EFFECT OF MuLV-RELATED GENES ON PLASMACYTOMAGENESIS IN BALB/c MICE

By MICHAEL POTTER,* JANET W. HARTLEY,‡ JUDITH S. WAX,§ and DANIEL GALLAHAN*

*From the *Laboratory of Genetics, National Cancer Institute, and ‡The National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205; and §Litton Bionetics, Kensington, Maryland 20795*

Plasmacytomas can be induced in high frequency in BALB/cAn mice by the intraperitoneal injection of mineral oils, branch chain alkanes such as pristane (2,6,10,14-tetramethylpentadecane), or the implantation of solid plastic materials (1). Most of the common inbred strains, e.g., C57BL/6, C3H/He, DBA/2, CBA/T6T6, AKR are resistant (2). Plasmacytomas can be induced in strain NZB mice that lack endogenous ecotropic viral loci. The incidence in this strain is lower than in BALB/c and the latent periods are much longer (2, 3). The pathogenesis of plasmacytoma development has not been established, but several factors have been shown to play a role. The granulomatous tissue in the peritoneum provides a microenvironment that is essential for plasmacytoma growth (4, 5). Nonrandom chromosomal translocations have been found in 45 of 46 plasmacytomas (6, 7) that disturb the regulation of the *c-myc* oncogene locus on chromosome 15 (8–11). The role of endogenous retroviruses in BALB/c has long been suspected as another possible pathogenic factor as type C budding particles have been found in primary plasmacytomas (12). N- and B-tropic type C viruses have been isolated from primary plasmacytomas (2, 13); also in one tumor a mink cell focus-forming virus (MCF)[1] has been recovered (13). B-tropic ecotropic type C murine leukemia virus (MuLV) and recombinants such as MCF viruses are capable of infecting other cells in the BALB/c host, thereby increasing the chances for mutagenic insertions into the host genomes in a fashion similar to that observed in bursal lymphomatosis in chickens (14, 15). The role of such spreading somatic cell infections as a factor in plasmacytomagenesis could be tested by eliminating ecotropic proviral loci. It has been shown that strains BALB/c and DBA/2 each have a single ecotropic MuLV locus. *Cv* (or *emv*-1) is on chr 5 in BALB/c (16) while the ecotropic locus in DBA/2 is on chr 9 (17). A second gene *Rmcf*^{s} that specifies sensitivity to infection with MCF viruses is closely linked to *Cv* on chr 5 in BALB/c (18). DBA/2 carries an allele of *Rmcf* that determines relative resistance to MCF viruses (*Rmcf*^{r}) (18); resistance is semi-dominant. *Rmcf* phenotype was determined by testing tissue cultures of fibroblasts from tail biopsies for susceptibility to infection by appropriate MCF viruses. *Rmcf* trait may be determined by a classical pair of alleles or one of the phenotypes could be

[1] *Abbreviations used in this paper:* chr, chromosome; IAP, intracisternal A particles; IdU, 5-iododeoxyuridine; MCF, mink cell focus-forming virus; MuLV, murine leukemia virus.

determined by the presence of a retroviral sequence. Because of the close linkage of *Rmcf*^{s} and *Cv* and since during introgressive back-crossing large segments of chromatin surrounding given marker alleles are introduced, it is possible to construct a congenic ecotropic MuLV free stock by introducing the *Rmcf*^{r} marker gene onto the BALB/c background. In the present experiments we have back-crossed *Rmcf*^{r} for six generations (N6) onto BALB/c and then at N6 *Rmcf*$^{s/r}$ heterozygotes were mated to each other and the progeny typed for induction of MuLV. Induction negative, MCF-resistant mice were mated and, as predicted, were found to be *Rmcf*$^{r/r}$; these are the source of the BALB/c.DBA/2 *Rmcf*^{rr}*Fv-1*^{bb} congenic stock. Liver DNA from these mice was hybridized to an ecotropic-specific probe (Fig. 1) and no band was detected, confirming that the DBA/2-derived DNA stretch on chr 5 introduced by introgressive back-crossing contained *Rmcf*^{r} and replaced the region in BALB/c that contained the *Cv* locus.

