RESISTANCE OF AKR MICE TO THE ONCOGENIC ACTION OF MOLONEY MOUSE SARCOMA VIRUS

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Mice of line AKR were almost completely resistant to 1000 oncogenic doses of Moloney mouse sarcoma virus. The (AKR ♀ × C57BL/6 ♂) F_1 (AKR♀ × BAL/B♂) F_1 hybrids were sensitive to the oncogenic action of the virus to the same degree as their parents (mice of lines BALB/c De or C57BL/6). The resistance of AKR mice to the oncogenic action of the virus was reduced by x rays (dose 350-450 R) or by administration of the virus to newborn and young mice. Varieties of Moloney sarcoma with greatly increased pathogenicity were isolated from the rat tumors or tumors induced in AKR mice.

Mice of line AKR exhibit a high incidence (about 90%) of spontaneous leukemias, the etiological agent of which is the Gross virus which persists in them permanently. Infection of the mice of this line with other oncogenic viruses leads essentially to a mixed oncovirus infection, the course and outcome of which may differ considerably from those of a "monoinfection" [2-4]. This is particularly true of combinations of oncogenic viruses in which Gross virus may play the role of "assistant" virus.

The object of the present investigation was to study the oncogenic action of Moloney sarcoma virus [8] in mice of different lines.

EXPERIMENTAL METHOD

Male and female mice weighing 10-12 g, of lines AKR, C57BL/6, and BALB/c De, and pregnant Wistar rats were obtained from the "Stolbovaya" Nursery of the Academy of Medical Sciences of the USSR. Moloney mouse sarcoma virus strain msv(m) was obtained from Professor N. P. Mazurenko's laboratory (Institute of Clinical and Experimental Oncology, Academy of Medical Sciences of the USSR). Cell-free extract (1 ml medium No. 199 to 1 g tumor) of a tumor of BALB/c De mice obtained by intramuscular injection of msv(m) virus, was used as the "virus." The original msv(m) virus or various dilutions of it was injected in a dose of 0.2 ml intramuscularly into the femoral muscle. Newborn or young rats received the virus in a dose of 0.1 ml. The animals were examined every 2-3 days to determine the time of appearance of the tumors and to assess their size.

EXPERIMENTAL RESULTS

The standard preparation of msv(m) virus in a dilution of 10^{-1} induced characteristic tumors at the site of injection in 100% of mice of lines BALB/c De and C57BL/6 after a latent period of 7-10 days. The tumors very quickly reached a size of 1.5-2 cm, and then just as quickly disappeared completely and were absorbed by the 15th-20th day. In a dilution of 10^{-3} the virus induced similar tumors in more than 50% of mice of both lines (Table 1).

Mice of line AKR were found to be almost totally resistant to msv(m) virus. Injection of 1000 or more oncogenic doses of virus intomice did not cause the formation of tumors similar to those observed

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TABLE 1. Sensitivity of Adult Mice of Various Lines to the Oncogenic Action of the Virus

Line of mice	Dilution of virus	Frequency of induction of tumors*	Latent period of appearance of tumors (in days)	Frequency of regression of tumors‡	Time of regression of tumor (in days)
BALBL/c De	10^{-1}	30/30	7—10	30/30	15—20
	10^{-3}	13/26	22—25	13/13	30—33
C57BL/6	10^{-1}	30/30	7—10	30,30	15—20
	10^{-3}	6/14	21—24	6/6	29—31
AKR	10^{-1}	27/106 †	7—10	27/27	14—15
	10^{-2}	0/12	—	—	—
F_1 (AKR × C57BL/6)	10^{-1}	6/6	7	6/6	24—29
F_1 (AKR×BAL B/c De)	10^{-1}	16/16	7	16/16	24—29

*Numerator gives number of animals developing tumors, denominator gives number of animals infected.
† Microtumors measuring from 0.1 to 0.2 cm.
‡ Numerator gives number of tumors absorbed, denominator gives total number of tumors obtained.

