EXPRESSION OF TYPE C ONCORNAVIRUS PROTEINS IN TUMORS OF CC57BR MICE

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Expression of the genome of endogenous type C oncornaviruses of mice in tumors induced by methylcholanthrene (MC) in mice of strain CC57BR, with a low incidence of leukemia, was studied by radioimmunodiffusion with precipitating test systems. The gs-1 antigen of p30 protein of type C murine viruses and the type-specific antigen of Gross virus (AGLV) were used as markers of the virus genome. Primary and transplantable sarcomas induced by MC were found to contain the gs-1 antigen, whereas AGLV appears only during passage of the tumors. Clear correlation was found between the quantity of p30 protein (titer of the gs-1 antigen) and the presence of AGLV: the gs-1 titer increased considerably simultaneously with the appearance of AGLV and disappearance of AGLV was always accompanied by a fall in the gs-1 titer. The results are evidence of coordinated expression of the two test proteins of endogenous type C murine oncornaviruses in chemically induced tumors of CC57BR mice.

KEY WORDS: radioimmunodiffusion; type C murine viruses; group-specific gs-1 antigen; type-specific AGLV antigen; chemical carcinogenesis.

The genome of mouse cells contains the genetic material of type C murine endogenous oncornaviruses. In mice of strains with high and low incidence of leukemia virus genes are expressed differently. In the first case the genome is expressed completely: mature virus particles are produced in the cells. In the second case the virus is not produced, but some structural proteins of type C viruses are synthesized in cells of lymphoid tissues. Many workers have shown that these tissues contain the basic structural p30 protein and the membrane gp69/71 glycoprotein [4, 7, 11, 12]. Under the influence of chemical and physical carcinogenic factors or during aging of the animals, the virus genome may be activated. During the investigation of sarcomas and lymphomas induced by chemical carcinogens, their cells have been shown to contain a large quantity of group-specific gs-1 antigen, the group determinant of p30 protein [6, 7, 10, 13]. Some workers have isolated a leukemogenic agent from chemically induced tumors [3, 9, 10]. The activation of the genome of endogenous viruses during chemical induction of tumors is of cardinal interest in the study of the mechanisms of carcinogenesis.

The object of this investigation was to study the expression of the genome of endogenous type C murine oncornaviruses in tumors induced by methylcholanthrene (MC) in CC57BR mice, a strain with low incidence of leukemia. The gs-1 antigen of the internal structural p30 protein and the type-specific antigen of Gross virus (AGLV), which is evidently the type determinant of the membrane glycoprotein of Gross virus [6], were used as markers of the virus genome. In the present investigation the dynamics of expression of AGLV and p30 was investigated in primary and transplantable sarcomas induced by MC and attempts were made to discover whether the expression of these two proteins is coordinated. The method of radioimmunodiffusion with precipitating test systems for gs-1 antigen and AGLV was used.

EXPERIMENTAL METHOD

Embryos and newborn and adult mice of the CC57BR strain, characterized by low incidence of leukemia, obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, and tumors induced in mice of this strain by MC were used. MC (from Mann Research Laboratories Inc., New York) was injected into CC57BR mice aged 4-6 weeks subcutaneously in the dorsal region in a dose of 0.3 mg in 0.1 ml olive oil. On average 20 weeks after the injection 14 of the 39 mice developed subcutaneous sarcomas at the site of injection

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TABLE 1. Expression of p30 Protein of Type C Murine Viruses and AGLV during Passage of Sarcomas Induced by MC

Tumor and No. of passage	Titer of gs-1 antigen	AGLV	Tumor and No. of passage	Titer of gs-1 antigen	AGLV
54-0	+ UD	—	62-0	+ 1:4	—
54-1	+ "	—	62-1	—	—
54-2	+ "	—	62-2	+ UD	—
54-3	+ "	—	62-3	+1:4	—
54-4	+ 1:64	+	63-4	+1:32	+
54-5	+ 1:32	+	62-5	+1:4	—
54-6	+1:128	+	62-8	+ 1:4	—
54-7	+ 1:64	+	62-9	+ 1:128	+
54-8	+ 1:32	+	62-10	+ 1:64	+
			62-11	+ 1:64	+
54-9	+ 1:128	+	62-18	+ 1:32	+
54-10	+ UD	—			
54-13	+ 1:128	+	63-0	+ 1:2	—
54-14	+ 1:64	+	63-1	—	—
54-15	+ 1:32	+	63-2	+ 1:4	—
54-16	+ 1:64	+	63-3	+ 1:4	—
			63-4	+ ND	+
58-0	+ UD	—	63-5	+ 1:64	+
58-1	+ UD	—	63-8	+ 1:64	+
58-2	+ 1:2	—	63-9	+ 1:32	+
58-3	+ 1:2	—	63-10	+ 1:64	+
58-4	+ 1:32	+	63-11	+ 1:32	+
58-5	+ 1:64	+	63-18	+ 1:64	+
58-6	+ 1:2	—			
58-7	+ 1:64	+	66-0	+ 1:2	—
58-11	+ 1:64	+	66-1	+ 1:64	+
58-13	+ 1:32	+	66-5	+ 1:16	+
			66-6	+ 1:32	+
59-0	+ UD	—	66-7	+ 1:64	+
59-1	+ 1:4	—	66-8	+ 1:128	+
60-0	+ 1:8	+	67-0	+ UD	—
60-1	+ 1:64	+	67-1	+ 1:128	+
			67-2	+ 1:32	+
			67-3	+ 1:128	+
			67-4	+ 1:64	