Typings of mice for alleles of *Fv-1* and *Rmcf* and for induction of MuLV were performed using tissue cultures prepared from tail biopsies of weanling or young adult mice (19). *Fv-1* typing was determined by XC tests on replicate tail cultures each infected with a dilution of standard N- or B-tropic MuLV chosen to give clear distinction between the parental strains (20). *Rmcf* typing was performed by the UV-mink procedure (18). Briefly, tail cultures were infected with selected MCF viruses: AKR-13 (N-tropic host range) and CB208 (B-tropic host range). The HIX strain of Moloney MuLV-derived MCF viruses was used for typing hybrids that would be resistant to both these viruses by virtue of *Fv-1* restriction. 3 d after infection the cultures were exposed to UV-irradiation and overlaid with mink lung cells (ATCC CCL64). 6 or 7 d later the cultures were scored for cytopathic foci; presence of *Rmcf*^{r} was indicated by a 10-fold or greater reduction in titer relative to the sensitive BALB/c parent.

For MuLV induction tests, subconfluent tail cultures were treated with 20 μg/ml of 5-iododeoxyuridine (IdU) for 48 h. Medium containing the inducer was removed, the cell sheet rinsed, and fresh medium containing SC-1 mouse cells added to permit amplification of titers of induced virus. After 10–12 d and at weekly intervals thereafter as required, the mixed cultures were passaged and tested for ecotropic virus production by the XC test or by immunofluorescence (21). Negative induction tests were terminated after three passages.

We also constructed BALB/c congenic stocks carrying the *Fv-1*^{n} alleles of DBA/2 origin. The *Fv-1* locus is thought to control the production of a host protein that interacts with ecotropic retrovirus and restricts an early step in retroviral synthesis (see reference 22). The outcome of this restriction is a drastic decrease in the ability of N-tropic viruses to integrate in *Fv-1*^{bb} cells or the B-tropic viruses to integrate in *Fv-1*^{nn} cells. If B-tropic viruses were important in plasmacytomagenesis, C.D2*Fv-1*^{nn} mice could limit the ability of these viruses to infect other cells.

We constructed several BALB/c (*Fv-1*^{bb}) congenic stocks carrying *Fv-1*^{n} alleles of DBA/2. After seven introgressive back-crosses N7 *Fv-1*$^{n/b}$ heterozygotes were mated to each other and two stocks were derived from these progeny: BALB/c.DBA/2 N7 *Fv-1*^{nn} and BALB/c.DBA/2 N7 *Fv-1*^{bb}. Both strains were albinos and were homozygous for the following BALB/c alleles: *Idh-1*^{a}, *Pep-3*^{a} (chr-1), *Pgm-1*^{a} (chr 5), *ES-3*^{a} (chr 11), *Igh*^{a} (chr 12). The BALB/c.DBA/2 N7 *Fv-1*^{bb},