TABLE 2. Sensitivity of AKR Mice of Different Ages to the Oncogenic Action of msv(m) Virus

Age of mice at moment of infection with msv(m) virus in dilution of 10^{-1}	Frequency of induction of tumors*	Frequency of regression of tumors†
1–3 days	29/32	0/29
2 weeks	30/31	9/30
3 "	12/17	12/12
Adult mice irradiated in doses of 350–450 r	32/55	32/32

*Numerator gives number of animals developing tumors, denominator gives number of animals infected.
† Numerator gives number of tumors absorbed, denominator gives total number of tumors obtained.

in mice of line BALB/c De or C57BL/6 in any single case (Table 1). All that happened was that in about 25% of AKR mice a zone of induration, or a microtumor, from 0.1 to 0.2 cm in diameter, appeared at the site of injection, only to disappear completely 14–15 days after injection of the virus.

The (AKR♀ × BALB/c De♂) or (AKR♀ × C57BL/6♂) F_1 hybrids were identical in their sensitivity of msv(m) virus to mice of line BALB/c De or C57BL/6, respectively. No difference was found between them either in the frequency of induction of tumors (100%) or in their maximum size (1.5 cm^3), their time of appearance (7–8 days), or their time of absorption (24th day) (Table 1).

X ray irradiation of adult AKR mice in doses of 350–450 R (Table 2) led to a sharp decrease in their resistance to msv(m) virus. Tumors measuring 1 cm^3 or more appeared in 59% of infected mice 1.5 weeks after injection of the virus; by the 30th–40th day the developing tumors had completely disappeared.

Induction of tumors in AKR mice by msv(m) virus could also take place through infection of newborn or young animals. When the virus was injected into newborn mice or mice aged 3–5 days, tumors developed in almost 100% of the animals. These tumors grew progressively, and the mice died by the 10th–13th day after infection (Table 2).

Infection of mice aged 2 weeks also led to induction of tumors in 100% of cases, but in 30% the tumors were absorbed.

After infection of mice aged 3 weeks, tumors appeared in 70% of the animals. They grew to the size of 0.5 cm^3, but by the 30th day after injection of the virus they were all completely absorbed (Table 2).

Previous investigations have shown that Freund's complete adjuvant (FCA) greatly stimulates virus oncogenesis in experiments with polyomar virus, Rous virus, and mouse leukemia viruses [1]. By using different doses and methods of administration of FCA, the writers attempted to modify the oncogenesis induced by msv(m) virus. The results obtained showed that FCA had no appreciable effect on the sensitivity of the animals or on the character of the oncogenesis induced by msv(m) virus.

A virus capable of inducing characteristic tumors in BALB/c De and C56BL/6 mice was isolated from tumors induced by msv(m) virus in irradiated or very young AKR mice. However, the virus isolated from AKR mice, and described as msv(m)-AKR by contrast with the standard msv(m) strain, proved to be highly pathogenic also to AKR mice. In the mice of this line standard preparations of msv(m)-AKR virus induced progressively growing tumors in 100% of mice, and caused the animals' death. Characteristic tumors in AKR mice could also be induced by virus isolated from tumors of (AKR × C57BL/6) F_1 mice.

Standard msv(m) virus did not induce tumors in adult or newborn hamsters; Wistar rats aged 5-7 days, infected with msv(m) virus, developed tumors in 20% of cases, and as a rule the tumors were absorbed. Only one of the seven rat tumors obtained could be successfully maintained by serial passage through young Wistar rats. A cell-free extract of one such tumor induced tumors in 100% of mice of all three lines (BALB/c De, C57BL/6, and AKR), causing death of the mice from progressively developing tumors. By passage of virus isolated from a rat tumor (a virus designated msv(m)-R) through mice of line AKR yielded a virus (in a titer of 10^{-3} or more), after the 3rd passage, which induced very large tumors in mice of lines AKR and BALB/c De as early as on the 2nd-3rd day, and they rapidly progressed to kill the animals on the 3rd-5th day. Virus msv(m)-R was found to be highly oncogenic not only to mice, but also to young rats (which died with very large tumors) and hamsters.

These results show, at least indirectly, the immunological nature of the resistance of AKR mice to msv(m) virus. This conclusion is supported by the writers' preliminary results showing that the sera of AKR mice infected with msv(m) virus contain neutralizing antibodies against this virus, and in fairly high titer (1:16). Furthermore, repeated injection of msv(m) virus into AKR mice in which microtumors had developed did not give rise to any further induction of these lesions.

Consequently, the immunological tolerance of AKR mice to Gross leukemia virus does not prevent them being resistant to the closely related virus msv(m). Experiments with F_1 hybrids clearly demonstrate the genetic mechanism of this resistance which, moreover, is found in a system completely preserving its status of tolerance [7].

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