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Natural History of M-MSV Tumors in Mice Carrying Endogenized Moloney Leukemia Virus*

L. Chieco-Bianchi, E. D'Andrea, A. De Rossi, P. Zanovello, F. Ronchese, and D. Collavo

A. Introduction

The conservation of endogenous type C DNA provirus sequences throughout evolution has raised the question of whether or not they exert normal physiological functions. Among the different hypotheses advanced has been suggested that a genetically transmitted virus could serve to protect the host, possibly via immune reactions, against related, more virulent viruses that may be acquired from the outside [14]. No substantial evidence for this hypothesis has been provided, however. Years ago during studies aimed at investigating in vivo interaction between endogenous and exogenous type C murine retroviruses, we noticed that AKR mice which had been injected with Moloney murine sarcoma virus (M-MSV) developed tumors with a longer latent period than that observed in mice of conventional strains. Furthermore, these late appearing tumors showed an unusual growth pattern, which was characterized by a slow but continuous progression until the host's death [2]. Subsequently, these findings were confirmed in larger studies using different mouse strains [4, 7, 8]. Figure 1 depicts the general pattern of tumor behavior that has emerged; mice characterized by early endogenous ecotropic virus activation are resistant to early M-MSV oncogenesis, but late appearing progressive tumors are observed in the majority of strains. While late tumor progression is probably due to immunological tolerance of cytotoxic T-lymphocytes (CTL) toward virus-coded antigens [6], the mechanism underlying resistance to early tumor induction is still poorly understood. BALB/Mo mice, which carry the exogenous Moloney leukemia virus (M-MuLV) as an endogenous virus integrated at a single locus (*Mov-1*) on chromosome 6 [1, 11], offer the unique opportunity of studying whether the full expression of genetically transmitted M-MuLV confers resistance against the antigenically related (M-MuLV) M-MSV complex. Indeed, in a first series of experiments [9], it was found that the natural history of induced tumors in these mice is quite similar to that observed in AKR type mice. In addition, resistance to early tumors appears related to the time course of M-MuLV activation as well as to the presence in the serum of normal mice of antibodies possessing specific binding capacity to M-MuLV surface determinants. The possibility that this particular tumor pattern might be influenced by these antibodies, or by virus-specific CTL activity, is discussed in this study.

B. Results and Discussion

I. Biological Activity of Natural Antibodies

The detection of natural antibodies specific for M-MuLV in the serum of adult BALB/Mo mice by means of a ^{125}I-labeled protein A binding assay prompted us to investigate its possible role in M-MSV oncogenesis.

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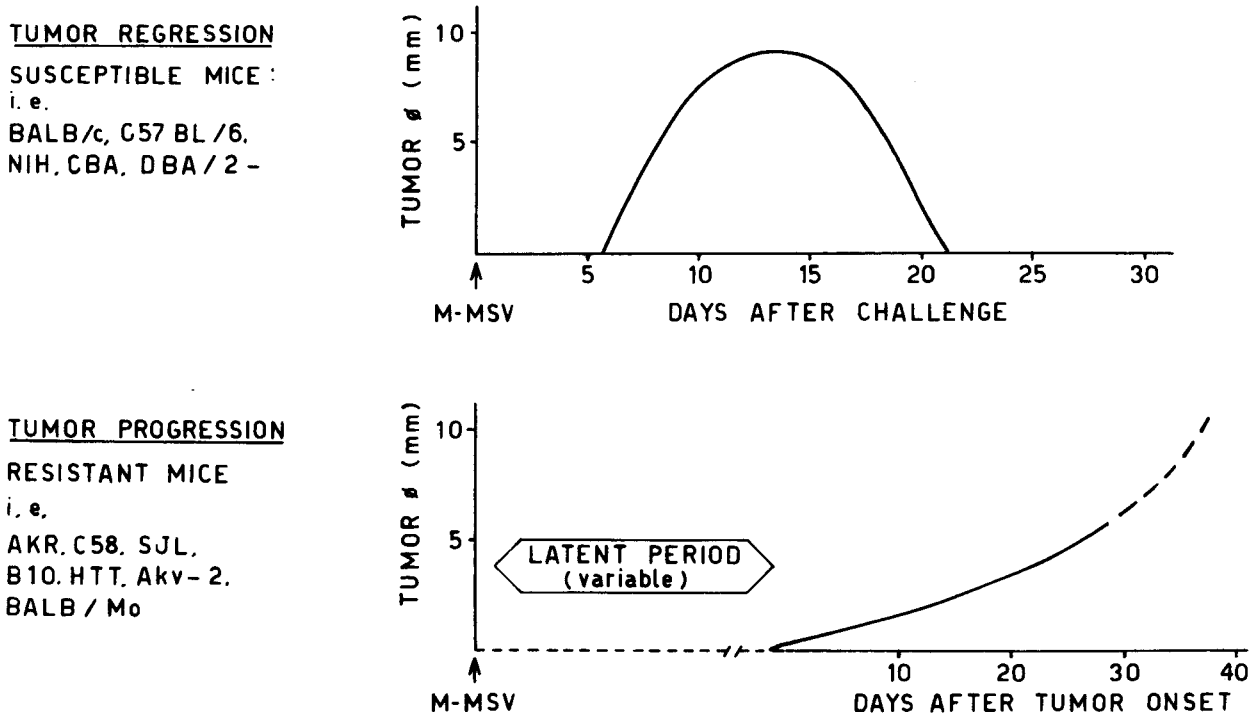

