DETECTION OF ENDOGENOUS TYPE C VIRAL ANTIGENS IN MICE DURING DEVELOPMENT

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AKR and BALB/c mice (12- to 20- day embryos, newborn, and adult) were investigated by a radioimmunodiffusion (RID) method with test systems for group-specific antigen of the principal p30 structural protein (gs-1) of type C murine viruses and for type-specific antigen of Gross virus (AGLV). The p30 protein was clearly detectable after the 12th day of intrauterine development of both strains of mice; it persists in the tissues of the embryos until birth, and is found in the tissues of mice of both strains after the first day of postnatal development. AGLV was not detected in embryonic or adult BALB/c mice or in AKR embryos. However, this antigen was found in young AKR mice starting from the first to second day after birth. It is concluded from the results that the expression of p30 protein and AGLV is independent, within the limits of sensitivity of the RID method.

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

The presence of the genome of endogenous type C viruses in the normal genome of sex and somatic cells has been demonstrated for nearly all mammals [4, 7]. This fact raises the question of the possible role of the endogenous viral genome in normal cellular functions in ontogeny. To determine the activity of the viral genome structural proteins of the virion, which can be synthesized in the cells independently of infectious virus production, can be used.

Expression of the group-specific (gs-1) antigen of the principal structural p30 protein of oncornaviruses has been investigated previously in different strains of mouse embryos but rather contradictory results have been obtained [1, 5, 8]. Some workers found gs-1 antigen in mice of various strains with low incidence of leukemia (BALB/c, CBA, B10D2, CC57BR) in the late stages of prenatal development [1], whereas others found it in 10- to 18-day NIH Swiss and 16- to 18-day embryos of wild mice, whereas this antigen was not found in BALB/c embryos at the same times of development [8]. A third group of authors detected gs-1 irregularly in AKR mouse embryos immediately before birth and constantly in newborn mice [5]. No definite conclusion can be drawn from these results on the principles governing synthesis of the principal structural p30 protein during development of mice. The study of the other structural components of endogenous viruses is only just beginning [9].

The object of this investigation was to study expression of p30 protein and of the type-specific antigen of Gross virus (AGLV), which is possibly the type determinant of the membrane glycoprotein of Gross virus, during ontogeny in AKR and BALB/c mice. Using the radioimmunodiffusion (RID) method [1] attempts were made to determine the stages of embryogenesis of mice of strains with high and low incidence of leukemia at which the synthesis of these proteins begins and whether it is coordinated.

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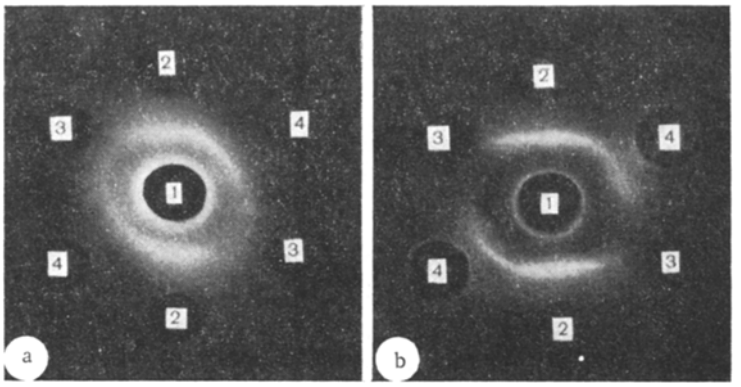

Fig. 1. Detection of gs-1 antigen of protein p30 of type C viruses in AKR and BALC/c embryos by the RID method. a: 1) Antiserum; 2) antigen of test system for gs-1 antigen; 3) physiological saline; 4) extract of 18-day AKR embryos. b: 1,2,3) The same as in a; 4) extract of 16-day BALB/c embryos.