Legend. 0) primary tumor; UD) extract gave positive reaction for gs-1 only in undiluted form; ND) titer of gs-1 antigen in extract not determined.

of the carcinogen. When the tumor reached a diameter of 15-20 mm (on average 25.5 weeks after injection of MC) it was removed. Some of the tumor tissue was inoculated subcutaneously in the dorsal region into adult CC57BR mice. The rest was used for preparing extracts to act as antigens in radioimmunodiffusion. The 50% extracts of primary and transplantable (in some cases up to 18 generations) tumors, of normal mouse spleens, and also of whole embryos separated from the membranes, were made up in physiological saline, pH 7.2, as described previously [2, 6]. If a negative result was obtained in the radioimmunodiffusion test, the 50% extract was concentrated with a current of air by 3-8 times in volume. The extracts were kept at −20°C. A primary culture of embryonic CC57BR mouse fibroblasts also was tested. Radioimmunodiffusion was carried out by the method of Abelev and Él'gort [1] with precipitating test systems for gs-1 antigen of p30 protein [5] and AGLV [6]. In radioimmunodiffusion the test systems were used for gs-1 in a dilution of 1:12-1:24 and for AGLV in a dilution of 1:8-1:16 compared with the corresponding visible test system. Extracts were titrated around serum of the test system for gs-1 with substitution of the antigen of this test system.

EXPERIMENTAL RESULTS

To obtain an idea of the background levels of the test antigens in normal CC57BR mice their expression was studied from the early stages of ontogeny. Extracts of whole embryos, starting from the 12th day of intrauterine development and until birth, an extract of skeletal muscle tissues and internal organs of newborn and suckling mice, and also extracts of the spleens of adult CC57BR mice all contained gs-1 antigen. However, gs-1 antigen was absent from the serum of mice at all ages studied. In normal CC57BR mice, of the two antigens tested by radioimmunodiffusion, only gs-1 could thus be detected, and the second marker antigen (AGLV) was not found at any stage in the development of these mice. The tissues of mice in which tumors had not developed 9 months (4 mice) and 15 months (3 mice) after injection of MC were indistinguishable from the tissues of normal mice in their content of antigens of type C murine oncornaviruses.

Of 14 primary sarcomas induced by MC in CC57BR mice all except one (MCh-53) contained detectable quantities of gs-1 antigen. AGLV was detected in the extracts of only one primary tumor, namely MCh-60. Of 14 tumors induced by MC, 8 were transplanted. The results of investigation of the transplanted tumors are given in Table 1.

As Table 1 shows, the gs-1 titers varied in AGLV-positive tumors also. This can evidently be explained on the grounds that for most effective demonstration in extracts of AGLV they were concentrated as much as possible (from 3 to 8 times). The gs-1 titers thus do not reflect the true content of antigen in the tumors and are therefore not strictly comparable with one another.

The absence of gs-1 antigen in normal mesenchymal tissue [13] is evidence that during carcinogenesis the endogenous virus genome is evidently activated in the mesenchymal cells, and these cells acquire expression of p30 protein. Another possible cause of the increased content of p30 protein in the sarcomas is their cell composition. According to preliminary data, the primary culture of CC57BR mouse fibroblasts contains detectable quantities of gs-1 antigen. A tumor arising from such gs-1-positive fibroblasts may contain this antigen in larger quantities than the normal mesenchymal tissues in the region of which they appeared.

In 6 of the 8 transplanted tumors a sharp increase in the titer of gs-1 antigen was observed at one of the passages, and during subsequent passage a temporary fall in the titer of this antigen was observed in 3 of them compared with the preceding passage, with a sharp increase in the following passage. An increase in the titer of gs-1 antigen was always accompanied by the appearance of AGLV in the tumor, whereas a decrease, on the other hand, was accompanied by disappearance of AGLV. This synchronization evidently indicates that some phenomenon affects the expression of the two proteins. There are two possible explanations: infection of all the tumors in the investigation during passage by exogenous virus of the Gross type or "derepression" of the complete genome of the endogenous virus in the tumor. However, conversion of an AGLV-positive tumor into AGLV-negative and the fall in its titer of gs-1 antigen are evidence against the possibility of infection of the tumor with foreign virus.

The correlation between the appearance of AGLV and the increase in titer of the gs-1 antigen in tumors induced by MC, in the writers' opinion, is evidence of the linked, coordinated expression of the two virus proteins (p30 and AGLV) in the tumors rather than of their independent expression. Strand et al. [12] reported the independent expression of the internal p30 protein and membrane gp69/71 of type C murine oncornavirus in the tissues of normal mice of a strain with low leukemia incidence. These data are in agreement with the concept of four groups of genes (gag, env, pol, and onc) coding the internal structural proteins, proteins of the virus envelope, reverse transcriptase, and transforming ability (oncogen) [8].

The present results with respect to detection of antigens of type C murine oncornaviruses during passage of tumors are evidence of the coordinated expression of the test antigens in tumor tissues by contrast with the independent expression of p30 and gp69/71 in normal tissues of mice with low incidence of leukemia [12]. The results suggest that the mechanisms of cell control over the expression of genes of endogenous viruses differ in normal and tumor tissues of mice.

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