Natural Killer Cell Suppression of Friend Virus-Induced Preleukemic Hemopoietic Stem Cells

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To determine whether hemopoietic cells infected with Friend polycythemia-inducing spleen focus-forming virus (SFFVp) are conserved or suppressed via natural surveillance in leukemia-resistant adult mice, we engrafted C57BL/6 recipients with isologous transgenic (donor origin marker) or natural killer (NK) cell-deficient B6 beige marrow cells exposed to SFFVp in vitro. Both groups of primary recipients were viremic and nonleukemic. Spleen cells from primary SFFVp-infected chimeras were engrafted into irradiated leukemia-susceptible secondary recipients to reveal dormant leukemia and grew as tumors of donor origin in 8 of 38 (21%) and 33 of 47 (70%) instances, respectively. Treatment of marrow donors and recipients with anti-asialo GM1 serum resulted in the depression of NK cell activity and the rapid development of dormant leukemia. We conclude that NK cells are an effective surveillance mechanism able to suppress SFFVp-induced preleukemic stem cells.

Natural killer (NK) cells (YAC-1 cells) are a hemopoietic subpopulation which can destroy tumor cells and virus-infected cells (19, 48). Since NK cells rapidly eliminate susceptible targets without prior sensitization, they may represent a first line of defense or immune surveillance mechanism able to be directed against murine leukemia virus (MuLV)-infected hemopoietic targets (6, 23). The ability to lyse host cells destined to progress from precancerous to overtly transformed populations, while sparing normal hemopoietic progenitors, would represent a stage-specific killer cell-target cell recognition system (7, 14).

Marrow cell grafts reconstitute NK cell function in irradiated recipient mice provided that the marrow microenvironment is intact (15). Thus, murine NK cells are marrow dependent (18, 27, 32) and able to renew themselves in histocompatible recipients (15, 16). An intriguing correlation is that adult mice depleted of functional NK cell activity following treatment with the bone-seeking isotope ⁸⁹Sr are also deficient in their ability to resist bone marrow allografts (3) and to suppress Friend virus (FV)-induced erythro-leukemogenesis (28). These phenomena include the recognition of hemopoietic histocompatibility antigens and are genetically controlled (2, 5, 51). Thomsen et al. (45) have demonstrated that NK cell activation following lymphocytic choriomeningitis virus infection is associated with the acquired ability of mice to inhibit the growth of syngeneic pluripotent hemopoietic spleen colony-forming stem cells (CFUs). The decreased transplantability of syngeneic CFUs latently infected with replication-defective Friend polycythemia-inducing spleen focus-forming virus (SFFVp) is a hallmark of preleukemic change (10). Multistage Friend disease can be induced by helper-free SFFVp in leukemia-sensitive (*Fv-2^{ss}*) B6.C hosts (C57BL/6-H-7^b [47N] congenic with C57BL/6 at *Fv-2* [4, 10, 50]) and is characterized by early effects upon erythroid progenitor cells (30) followed

weeks later by the evolution of transplantable and often curable FV-induced tumors (10, 34, 49, 50). The NK cell deficiency in ⁸⁹Sr-treated C57BL/6 (+/+) mice (17) and the partial abrogation of potent *Fv-2*-determined (31, 37) leukemia resistance suggest that NK cell effectors are responsible for the rejection of SFFVp-induced preleukemic stem cells.

Replication of SFFVp-MuLV in adult +/+ and NK cell-deficient *bg/bg* mice. We previously described an experimental system in which adult leukemia-resistant +/+ mice could be chronically infected with FV complex (11). Since the majority of such mice were NK cell competent, it was important to determine whether NK cells can act in concert with *Fv-2^{rr}* (i.e., leukemia resistance) to protect +/+ hosts against SFFVp disease. To determine the replicative and leukemia-inducing potentials of standard SFFVp-MuLV in untreated adult NK cell-deficient C57BL/6 beige (*bg/bg*) mice, we inoculated groups of 20 +/+ and 20 *bg/bg* adult mice intravenously with NB-tropic (8) SFFVp-MuLV (5×10^2 focus-forming units- 10^3 XC PFU per animal). Ten days later, eight mice within each group were sacrificed. No spleen enlargement or focus formation (8) was observed. Pooled plasma samples within each group were assayed in adult Swiss mice for the presence of SFFVp. Both +/+ and *bg/bg* mice replicated SFFVp transiently. Estimated peak MuLV titers (3×10^3 to 8×10^3 XC infectious centers per 10^6 spleen cells) were observed in both +/+ and *bg/bg* mice on day 30 (data not shown). Beyond this time, there was a steady decline in XC infectious centers retrieved from +/+ mice. No evidence of MuLV persistence could be obtained beyond day 60. In contrast, *bg/bg* mice were unable to clear MuLV during 120 days of observation, although a histologic examination of spleen tissue revealed no malignant change. Defects in *bg/bg* macrophage activation and sluggish T-lymphocyte responses might contribute to host sensitivity to MuLV (22, 25, 35, 36). In summary, the beige defect in NK (and other) cell functions does not reverse *Fv-2^{rr}*-mediated resistance to leukemia induction in intact mice but does

