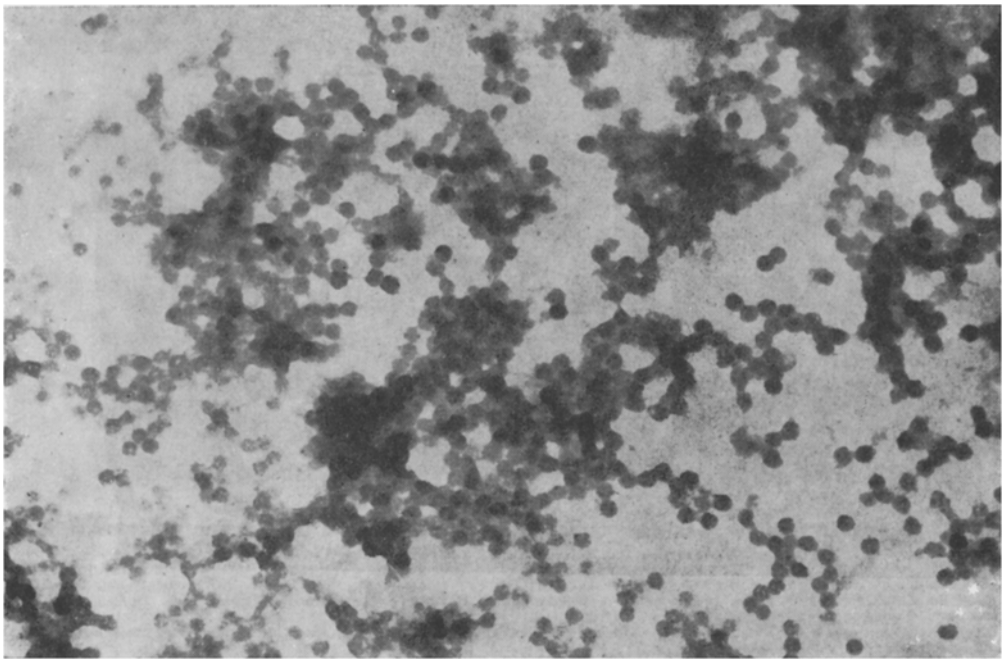

Fig. 1. Preparation from zone of gradient with density 1.22-1.24 g/ml, negatively stained with 1% aqueous uranyl acetate. Scanning electron micrographs. Magnification 30,000 ×.

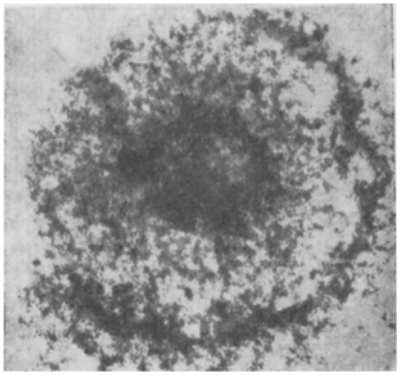

Fig. 2. Type A nucleoid from the same preparation lying separately. Magnification 500,000 ×.

min at 100°C and analyzed by electrophoresis in flat gradient (8-20%) polyacrylamide gel 1.5 mm thick, with 6% concentrating gel. A continuous buffer system [9] was used for electrophoresis. The electrode buffer and the gels contained 0.1% DDS. The gel was dried; ORWO SH 11 film was used for autoradiography.

EXPERIMENTAL RESULTS

A high degree of purity, concentration, and integrity of the internal structure of the type A particles was observed in negatively stained preparations from zones of the sucrose gradient with a density of 1.22-1.24 g/ml (Fig. 1). The spherical particles had an external diameter of 65-70 nm with their ribonucleoprotein packed in a definite manner (Fig. 2).

Analysis of the iodinated proteins of ACP by electrophoresis led to the identification of four proteins with molecular weight of 60,000 (p60), 45,000, 42,000, and 20,000; the principal protein of ACP is p60 (Fig. 3). The ACP protein spectrum differed from that of structural proteins of D-type oncogenic viruses, for which glycoproteins with molecular weight of 68,000-70,000 and 20,000 have been described, and also proteins with molecular weights of 27,000 (p27), 14,000, 12,000, and 10,000 [13].

Antiserum obtained against ACP reacted in the gel immunodiffusion test with purified ACP preparations in a final dilution of 1:32. This antiserum, like that against disintegrated D-type VIlB oncogenic virus, precipitated all the principal core proteins of D-type virions (Fig. 3).