Fig. 1. Patterns of tumor behavior following M-MSV inoculation in adult mice of different strains

We first assayed the binding capacity of serum pooled from 3-month-old BALB/Mo mice on SC-1 mouse cells chronically infected with M-MuLV by immunofluorescence, using sera from normal or M-MSV injected adult BALB/c mice as controls. As shown in Table 1, immunofluorescence positive (IF+) cells were detected with BALB/Mo serum and with M-MSV immune BALB/c serum, while cells incubated with normal BALB/c serum were negative. Thus, BALB/Mo serum apparently pos-

Table 1. Reactivity of BALB/c and BALB/Mo sera on M-MuLV chronically infected SC-1 cells as evaluated by immunofluorescence assay[a,b]

Serum sample tested	Serum dilution		
	1:10	1:20	1:40
BALB/c	0.1	0.1	0.1
BALB/Mo	50 – 60	30 – 50	10 – 30
BALB/c-M-MSV[c]	80 – 100	60 – 80	50 – 60

[a] The immunofluorescence assay was performed by incubation of acetone-fixed cells with serum from tested mice followed by incubation with goat anti-mouse Ig fluorescinated serum (National Cancer Institute, Bethesda, USA) at 1:40 dilution

[b] Values refer to the percentage of immunofluorescence-positive cells

[c] Donor mice were immunized by i.m. injection of M-MSV (2×10^6 FFU/0.10 ml), and subsequently i.p. boosted twice at 2 – 3 week intervals with the same virus dose

Table 2. Virus neutralization by sera of normal and M-MSV injected BALB/c and BALB/Mo mice[a]

Serum sample tested	Serum dilution		
	1:20	1:40	1:80
BALB/c	0.01	0.01	0.01
BALB/Mo	20.60	7.60	0.01
BALB/c-M-MSV[b]	98.25	84.30	52.70
BALB/Mo-M-MSV[b]	26.80	9.80	0.01

[a] The assay was performed by M-MSV focus reduction on SC-1 cells as reported [10]; 200 FFU/0.20 ml were incubated with serial dilutions of sera at room temperature for 1 h before culture infection. Values refer to the percentage of neutralization calculated as $=[1-(Vn/Vo)]\times100$

[b] See Table 1 footnote c

Table 3. Blocking of virus neutralization by normal BALB/Mo serum[a]

Serum sample tested	Serum dilution		
	1:30	1:60	1:120
BALB/c-M-MSV	97.80	74.80	44.50
BALB/c-M-MSV + BALB/c	98.00	73.40	44.00
BALB/c-M-MSV + BALB/Mo	91.00	49.70	11.20

[a] Blocking of neutralization was carried out by incubating for 1 h at room temperature M-MSV with pooled sera, at 1:20 dilution, from normal, 2-month-old, BALB/c or BALB/Mo donors, followed by an additional 1 h incubation with two-fold serial dilutions of BALB/c-M-MSV immune serum (see Table 1, footnote c). Values refer to the percentage of neutralization calculated as $=[1-(Vn/Vo)] \times 100$

sesses binding capacity for M-MuLV induced cellular antigens as well.