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FIG. 1. Experimental design for studying the establishment of preleukemia (dormant tumors) by SFFVp in *Fv-2^{+/+}* and NK cell-deficient *bg/bg* chimeras. *Fv-2^{+/+}* mice were lethally irradiated (8 Gy from a dual-¹³⁷Cs-source small-animal irradiator [Gammacell 40; Atomic Energy of Canada, Ltd., Commercial Products, Ottawa, Ontario, Canada]) and grafted with 10^7 *MyK* or *bg/bg* bone marrow cells exposed in vitro to FV complex or vehicle; 30 to 80 days later, a spleen fragment was removed from each animal and assayed for virus content (8, 13) as well as for the frequency of CFUs (20, 46) and erythropoietin-dependent erythroid progenitor cells (day 14 burst-forming units) (BFUe) (20). Bone marrow from primary recipients that were disease free over the 30 to 80 days was retransplanted into lethally irradiated B6.C secondary recipients. Several assays were used to discriminate between uneventful reconstitution (i.e., "normal" SFFVp-infected CFUs [sfv-CFUs on figure]) and repopulation with SFFVp-induced erythroleukemia cells (sfv-TCFUs on figure) (12). This same protocol for creating primary *MyK*→*+/+* SFFVp-infected chimeras was adapted to include the administration of anti-asialo GM1 serum (0.2 ml of a 1:10 dilution) (Wako Chemicals, Dallas, Tex.) to deplete *MyK* donors and *+/+* recipients of NK cell functions (15). FFU, Focus-forming units; Epo, erythropoietin; Hct, hematocrit.

TABLE 1. Ability of SFFVp-MuLV to induce erythroleukemia in irradiated adult C57BL/6 mice repopulated with *MyK* or *bg/bg* marrow cells

Study group ^a (no. of mice)	Disease status ^b between days 30 and 80 postinoculation (% hematocrit)	Estimate of virus recovered ^c		
		SFFV focus-forming units/ml of plasma (range)	MuLV XC infectious centers per spleen	BFUe/spleen ^d
1. <i>MyK</i> → <i>+/+</i> + SFFVp-MuLV (48)	6 Leukemic (80)	5.3×10^4	3.1×10^8	3.8×10^4
	4 Regressed erythro- blastosis (70-42)	8.0×10^3	1.1×10^8	8.5×10^3
	38 Normal (44)	6.8×10^4 (2×10^4 – 20×10^4)	7.5×10^7	5.0×10^3
2. <i>bg/bg</i> → <i>+/+</i> + SFFVp-MuLV (56)	5 Leukemic (62)	3.5×10^4	4.5×10^8	1.0×10^4
	4 Regressed erythro- blastosis (80-43)	4.0×10^3	7.3×10^7	NT ^e
	47 Normal (40)	5.0×10^3 (1×10^3 – 20×10^3)	5.6×10^7	4.2×10^3 ^f
3. <i>bg/bg</i> → <i>bg/bg</i> + SFFVp-MuLV (30)	6 Leukemic (61)	1.3×10^3	NT	NT
	24 Normal (38)	4.0×10^2 ($<1 \times 10^3$ – 10×10^2)	NT	NT
4. <i>bg/bg</i> → <i>bg/bg</i> + <i>Fv-2</i> -adapted SFFVp (11)	11 Leukemic (70)	$\geq 10^3$	$\geq 10^8$	$>50 \times 10^3$

^a Prior to engraftment, donor marrow cells were exposed to SFFVp-MuLV (10^4 focus-forming units– 6×10^3 XC focus-inducing units per 10^7 cells). These values also represent the amounts of cells and virus administered to each animal. All *+/+* or *bg/bg* irradiated adults (20 each) that did not receive a marrow graft died of hemopoietic failure between days 18 and 30.

^b In all instances, the form of leukemia induced was associated with the appearance of erythroblasts as the predominant immature cell type in the peripheral blood and the appearance of BFU-EI within the spleen. These findings, together with the severe polycythemia observed, are consistent with SFFVp-induced Friend disease. Leukemia regression observed in a small percentage of animals was associated with a dramatic decline in the hematocrit and spleen size.

^c Plasma samples from individual mice were harvested 30 to 40 days postengraftment. A pool within each group was appropriately diluted and inoculated intravenously into adult *Fv-2^{ss}* mice. Individual samples were also tested (see ranges). Spleen foci induced by SFFVp were scored on day 10 (8). Nucleated cells contained within the spleens of primary chimeric mice were monitored for the production of NB-tropic MuLV (11) at the time of retransplantation into B6.C secondary recipients.

^d Washed spleen cell preparations were assayed for the presence of BFUe (20). The estimated of BFUe frequency within the spleens of untreated adult *+/+* mice was approximately 20 per 5×10^5 cells (a total of 4×10^3 BFUe per spleen).

^e NT, Not tested.

^f Among this population of BFUe (a pool of 50), we noted that all committed myeloid progenitors contained giant sudanophilic granules characteristic of the beige mutation. Erythromyeloid progenitors (granulocyte-erythrocyte-macrophage-megakaryocyte) were thus confirmed.