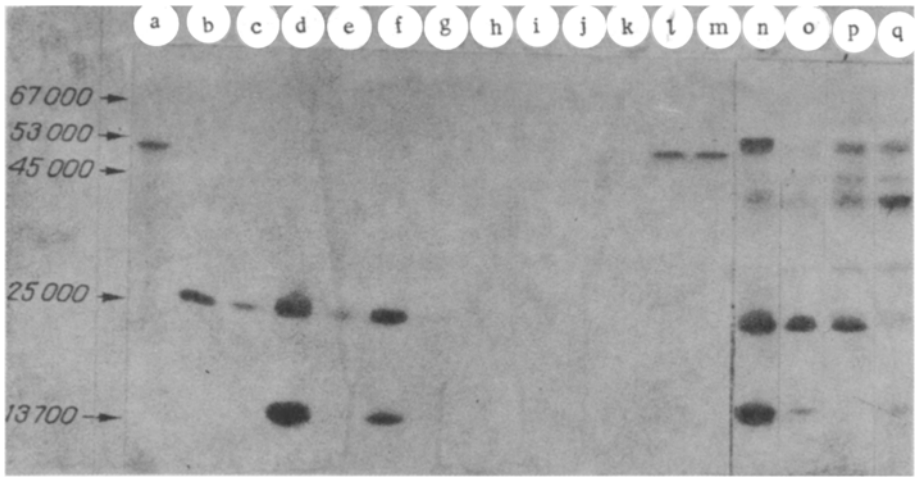

Fig. 3. Autoradiograph of distribution of ^{125}I-labeled ACP proteins and during electrophoresis in DDS-PAG. The markers used were (a): BSA 67,000, ovalbumin 45,000, heavy chains 53,000 and light chains 25,000 daltons of γ-globulins, pancreatic ribonuclease 13,700 daltons. MPMV proteins precipitated by antisera against p27 MPMV (b); against p27 in the presence of 10 μg unlabeled MPMV (c); against disintegrated VI1B (d); against disintegrated VI1B in the presence of 10 μg unlabeled MPMV (e); against ACP (f); against ACP in the presence of 10 μg unlabeled MPMV (g). ACP proteins precipitated by antiserum against disintegrated MMTV (h); against p30 of RIV (i); against p27 of MMTV (j); against disintegrated VI1B (k); against ACP (l); against ACP in the presence of 10 μg unlabeled MMTV (m). VI1B proteins precipitated by antiserum against disintegrated VI1B (n); against ACP (o); against p27 of MTV (p); against normal rabbit serum (q).

Immunoprecipitation of iodinated ACP proteins showed that commercial antiserum against p27 of MPMV and antiserum against disintegrated VI1B did not precipitate the major proteins of ACP. Homologous antiserum precipitated only p60 (Fig. 3). On the addition of an excess of unlabeled MPMV to the incubation mixture partial elimination of the corresponding bands took place on immunoprecipitation of the labeled MPMV proteins, but no competition was observed between MPMV proteins and p60 of ACP. During elimination of antibodies against MPMV from the antiserum by careful absorption with MPMV, it preserved its ability to precipitate p60 of ACP (Fig. 3). Various antisera against structural proteins of oncogenic viruses of different origin were tested for the presence of antibodies capable of precipitating p60 of ACP. The principal structural proteins of ACP was precipitated only by antiserum obtained against ACP. Proof that the p60 which was found was a protein specific for ACP from transplantable human HeLa-like cells (Hep-2) was given by the following facts: In preparations of ACP free from cellular components the principal protein with a molecular weight of 60,000 was found; the homologous antiserum precipitated p60 of ACP after its absorption by MPMV, human embryonic lung extract, and human donor's serum (Fig. 3).

Ability of ACP from Hep-2 cells to induce the formation of antibodies against structural proteins of D-type nucleoid may be connected with incorporation of the precursor of proteins of the gag gene of D-type oncogenic viruses into the composition of ACP, as is observed in mouse systems [12, 14] or with contamination of ACP by D-type nucleotides. However, the principal structural protein of ACP (p60) has no common antigenic determinants with structural proteins of D-type virions. The results of the present experiments evidently confirm data obtained previously showing the independent origin of ACP and D-type oncogenic viruses.

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THE POSSIBILITY OF STUDYING LIPID PEROXIDATION IN SURVIVING TISSUES

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Considerable disturbances of metabolism of lipid peroxides take place in experimental hypercholesteremia in animals [3, 4] and in coronary atherosclerosis in man [5, 7, 8]. According to existing views, lipid peroxides and radical intermediates of lipid peroxidation may have a harmful action on biopolymer molecules and on supramolecular structures of the cell [3] and may thereby facilitate the primary lesion in the blood vessel wall [4]. It is thus very important to study disturbances of lipid peroxide metabolism in the human aorta and other tissues in atherosclerosis. Cadaver material obtained at early autopsy (after 1-3 h) in cases of sudden death can be used for such investigations. However, it is difficult to determine the time elapsing after death during which the test material remains in its native state.

The object of this investigation was to study the effect of postmortem changes arising in the liver and aorta of mammals on the content of lipid peroxidation (LPO) products and on activity of the antioxidant enzymes — superoxide dismutase and glutathione peroxidase II — in these tissues.

EXPERIMENTAL METHOD

Experiments were carried out on 24 male chinchilla rabbits weighing 2-2.5 kg and on 16 male August rats weighing 110-120 g. All the rabbits were killed simultaneously by a blow on the head and the cadavers were kept at 4-8°C for 9 h. Ischemia of the liver was produced in the rats by compression of the vascular pedicle (maximal duration of ischemization 2).* The liver and aorta were perfused with cold isotonic KCl solution, cut into small pieces (the aorta after preliminary separation of intima and media), and homogenized in a glass homogenizer with Teflon pestle or in a glass Potter's homogenizer. The tissue homogenates were centrifuged (750g, 10 min) on a K-70 centrifuge (from Janetzky, East Germany); microsomes and

*The experiments on ischemization of the rat liver were carried out jointly with M. N. Bilenko and L. B. Dudnik, of the Laboratory for Transplantation of Organs and Tissues, Academy of Medical Sciences of the USSR.

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