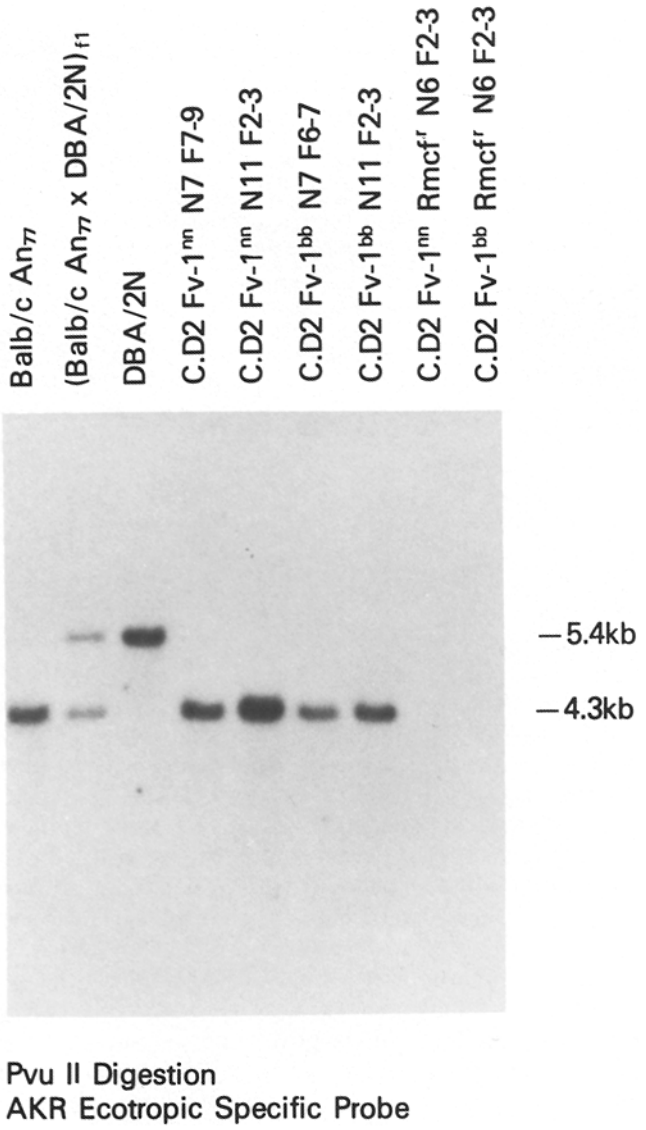

FIGURE 1. Southern blot hybridization of BALB/c.DBA/2 liver DNAs to ecotropic viral probe. 10 μg of DNA was digested to completion with restriction enzyme Pvu II. Probe was 400 bp of ecotropic specific envelop region of cloned Akv-1. Probe DNA was labeled by nick translation and hybridized at a concentration of 2×10^6 cpm/ml. Blots were washed in 0.3 × SSC at 65°C and exposed to film.

however, expressed Qa2$^+$, suggesting they carried part of chr 17 MHC locus of DBA/2 (23) or another regulating locus (see reference 24). At N11 the homozygous stocks BALB/c.DBa/2 N11 *Fv-1*^{nn} and BALB/c.DBA/2 N11 *Fv-1*^{bb} were again derived, neither of these was Qa2$^+$. A double congenic stock carrying *Fv-1*^{n} and *Rmcf*^{r} and lacking *Cv* was also constructed (BALB/c.DBA/2 *Rmcf*^{r} *Fv-1*^{nn} *Cv*^{0}).

TABLE I

Induction of Plasmacytomas in BALB/c.DBA/2 Congenic Mice

BALB/c.DBA/2 Strain or Congenic	No. mice	No. back-crosses*		Fv-1	Cv-1	Rmcf	% plasmacytomas at day indicated after 1st injection of pristane					Total
							150	200	250	300	350	
									%			
Fv-1^{nn} N7 F7-9	65	N7	7–9	nn	+/+	s/s	6.5	14	21	30	(35)	35
Fv-1^{bb} N7 F6-7 (Qa2$^+$)	61	N7	6–7	bb	+/+	s/s	0	6.4	15	26	(30)	30
Fv-1^{nn} N11 F2-3	65	N11	2–3	nn	+/+	s/s	1.5	6.1	23	27	—	27
Fv-1^{bb} N11 F2-3	55	N11	2–3	bb	+/+	s/s	1.8	23	42	51	(52)	52
Fv-1^{nn} Rmcf^r N6 F2-4	59	N6	2–4	nn	0/0	r/r	0	6.7	22	25	(39)	39
Fv-1^{bb} Rmcf^r N6 F2-4	57	N6	2–4	bb	0/0	r/r	12.0	33	45	63	—	63
BALB/cAnπ	245			bb	+/+	s/s	4.0	26	49	57	61	61
(BALB/c × DBA/2)F$_1$	140	F1	—	n/b	+/0	s/r	0	0	0	0	0	0
(DBA/2 × BALB/c)F$_1$ × BALB/c	100	N1	—	n/b	+/0	s/r	1	4	7	10	11	11
				b/b	+/+	s/s						

* First column indicates back-cross generation; second column indicates no. of inbred generations.