We then performed virus neutralization assay by evaluating M-MSV focus reduction on SC-1 cells. Table 2 shows that, compared to M-MSV immune BALB/c serum, very little if any neutralizing activity was exerted by normal BALB/Mo serum and, more interestingly, by the putative M-MSV immune BALB/Mo serum. However, preincubation of M-MSV with normal BALB/Mo serum, followed by incubation with M-MSV immune BALB/c serum, remarkably reduced the neutralizing activity of the latter (Table 3). This finding is similar to that observed with monoclonal antibodies specific for gp52 of mouse mammary tumor virus [13], and suggests that natural antibodies found in BALB/Mo mice may recognize viral antigenic determinants distinct from, but adjacent to, the target site for neutralizing antibody. Thus, the observation that BALB/Mo serum binds to M-MuLV and yet competes with neutralizing activity of M-MSV immune serum would indicate its potential blocking capability. Indeed, in preliminary experiments, facilitation in M-MSV tumor growth was observed in BALB/c mice treated with repeated injections of normal BALB/Mo serum.

II. Generation of Virus-Specific CTL

We have repeatedly observed that M-MuLV neonatally injected mice, challenged as young adults with M-MSV, do not regress their sarcoma and are unable to generate virus-specific CTL [3, 5, 6]. Since activation of endogenous M-MuLV in BALB/Mo mice takes place only after 1–2 weeks of postnatal life [9, 12], we considered it of interest to ascertain whether virus-specific CTL could be generated in adult BALB/Mo mice challenged with M-MSV.

However, since the BALB/Mo mouse line was originally derived from an M-MuLV infected (BALB/c×129) blastocyst, the possibility that residual heterozygosity deriving from the 129 (H-2^b) strain might interfere with CTL activity due to a lack of H-2 restriction had to be considered. Accordingly, F1 mice were produced by mating BALB/c (H-2^d) females with BALB/Mo males; these F1 hybrids, albeit heterozygous for the *Mov-1* locus, are quite similar to the parental BALB/Mo mice as far as time course and levels of M-MuLV expression, and M-MSV tumor response are concerned (unpublished data). Therefore, spleen cells from BALB/c and (BALB/c×BALB/Mo)F1 donors previously injected with M-MSV were restimulated in vitro with LSTRA (H-2^d) Moloney leukemia or normal (BALB/c×BALB/Mo)F1 spleen cells, and then assayed on ^{51}Cr-labeled LSTRA targets. Table 4 shows the results of a typical experiment. Spleen cells from the M-MSV injected F1 mice did not lyse LSTRA leukemic targets, while those from BALB/c mice gave high cytotoxicity. Furthermore, restimulation of BALB/c effector cells with spleen cells obtained from 8-week-old normal F1 donors was highly effective in producing CTL activity, and demonstrated that F1 spleen cells express antigenic determinants relevant for CTL generation. Thus, in agreement with results obtained by infecting newborn mice with exogenous MuLVs [3, 5, 6], it appears that a state of immunological tolerance involving a CTL subpopulation is also present in F1 mice (and by inference in their BALB/Mo parent).

Table 4. CTL generation from spleen cells of BALB/c and (BALB/c×BALB/Mo)Fl mice injected with M-MSV[a]

Effector cells	Stimulator cells	Target cells	% specific ^{51}Cr release at effector/target cell ratio of		
			30:1	10:1	3:1
BALB/c	LSTRA	LSTRA	54	40	21
BALB/c	(BALB/c×BALB/Mo)Fl	LSTRA	68	35	25
(BALB/c×BALB/Mo)Fl	LSTRA	LSTRA	1	1	1

[a] Eight-week-old mice were splenectomized 14 days after M-MSV inoculation. Cytotoxic T-lymphocyte activity was evaluated after restimulation in vitro with LSTRA cells (a BALB/c transplantable leukemia originally induced by M-MuLV) or (BALB/c×BALB/Mo)Fl spleen cells pretreated with mitomycin C [5]

In conclusion, these data indicate that natural anti-M-MuLV antibodies found in BALB/Mo mice are not involved in resistance to early tumor induction by M-MSV, but instead, together with virus-specific CTL unresponsiveness, might be responsible for lethal evolution of the induced tumors.

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