SPONTANEOUS REGRESSION OF RAUSCHER'S LEUKEMIA IN AKR MICE

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Adult AKR mice are highly sensitive to the induction of erythroleukemia when infected with Rauscher leukemia virus. However, in about 65% of cases the leukemia in these mice regresses spontaneously. The frequency of regression depends on the age of the infected mice, but repeated injection of Rauscher virus into mice in which regression of leukemia has taken place no longer induces leukemia.

In an earlier paper [4] the practically total resistance of adult mice of the leukemia-sensitive line AKR to Moloney mouse sarcoma virus was demonstrated. The writers continued to study the spectrum of sensitivity of AKR mice to other oncogenic viruses, including Rauscher mouse leukemia virus [6]. Experimental results demonstrating the course of Rauscher leukemia in AKR mice are described below.

EXPERIMENTAL METHOD

Mice of lines AKR and BALB/cDe, weighing 12-14 g and of both sexes, were obtained from the "Stolbovaya" nursery of the Academy of Medical Sciences of the USSR.

Plasma of BALB/cDe mice with a developed Rauscher erythroleukemia (12-14th day after intraperitoneal injection of the suspension of leukemic spleen into the mice) was used as the source of Rauscher leukemia virus (RLV). All titrations and determinations of the virus were carried out on mice of the sensitive line BALB/cDe, aged 2-3 months, by standard methods. The methods of titration of the virus have been fully described previously [1, 2].

EXPERIMENTAL RESULTS

After intravenous injection of 10^3-10^4 leukemogenic doses of RLV into 3-month AKR mice, enlargement and an increase in weight of the spleen characteristic of Rauscher leukemia were observed in practically all (38 of 42) the animals sacrificed 10-12 days after infection. Whereas the weight of the spleen of the control AKR mice of that age never exceeded 150 mg, the mean weight of the spleen of the experimental mice of this series was more than 400 mg, the weights ranging from 350 mg to 1 g. Histological examination of the enlarged spleen of the infected mice showed the characteristic picture of Rauscher erythroleukemia [2, 6, 9]. This increase in weight continued (with fluctuations from 200 to 900 mg) until the 18th day after infection (in all of the 10 mice; mean weight of the spleen in this group 470 mg). Active RLV was isolated in 100% of cases from the plasma and spleen of these mice.

An increase in weight of the spleen was observed in only 7 of the 17 mice sacrificed between the 21st and 27th days after infection. However, in this case the mean weight of the enlarged spleen was 260 mg, and differed sharply from the control in only 1 case (weight of the spleen 595 mg). In this series RLV was definitely isolated from only one mouse (from a 10% extract of the spleen which was greatly increased in weight).

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TABLE 1. Weight of Spleen and Number of Leukemic Plaques in It in AKR and BALB/cDe Mice Infected with RLV

Series of expts.	Line and age of mice	Weight of spleens (in mg) and number of leukemic plaques in them*	
		8th-10th day after infection (first period)	21st-27th day after infection (second period) ‡
I	AKR 4 weeks 2 months	410±45 † (c)	890, 240, 285, 250, 190 Mean (c) (16) (41) (8) (0) 175, 225, 1300, 150, 175, 240 ** (10) (11) (c) (0) (10) (10) 185, 170 (4) (6)
		Wt. of spleens of control mice not more than 90 mg	
II	AKR 2-4 months	377±23,5 † (64±17,3)	145, 160, 350, 180, 1200 Mean (26) (16) (37) (34) (c) 120, 265, 150, 2200, 135, 175 ** (0) (2) (0) (c) (4) (11) (c)
		Wt. of spleens of control mice not more than 100 mg	
III	BALB/c De 4 months	239±27,5 † (2±1,0)	>2000 † (c)
		Wt. of spleens of control mice not more than 200 mg	
IV	AKR 5-6 months	146±9,6 † (13±4,8)	144±12 † (2±1,4)
		Wt. of spleens of control mice not more than 150 mg	
V	AKR 3-4 mos. (received RLV previously)	156±21,5 † (2±0,7)	

* Number of leukemic plaques in a given spleen shown in parentheses under weight; (c) denotes multiple confluent plaques, which were impossible to count accurately (as a rule there were more than 100 plaques per spleen).

† Mean weight of spleens and mean number of plaques per spleen are shown.

‡ In series I and II of experiments at the second period the figures for each mouse are shown separately.

** In the calculation of the mean, spleens weighing more than 500 mg (and with confluent plaques) were disregarded (see text).

Of the 28 mice sacrificed 1.5-3 months after infection, in 5 the weight of the spleen was greater than in the control, but the maximum weight was only 200 mg. It was impossible to isolate RLV from the plasma or splenic extracts of any of the mice of this series. The histological picture of these spleens was virtually indistinguishable from normal.

To obtain quantitative data on the sensitivity of AKR mice of different ages to RLV and the regression of leukemia in these animals the method of direct counting of the number of leukemic "plaques" on the surface of the spleen of infected mice was used. This method has often been tested and is extensively used in many different laboratories of the world [5]. Accordingly, mice were injected intravenously with one of two dilutions of the original virus (1 : 25 or 1 : 50). The mice were sacrificed either 8-10 days (first period) or 21-27 days (second period) after infection and their spleens were fixed in 10% neutral formalin solution. The number of whitish leukemic plaques easily visible to the unaided eye was counted on the surface of these spleens after 24 h.