TABLE 1. Structural Proteins of Type C Viruses in Mouse Tissues during Ontogeny

Stage of development	AKR mice		BALB/c mice	
	p30	AGLV	p30	AGLV
Embryos:				
12 days	1/1	0/1	1/1	0/1
13 "	7/7	0/7	2/2	0/2
14 "	3/3	0/3	1/1	0/1
15 "	5/5	0/5	3/3	0/3
16 "	1/1	0/2	1/1	0/2
17 "	1/1	0/2	1/1	0/2
18 "	4/4	0/4	3/3	0/3
19 "	5/5	0/5	2/2	0/2
20 "	5/5	0/5	2/2	0/2
Postnatal development				
1 days	9/9	0/10	1/2	0/2
1—2 days	—	1c/4	—	0/1
3 "	1/1	0/1	1/2	0/4
4 "	1/1	0/1	1/2	0/1
7 "	3/3	1b/3	3/4	0/4
9 "	—	1c/1	2/2	0/2
13 "	2/2	1c/3	—	—
1 month	—	1c/1	3/3	0/3
2 months	1/1	1c/1	—	—
4—5 "	—	8bc/8	2/2	0/2
6—7 "	1/1	1c/1	—	—
9 "	—	1b/1	2/2	0/2
12 "	—	1c/1	—	—

Legend. Numerator gives number of positive cases, denominator total number of cases tested; a) extracts of structural tissues of skeletal muscles; b) extracts of spleens; c) serum or plasma.

EXPERIMENTAL METHOD

Embryos at the 12th to 20th day of gestation, and newborn and adult mice of the low-leukemia BALB/c strains, obtained from the nursery of the Academy of Medical Sciences of the USSR (Stolbovaya) and mice of the high-leukemia AKR strain, provided by Professor N. N. Medvedev and colleagues of the group for inbred animals, N. F. Gamaleya Institute of Epidemiology and Microbiology, were used for the experiments. The time of fertilization of the females (day 0 of pregnancy) was determined from the appearance of a vaginal plug, and the age of the embryos was assessed in some cases from their weight and size [11]. The embryos were removed

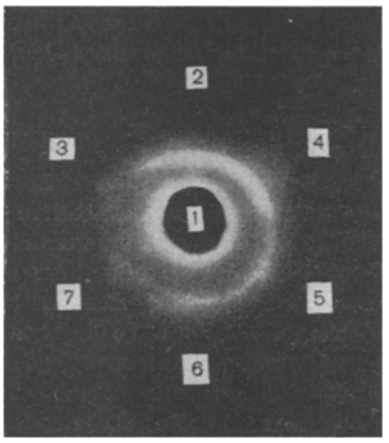

Fig. 2. Detection of type-specific antigen of growth virus in AKR mice by the RID method: 1) antiserum; 2) antigen of test system for AGLV; 3) physiological saline; 4) serum of 13-day AKR mice; 5, 6,7) serial twofold dilution.

from the uterus, freed from membranes, and moistened with physiological saline. Antigens for the RID reaction [1] were 50% extracts of the whole embryos or organs in physiological saline, pH 7.2, made up as described previously [2]. If a negative result was obtained in the RID the 50% extracts were concentrated 4-8 times by volume. Besides extracts, sera or heparinized plasma of the mice were used. The RID was carried out with test systems for p30 and AGLV described below. The scheme of the reaction was standard (Fig. 1). The precipitate was treated with ^{125}I-labeled donkey antibodies against rabbit IgG. The following precipitating test systems were used.

1. The test system for gs-1 antigen of protein p30 consists of rabbit antiserum against Rauscher virus [2] and extract of OP lymphosarcoma of CC57BR mice, which belongs serologically to tumors of the Gross type [3]. These reagents, in equivalent proportions, form one precipitation line in agar. In the RID the test system was used in dilutions of 1:16-1:24.

2. The test system for type-specific antigen of Gross virus (AGLV) was described in detail earlier [3]. It consists of the serum of a rabbit immunized with intact Gross virus and extract of OP lymphosarcoma of CC57BR mice. The components of the test system in equivalent proportions form one precipitation line identifying AGLV. This test system was used in the RID in dilutions of 1:12-1:16.

