The Effect of Immunosuppression on Oncogenesis by Murine Sarcoma Virus[1] (36928)

MARTIN ZISBLATT AND FRANK LILLY
(Introduced by E. J. Hehre)
Department of Genetics, Albert Einstein College of Medicine, Bronx, New York 10461

The growth limitation and ultimate regression of primary tumors induced by murine sarcoma virus (MSV) in adult mice appear to be due to an immunological response directed against antigens of the virus particle and/or virus-induced antigens on the surfaces of infected cells. Evidence supporting this conclusion includes: (a) tumors induced in newborn immunologically immature mice grow progressively (1, 2); (b) the ability of mice to resist the progressive growth of these tumors develops in an age-dependent manner which correlates with the development of immunological competence (1, 3); (c) neonatal thymectomy, which interferes with the maturation of the immune system, renders mice more susceptible in adulthood to the progressive growth of these tumors (4, 5); and (d) the administration of thymosin, which partially restores immunological function in neonatally thymectomized mice, accelerates the development of resistance to progressive tumor growth (1). Furthermore, treatment with immunosuppressive agents such as ionizing radiation (2), antithymocyte serum (6), cortisone (7), and cyclophosphamide (3, 8) results in an increased susceptibility of mice to progressive tumor growth. In contrast to the effectiveness of these treatments in increasing the incidence of *progressive tumor growth*, their effect on susceptibility to *tumor induction* by MSV remains unclear. Although no increase in tumor incidence was observed following sublethal total body X-irradiation (2), several authors have reported that treatment with either antilymphocyte serum (9), antithymocyte serum (6), or cortisone (7) renders mice more susceptible to tumor induction by MSV. In view of these conflicting reports, a study was undertaken to further define the role of immunocompetence in resistance to oncogenesis by MSV.

Materials and Methods. Mice. Male and female CBA/Wh mice of 5 to 6 wk of age were obtained from a breeding colony maintained in our laboratory. Female A/HeJ mice of 4 to 5 wk of age were obtained from the Jackson Laboratory (Bar Harbor, ME) and were housed in our laboratory for at least 1 wk prior to use.

Virus. Murine sarcoma virus (Moloney) Lot. Nos. 175R and 192R used in this study was a gift from Dr. J. B. Moloney of the National Cancer Institute. The CBA/Wh mice were inoculated with 0.1 ml of the specified dilution of MSV-M Lot No. SVRP-175R and A/HeJ mice were challenged with 0.1 ml of a 1:10 dilution of MSV-M Lot. No. SVRP-192R. All injections were via the subcutaneous–intramuscular route as described by Blumenshein and Moloney (10).

X-irradiation of mice. X-Irradiation was performed the day prior to infection with MSV. Mice were irradiated in partitioned Lucite chambers, and dose rate calibrations were performed immediately prior to irradiation using a Victoreen 100R Dosimeter placed in a covered Lucite chamber. 400 R of whole body X-irradiation was delivered with a Picker 280 kV Vanguard therapy unit under the following conditions: 280 kV; 20

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mA; 1.49 mm Cu filtration; target to skin distance of 44–46 cm, and a dose rate of 134–145 R/min in different experiments.

Antilymphocyte serum. Rabbit anti-mouse lymphocyte serum (ALS), obtained from Dr. M. A. Hardy of this institution, was prepared against BALB/c lymph node cells according to the method of Gray *et al.* (11). The cytotoxic activity of this serum was tested by Sanderson's modification (12) of the dye exclusion test using ^{51}Cr, and the 50% endpoint on lymph node cells derived from CBA/Wh mice was at a dilution of 1:780. Immunosuppression was achieved by the intraperitoneal inoculation of 0.25 ml of ALS on each of 4 consecutive days. These injections began 3 days prior to the virus inoculation and ended on the day of virus inoculation. Due to the toxicity associated with injections of high doses of ALS, a few mice died shortly after undergoing the described immunosuppressive treatment. Those mice dying without palpable tumors before the mean latency period for tumor appearance at the specified virus dilution are not considered in the results.

Results. In both intact and immunosuppressed mice the incidence of tumors appearing after infection with MSV was dependent upon the dose of virus inoculated (Table I). The infection of mice with low doses of virus resulted in a lower tumor incidence as well as an increase in both the mean and the range of the latency periods until tumor appearance. In addition, a comparison of the tumor incidence among normal, irradiated, and ALS-treated mice shows that the immunosuppressed mice were more susceptible to the induction of tumors than were the untreated mice. This difference in susceptibility was clear only at the lower virus doses (10^{-3} to 10^{-5} dilutions). The oncogenic capacity of a 10^{-4} dilution of MSV in immunosuppressed mice corresponded to that of a 10^{-3} dilution in intact mice. A Reed–Muench analysis (13) of the data indicated the 50% infectious dose (ID_{50}) of this preparation of MSV to be $10^{-3.65}$ in intact mice, $10^{-4.55}$ in irradiated mice, and $10^{-4.71}$ in mice treated with ALS.