The results given in Table 1 show that AKR mice aged between 4 weeks and 2 months were highly sensitive to induction of leukemia. As a rule the plaques were confluent on the 8th-10th day after infection

in these animals and the weight of their spleen was 3-5 times greater than in the control. Approximately equally high sensitivity to induction of leukemia was still found in mice at the age of 2-4 months: in about 50% of cases the plaques on the spleen were confluent and the weight of the spleen was 2-4 times greater than in the control.

The picture was quite different when the spleens of mice of these ages sacrificed on the 21st-27th day after infection were examined. The number of plaques (averaging from 29 to 1 per spleen in the various series), as well as the weight (mean not more than 150 mg) of most spleens were sharply reduced. In some cases (6 of 27), however, the weight of the spleen and number of plaques in the mice of these groups not only were not reduced but, on the contrary, showed a further sharp increase.

Older mice of line AKR (age 5-6 months) were much less sensitive to RLV. The weight of the spleen in these animals on the 8th-10th day after infection was virtually indistinguishable from the control and the number of plaques was much smaller than in the mice of the two age groups discussed above. Hardly any spleens of greatly increased weight (over 500 mg) and with confluent plaques were observed in mice of this age sacrificed 21-27 days after infection (in only 1 of 15 animals).

In mice which had been infected with RLV previously, at the age of 2 months, and in which the leukemia had regressed, sensitivity to induction of leukemia was less still (the number of leukemic plaques averaged 1-4).

A series of experiments with BALB/cDe mice aged 2-4 months is also shown in Table 1. The number of plaques in the spleens of these mice, sacrificed at the first period after infection, was significantly lower than in AKR mice of the same age. However, on the 21st-27th day, confluent plaques were observed on the spleens of 100% of the BALB/cDe mice, and the weight of each spleen exceeded 2 g. It is clear that when large spleens were found in the AKR mice sacrificed on the 21st-27th day after infection, this reflected progressive Rauscher leukemia identical in form with that occurring in BALB/cDe mice.

To determine the frequency of development of progressive lethal Rauscher erythroleukemia in AKR mice infected with RLV, a batch of 100 mice aged 2 months was allowed to live until natural death. Before 1 month had passed 12 mice had died with characteristic features of Rauscher erythroleukemia. Subsequently (until 5 months inclusive) a further 16 mice of this batch died with signs of progressive erythroleukemia. Another 8 mice were sacrificed at various times in a state of agony with very large spleens. In all these 8 cases, active RLV was isolated from the plasma of the mice. It can accordingly be concluded that by the age of 5 months the progressive form of erythroleukemia develops in 36% of adult AKR mice infected. The only point to make is that in this series of experiments at times from 10 to 18 days after infection, marked increases in size of the spleen, which was transient in character, could be detected in practically all the mice by palpation.

In agreement with other investigators and with the writers' own findings, all 3-month BALB/cDe mice infected with the same doses of RLV as the AKR mice died from 20 to 30 days after infection with characteristic features of progressive erythroleukemia (the weight of the spleen in these animals was 2 g).

On infection of newborn or young (under 4 weeks) AKR mice intraperitoneally with RLV, a progressive lethal form of erythroleukemia developed in 100% of cases.

Previous investigations showed that Freund's complete adjuvant (FCA) greatly stimulated virus oncogenesis in the case of polyoma virus, Rous virus, and mouse leukemia viruses [2, 3]. However, injection of FCA into adult AKR mice intraperitoneally 1 week before infection with RLV did not stimulate the development of leukemia. A progressive lethal form of Rauscher erythroleukemia developed in these mice at the periods specified in only 6% of cases (2 of the 32 animals).

Consequently, the course of Rauscher erythroleukemia in AKR mice differed sharply from that observed after infection of mice of other sensitive lines. In about 65% of cases Rauscher leukemia in adult AKR mice was regressive despite 100% sensitivity of these mice to induction of the process. The degree of sensitivity of AKR mice to RLV is not lower, and may even be higher, than in mice of the sensitive line BARB/ cDe.

Until very recently spontaneous (not induced by chemotherapy or immunotherapy) regression of virus-induced mouse leukemias in a sensitive line was regarded as impossible. It was only in 1969 that Rich et al. [7, 8] described spontaneous regression of a leukemia induced by a special strain of Friend leukemia. Regardless of the special nature of the strain, the mechanism of regress was evidently immuno-

logical. Preliminary results suggest that in the present case the mechanism of regression also was connected with the immune response: the frequency of regression depends on age; reinjection of RLV into mice after regression of leukemia does not lead to induction of the disease; virus-neutralizing antibodies are found in high titer in the serum of mice after regression of leukemia.

Consequently: 1) having regard for the virtually complete immunological tolerance to gross virus and to the leukemia induced by it, and 2) in view of their high sensitivity to induction of erythroleukemia on infection with RLV, which is closely related to gross virus, it can be concluded that in AKR mice there is a mechanism responsible for the high frequency of spontaneous regression of Rauscher leukemia.

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