The mice were given three 0.5-ml injections of pristane spaced 2 months apart to induce plasmacytomas, and after 120 d were examined every 2 wk for the development of ascites. A drop of ascitic fluid was obtained by inserting i.p. a 25-gauge needle with semitransparent sleeve that permitted visualization of a successful tap. Smears of ascites fluid were stained with Wright-Giemsa stain and the diagnosis of a plasmacytoma was made by finding at least 10 characteristic hyperchromatic tumor cells per slide. As may be seen from Table I, BALB/c were highly susceptible while BALB/c × DBA/2 F1 hybrids resembled the DBA/2 parent and were resistant. 11% of the first generation back-cross mice to the BALB/c parent developed plasmacytomas. These results indicate DBA/2 carries two or three dominant genes that determine resistance to plasmacytoma induction. C.D2 N7 *Fv-1*^{bb} *Rmcf*^{rr} and C.D2 N11 *Fv-1*^{bb} were highly susceptible to plasmacytoma induction and the frequency was similar to those obtained in BALB/c (25). The other stocks gave lower, intermediate numbers of tumors. All three *Fv-1*^{nn} stocks showed this intermediate resistance. The *Fv-1*^{bb} N7 (Qa2$^+$) mice were also partially resistant.

The results indicate that the ecotropic MuLV locus is not essential for the development of plasmacytomas. Further, the *Qa2* and *Fv-1*^{n} genes of DBA/2 origin appear to be linked to genes governing resistance to plasmacytoma development. In light of the present data, it is difficult to implicate *Fv-1*^{n} *per se* as a gene that determines resistance to plasmacytoma induction. We are currently investigating whether the effects of these two genes is additive.

The results also indicate the potential for generating MCF viruses is also not required for plasmacytoma development. This does not, however, eliminate other endogenous retroviruses, e.g., the xenotropic type C viruses or intracisternal A particles (IAP) that could be activated intracellularly and through insertions be mutagenic. Evidence that the *mos* oncogene is activated by IAP reinsertion has been described in two tumors (26, 27). In both of these cases though, the tumors have been transplanted for considerable periods.

Summary

The role of spreading somatic cell infections with ecotropic MuLV viruses in the induction of plasmacytomas in BALB/cANπ mice was determined by con-

structing congenic mice that lacked the gene locus *Cv* that codes for ecotropic virus. DBA/2 mice that lack *Cv* on chromosome (chr) 5 carry a closely linked gene *Rmcf*^{r} that determines resistance to infection with mink cell focus-forming viruses (MCF). *Rmcf*^{r} was retrogressively back-crossed onto BALB/c for six successive generations to produce N6 mice. N6 mice were mated to each other to produce BALB/c.DBA/2 *Rmvf*^{r}/*Rmcf*^{r} homozygotes. This stock of mice lacked *Cv*, as demonstrated by DNA hybridization and were as fully susceptible to developing plasmacytomas as the parental BALB/c. A second congenic stock BALB/c.DBA/2 *Rmcf*^{r}/*Rmcf*^{r} *Fv-1*^{n}/*Fv-1*^{n} was also developed, but the mice of this stock showed a reduced incidence of plasmacytomas, as did BALB/c.DBA/2 *Fv-1*^{n}/*Fv-1*^{n} mice. These findings indicated *Fv-1* or a gene closely linked to it conferred partial resistance to plasmacytomagenesis. In constructing the BALB/c.DBA/2 *Fv-1*^{n}/*Fv-1*^{n} stock, a "control" congenic BALB/c.DBA/2 *Fv-1*^{b}/*Fv-1*^{b} was also developed at N6. Surprisingly, this stock carried the Qa2$^+$ trait. These mice were also partially resistant to plasmacytomagenesis, suggesting a gene on chromosome 17 (the location of *Qa2*) or a gene located elsewhere that regulates Qa2 expression is linked to a gene controlling partial resistance to plasmacytoma development.

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