In addition to the striking effect of host immunological competence on tumor incidence, both the mean and the range of the latency periods until tumor appearance were usually lower in immunosuppressed mice than in immunocompetent mice, indicating

TABLE I. Effect of Immunosuppressive Treatment on the Susceptibility of CBA/Wh Mice to Oncogenesis by Murine Sarcoma Virus (Moloney).

Virus dilution	Treatment	Tumor incidence[a]		Latency period (days)	
		No.	%	Mean ± SD	Range
10^{-1}	None	20/20	100	5.6 ± 1.4	4–9
	XR	21/21	100	4.7 ± 0.6	4–6
	ALS	17/17	100	4.2 ± 0.5	4–6
10^{-2}	None	18/20	90	7.7 ± 2.0	5–13
	XR	22/22	100	6.4 ± 1.3	4–8
	ALS	16/16	100	5.1 ± 1.3	4–8
10^{-3}	None	15/20	75	11.9 ± 5.1	7–26
	XR	22/23	96	7.5 ± 0.9	6–9
	ALS	12/13	92	9.8 ± 5.4	5–26
10^{-4}	None	3/24	13	14.3 ± 7.5	10–23
	XR	19/24	79	15.2 ± 7.6	7–34
	ALS	8/10	80	9.9 ± 2.1	7–13
10^{-5}	None	0/24	0	— —	—
	XR	4/22	18	21.5 ± 14.9	10–43
	ALS	4/11	36	28.8 ± 16.0	15–49

[a] (No. of mice developing tumors)/(no. of mice inoculated with MSV).

TABLE II. Effect of Immunosuppression on the Survival of Adult CBA/Wh Mice with Murine Sarcoma Virus (Moloney)-Induced Tumors.

Virus dilution	Treatment	Survival[a] on Day 60		Survival period (days)[b] of mice dying before 60 days	
		No.	%	Mean ± SD	Range
10^{-1}	None	15/20	75	39.8 ± 13.8	25–38
	XR	0/21	0	10.8 ± 1.8	8–14
	ALS	0/17	0	18.5 ± 7.3	10–21
10^{-2}	None	16/18	89	— —	21, 56
	XR	0/22	0	14.6 ± 5.0	9–28
	ALS	0/16	0	18.9 ± 8.1	9–33
10^{-3}	None	13/15	87	— —	51, 59
	XR	0/22	0	18.9 ± 7.2	11–39
	ALS	1/12	8	27.8 ± 12.2	11–51
10^{-4}	None	2/3	67	— —	43[c]
	XR	5/19	26	39.1 ± 13.0	13–59
	ALS	0/8	0	27.2 ± 10.6	11–42
10^{-5}	None	—	—	— —	—
	XR	3/4	75	— —	22[c]
	ALS	3/4	75	— —	22[c]
No virus control	XR	33/34	97	— —	12[c]
	ALS	17/18	94	— —	8[c]

[a] (No. of mice surviving on Day 60 after virus inoculation)/(no. of mice developing tumors) (see Table I).

[b] Survival from day of virus inoculation of those animals dying before day 60.

[c] Single animal.

that the oncogenic potential of a specified dose of virus was higher in immunosuppressed mice.

The effect of immunosuppression on the growth of MSV-induced tumors is shown in Table II. Whereas death within the first 60 days after virus inoculation was exceptional in intact mice, immunosuppressed mice succumbed to progressive tumor growth and few survived this period. As shown in Table II, both the percentage of immunosuppressed mice alive at day 60 and the length of survival of those mice dying before day 60 increased as the dose of virus inoculated decreased. Although not shown in Table II, the length of survival assessed from the day of tumor appearance was also found to be dependent on the amount of virus in the initial inoculum, possibly reflecting the infection of more cells by higher doses of virus, thus presenting a more formidable neoplastic challenge to the animal than occurred with lower virus doses.

Having established that irradiation of the host prior to infection with MSV increased the susceptibility to both the induction and progressive growth of this tumor, it was of interest to determine if resistance to tumor induction would return with the passage of time after irradiation. At specific intervals ranging from 1 to 21 days after receiving 400 R of total body X-irradiation, A/HeJ mice were challenged with 0.1 ml of a 1:10 dilution of MSV. Inoculation of unirradiated mice resulted in only 11/19 (58%) developing tumors, 6 of which never attained the dimensions of the usual tumors and regressed rapidly. In contrast, inoculation of mice irradiated up to 2 wk previously resulted in a high incidence of tumors (Table III), and it was not until 3 wk after irradiation that resistance to tumor induction became apparent as demonstrated by a lower tumor incidence and increased latency periods. Coincident with this recovery of resistance to tumor induction, the ability of mice

TABLE III. Development of Resistance to Murine Sarcoma Virus (Moloney) Following Prior Irradiation in Adult A/HeJ Mice.

