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

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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)† 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‡ 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*) (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

† 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 \( Rmcf \) 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 \( Rmcf \) marker gene onto the BALB/c background. In the present experiments we have back-crossed \( Rmcf \) for six generations (N6) onto BALB/c and then at N6 \( Rmcf \) 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 \( Rmcf \); these are the source of the BALB/c.DBA/2 \( Rmcf \) ecotropic MuLV free 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 \( Rmcf \) 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 \( Rmcf \) 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 \( \mu \)g/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 \( Fv-1^n \) cells or the B-tropic viruses to integrate in \( Fv-1^m \) cells. If B-tropic viruses were important in plasmacytomagenesis, C.D2\( Fv-1^m \) 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 \( Fv-1^m \) 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 \( Fv-1^{bb} \),
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<sup>mm</sup> and BALB/c.DBA/2 N11 Fv-1<sup>bb</sup> were again derived, neither of these was Qa2*. A double congenic stock carrying Fv-1<sup>mm</sup> and Rmcf<sup>+</sup> and lacking Cv was also constructed (BALB/c.DBA/2 Rmcf<sup>+</sup> Fv-1<sup>mm</sup> Cv<sup>+</sup>).
### Table I

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

| BALB/c × DBA/2 Strain or Congenic | No. of mice | No. back-crosses* | Fv-1 | Cv-1 | Rmcf | % Plasmacytomas at day indicated after 1st injection of pristane Total |
|-----------------------------------|-------------|-------------------|------|------|------|---------------------------------------------------------------|
| Fv-1<sup>+</sup> N7 F7-9          | 65          | N7 7–9            | nn   | +/+  | s/s  | 6.5 14 21 30 (35) 55                                          |
| Fv-1<sup>+</sup> N7 F6-7 (Qa2<sup>+</sup>) | 61          | N7 6-7            | bb   | +/+  | s/s  | 0 6.4 15 26 (30) 50                                          |
| Fv-1<sup>+</sup> N11 F2-3        | 65          | N11 2–3           | nn   | +/+  | s/s  | 1.5 6.1 23 27 — 27                                           |
| Fv-1<sup>+</sup> N11 F2-3        | 55          | N11 2–3           | bb   | +/+  | s/s  | 1.8 23 42 51 (52) 52                                          |
| Fv-1<sup>+</sup> Rmcf<sup>+</sup> N6 F2-4 | 59          | N6 2–4            | nn   | 0/0  | t/r  | 6.7 22 25 (39) 39                                          |
| Fv-1<sup>+</sup> Rmcf<sup>+</sup> N6 F2-4 | 57          | N6 2–4            | bb   | 0/0  | t/r  | 12.0 35 45 63 — 63                                          |
| BALB/c × DBA/2                   | 245         | —                 | +/+  | h/h  | s/s  | 4.0 26 49 57 61 — 61                                          |
| BALB/c × DBA/2 × BALB/c          | 140         | F1 —              | n/b  | +/+  | s/r  | 0 0 0 — 0 0 — 0                                             |
| (BALB/c × DBA/2 × BALB/c) × BALB/c | 100        | N1 —              | h/h  | +/+  | s/s  | 1 4 7 10 11 11 — 11                                          |

*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<sup>+</sup> Rmcf<sup>+</sup> and C.D2 N11 Fv-1<sup>+</sup> 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<sup>-</sup> stocks showed this intermediate resistance. The Fv-1<sup>-</sup> N7 (Qa2<sup>+</sup>) 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<sup>-</sup> 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<sup>-</sup> 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/cAN<sup>+</sup> 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' that determines resistance to infection with mink cell focus-forming viruses (MCF). Rmcf' 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 Rmcf'/Rmcf' 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'/Rmcf' Fv-1'/Fv-1' was also developed, but the mice of this stock showed a reduced incidence of plasmacytomas, as did BALB/c.DBA/2 Fv-1'/Fv-1' 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'/Fv-1' stock, a "control" congenic BALB/c.DBA/2 Fv-1b/Fv-1b 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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