EXPERIMENTAL RESULTS

The results of the experiments are summarized in Table 1. Mouse embryos of the low-leukemia AKR strain from the 12th day of gestation until birth and newborn mice between 1 and 7 days after birth contained the principal structural p30 protein of type C murine oncornavirus in their tissues (Fig. 1a). Similar results were obtained with embryos and newborn mice of the low-leukemia strain BALB/c (Fig. 1b). Consequently, expression of the principal structural p30 protein of oncornavirus in both high-leukemia AKR and low-leukemia BALB/c mice can be clearly identified after the 12th day of gestation. The p30 protein persists in the tissues of embryos of both high- and low-leukemia mice until birth and is found in the tissues of newborn mice of both strains after the first day of postnatal development. Embryos were not investigated before the 12th day, for they could not be separated from the surrounding membranes. The placentas of AKR mouse embryos also were positive for gs-1 antigen, but the gs-1 antigen was found irregularly in the placentas of BALB/c mice. The negative results of Huebner et al. [8], obtained by investigating BALB/c mouse embryos for gs-1 antigens, and the irregular discovery of gs-1 antigen by Hilgers et al. [5] in AKR mouse embryos immediately before birth can evidently be explained by the use of less sensitive methods of investigation.

AGLV was not found by the RID method in embryos of AKR and BALB/c mice, but the placentas of AKR mice contained this antigen in high titer at all times of pregnancy tested. AGLV began to be detectable in the tissues of AKR mice on the 1st to 2nd day after birth. At the end of one month the antigen content in AKR mice reached the adult level, at which it remained approximately unchanged for one year (mice over one year old were not studied (Fig. 2). Within the limits of sensitivity of the RID method independent expression of p30 protein and of type-specific antigen of Gross virus (AGLV) was thus observed. Strand et al. [13] found in-

dependent expression of viral proteins p30 and gp69/71, by determining the content of these proteins in the tissues of adult mice of different strains by competitive radioimmunoprecipitation. Strand and August [12] concluded from the results of their study of expression of p30 and gp69/71 proteins during development of BALB/c, AKR, and C3H mice that the genome of type C viruses is unrelated to cellular differentiation. Meanwhile, Lerner et al. [9], using an immunofluorescence method to examine histological sections of organs of NZB, NZW, and C67BL/st mice and their embryos, concluded that expression of gp69/71 is linked with particular cells (lymphocytes and epithelial cells) and the stage of development of the embryo. They concluded that the control of expression of the genome of type C viruses is linked with differentiation. Hine et al. [6] consider that the membrane glycoprotein detectable in virus-negative mouse tissues is a product of the genome of endogenous xenotropic virus.

The dynamics of appearance of AGLV as observed in the present experiments agrees with that of appearance of complete type C virus in AKR mice as described by Rowe and Pincus [10]. However, it cannot be concluded from this that the expression of AGLV is connected with production of the virus in AKR mice. The failure to detect AGLV by the RID method in the prenatal stages of development of AKR mice perhaps is attributable to the low concentration of antigen. This explanation is supported by the detection of titers of gs-1 and AGLV in the same extract with antiserum of the corresponding test system. The titer gs-1 in different extracts of OP lymphosarcoma was 1:16 to 1:64, and the titer of AGLV in the same extracts was 1:4 to 1:16. Embryonic extracts has a titer of gs-1 antigen only one-eighth as high as OP tumors. If, therefore, the ratio between the test proteins in the embryonic tissues was approximately the same as in the virus-producing tumor, the quantity of AGLV in the embryonic extracts must lie at the limit of sensitivity of the RID method.

Episodic investigations of 12- to 20-day embryos of other low-leukemia strains (CBA, CC57BR, C3H/Sn) gave results similar to those obtained with BALB/c embryos: gs-1 was found at all times of testing, but it was found irregularly in the placentas. AGLV was absent in these embryos.

AGLV was found by the present writers in the gastric content of day-old AKR mice, evidence of the presence of antigen in the milk of AKR mice. In this connection it is not clear whether the AGLV found in the tissues of newborn mice is a product of the endogenous viral genome or a component of virus obtained from the mother's milk. This problem can evidently be solved by the study of newborn mice isolated from their mothers immediately after birth.

The question of the regular expression of the structural proteins of virus during cell differentiation can evidently be solved by an immunofluorescence method of histological sections.

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