Treatment	Time elapsed[a] (days)	Tumor incidence[b]		Latency period (days)	
		No.	%	Mean ± SD	Range
XR	1	15/15	100	5.2 ± 1.6	4–9
XR	7	15/15	100	5.3 ± 0.5	5–6
XR	14	14/15	93	4.6 ± 0.8	4–7
XR	21	11/15	73	7.9 ± 1.8	5–11
None	—	11/19	58	6.5 ± 1.7	4–10

[a] The period of time between irradiation and virus inoculation.

[b] (No. of mice developing tumors)/(no. of mice inoculated with MSV).

to resist progressive tumor growth also returned in a time-dependent manner. As shown in Table IV, animals inoculated 1 wk after irradiation demonstrated an increased ability to resist progressive tumor growth by comparison with those inoculated 1 day after irradiation, and virus inoculations occurring 3 wk after irradiation produced few progressively growing tumors.

Discussion. The results presented indicate that the immunocompetence of the host is a prime determinant of its resistance to both the development and progressive growth of MSV-induced tumors. The difference between our results and previously reported data showing no increase in susceptibility to tumor induction following sublethal irradiation (2) could possibly be due to the use of different strains of mice or the use of 400 R of total body X-irradiation in our studies instead of the 350 R used in previous studies. However, this difference is most likely due to the use of low virus doses (10^{-4} and 10^{-5} dilutions) at which decreased resistance to tumor induction was most apparent in immunosuppressed animals. Whereas in the previous study the lowest virus dose used (10^{-3} dilution) induced approximately a 50% tumor incidence in both untreated and X-irradiated mice, a dilution of 10^{-4} in our studies resulted in only 13% of the untreated mice developing tumors as compared to 80% of the immunosuppressed mice developing tumors.

This increase in susceptibility to tumor induction following immunosuppression can be explained by either of two hypotheses which are not mutually exclusive. First, assuming that resistance to tumor induction is based on the development of antivirion immune reactivity after infection, the inoculation of high doses may present an animal with more virus than can be effectively neutralized. If this excess is large enough, no difference in susceptibility would be observed between intact

TABLE IV. Survival of Normal and Irradiated Adult A/HeJ Mice with Murine Sarcoma Virus (Moloney)-Induced Tumors.

Treatment	Time elapsed[a] (days)	Survival[b]		Survival period (days) of mice dying before 60 days	
		No.	%	Mean ± SD	Range
XR	1	0/15	0	24.8 ± 6.7	13–34
XR	7	0/15	0	35.8 ± 10.6	17–53
XR	14	6/14	43	47.0 ± 5.2	36–56
XR	21	9/11	82	— —	21, 39
None	—	11/11	100	— —	—

[a] The period of time between irradiation and virus inoculation.

[b] (No. of mice surviving 60 days after virus inoculation)/(no. of mice developing tumors) (see Table III).

and immunosuppressed mice. However, at lower doses of virus, low levels of immunity might be sufficient to account for the observed decrease in oncogenicity by virus neutralization. This view is supported by the reports indicating that most MSV-transformed cells continuously release infectious virus (4), and that the amount of virus present in these tumors is related to the initial virus dose used to induce the tumor (10). This released virus then infects neighboring cells and contributes to continued tumor growth (14). Thus the neutralization of infectious virus might well interfere with tumor growth.

A second hypothesis is that immunity to tumor development depends upon an immune response directed against virus-induced tumor-specific transplantation antigens on the surfaces of infected cells. Infection by large doses of virus could then present both intact and immunosuppressed mice with more tumor cell antigen than either could effectively cope with, resulting in tumor growth. Challenges with lower doses of virus would however result in the presence of fewer transformed cells (*i.e.*, less antigen) and a normal immune response against these cells might prevent their development into a palpable tumor. Although the operation of either of these mechanisms alone could account for the the development of tumor immunity, it seems most probable that resistance is due to a combined action of the two together.

The role of the immune system in the limitation of susceptibility to the induction and progressive growth of virus-induced tumors is not unique to the murine sarcoma virus system, but is also known to be significant in determining the outcome of infection of experimental animals with other oncogenic viruses. Recent evidence has also indicated that the level of immunocompetence is a significant factor in the incidence of tumors in man. It has become apparent that patients undergoing therapy with immunosuppressive drugs (15–17) or in states of depressed immunological competence (18–20) are significantly more prone to the development of malignancies than the general population. The results presented for MSV correlate well with these findings and emphasize the role of the immune response in regulating both initial susceptibility and outcome of infection with oncogenic viruses.

Summary. In adult CBA/Wh mice the incidence and mean latency periods of tumors appearing following infection with MSV were dependent upon the dose of virus inoculated and the immunological state of the host. Animals of reduced immunological competence had a 10-fold higher susceptibility to tumor induction as well as a decreased latency period to tumor appearance compared with controls. Moreover, whereas these tumors regressed in intact adult mice, they tended to grow progressively in immunosuppressed hosts. These results indicate that the immunological competence of the host is a prime determinant of susceptibility to the oncogenic action of murine sarcoma virus.

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