FIG. 2. Immunoprecipitation of SFFVp-MuLV proteins from labeled cell extracts. Analysis of L-[35 S]methionine-labeled proteins encoded by SFFVp and NB-tropic helper virus (MuLV) was done by polyacrylamide gel electrophoresis (29). Viral proteins were immunoprecipitated from metabolically labeled cell extracts with antiserum to MuLV gp70 *env* (goat antiserum no. 145 to Scripps Cancer Research Foundation 60A strain MuLV gp70 was a gift from M. Vogt, The Salk Institute, La Jolla, Calif.) (lanes 1 to 5, 7, 10, 13, 16, and 20) or with goat antiserum (to Moloney strain MuLV p30, prepared by R. Mueller-Lantzsch and H. Fan and provided by M. Vogt) that recognizes *gag* gene-encoded antigenic determinants (lanes 8, 11, 14, 17, and 21). Control reactions included normal goat serum with appropriate cell extracts (lanes 6, 9, 12, 15, and 19). Lanes: 1 and 3, normal rabbit kidney cells infected with C57BL-adapted (i.e., *Fv-2''* adapted) SFFVp; 2, 4, and 5, NIH 3T3 cells infected with a NB-tropic SFFVp-MuLV used for all animal inoculations; 6 to 8, primary bone marrow cell culture (≤ 18 h); 9 to 11, primary spleen cell culture; 12 to 14, cultured hepatocytes and passenger cells, all obtained from a leukemic *bg/bg* $\rightarrow$ $+/+$ chimeric mouse; 15 to 17, spleen cells cultured for 1 week; 19 to 21, ability of SFFVp-MuLV released from primary spleen cell cultures to recruit NIH 3T3 cells and establish a pattern of virus-encoded protein expression identical to that of the original inoculated virus preparation. FrSFFV = Friend strain of SFFVp.

affect the ability of the mice to clear the virus, particularly the MuLV component.

Leukemogenic potential of SFFVp in viremic *MyK* $\rightarrow$ $+/+$ versus *bg/bg* $\rightarrow$ $+/+$ marrow chimeras. The solid resistance of *bg/bg* mice to SFFVp made it possible to test the hypothesis that NK cells can suppress the progression or induction of syngeneic stem cells to the preleukemic state. Experiments were designed as outlined in Fig. 1 to investigate the relationship between marrow-reconstituted NK (YAC-1) cell functions and resistance to the primary induction of erythroleukemia and polycythemia by SFFVp. We repopulated lethally irradiated adult mice (6- to 8-week-old $+/+$) with *bg/bg* marrow or transgenic C57BL/6 (*MyK*) marrow (38) cells exposed to FV complex in vitro. *MyK* mice are female

$+/+$ mice harboring a fusion gene (MK') which includes the structural gene of herpesvirus thymidine kinase and which was used to identify transplanted cells as being of donor origin later in the study. Study groups 1 and 2 (Table 1) compare the effects of the beige mutation in the donor marrow upon the sensitivity to SFFVp disease. While all *MyK* $\rightarrow$ $+/+$ chimeras were chronically infected with SFFVp-MuLV, only 12% developed fatal SFFVp-induced erythroleukemia. An average fivefold enlargement of the spleen was associated with a significant increase in the numbers of erythropoietin-independent erythroid progenitors (day-14 burst-forming units) (BFU-EI). A total of 8% of the viremic *MyK* $\rightarrow$ $+/+$ mice expressed regressing erythroblastosis. BFU-EI frequency in regressed spleens declined

TABLE 2. Effect of NK cell depletion upon the ability of SFFVp-MuLV to induce erythroleukemia and dormant tumors in adult C57BL/6 mice

Study group (no. of normal primary chimeras)	Strain	Results of histological examination ^a (spleen wt in g) for lethally irradiated secondary recipients on day 30	No. (%) of donors ^b designated tumor dormant
1. <i>MyK</i> →+/+ (38)	B6.C	Active hemopoiesis, no invasion of white pulp (0.2 g) or subcapsular malignant erythropoiesis (≥0.68 g)	8 (21)
2. <i>bg/bg</i> →+/+ + SFFVp-MuLV (47)	B6.C	Active hemopoiesis (0.08 g) or polycythemia with malignant erythropoiesis (≥0.55 g)	33 (70)
	+/+	Active hemopoiesis with only beige neutrophils (i.e., sudanophilic granules) (0.1 g)	0 (0%); Hosts resistant to outgrowth of dormant tumor cells
3. <i>bg/bg</i> → <i>bg/bg</i> (24)	B6.C	Uneventful reconstitution (20% died of apparent hemopoietic failure) or rapid outgrowth of erythroid tumor of donor origin	17 (63)
4. <i>MyK</i> →+/+ + anti-asialo GM1 serum ^c	B6.C	Uneventful reconstitution (tumor outgrowth predominated)	14 (78)
5. No graft ^d	Irradiated B6.C + SFFVp (4×)	Ineffective hemopoiesis (no colonies, no virus recruitment) (≤0.08 g)	

^a Recipients became leukemic with severe polycythemia (hematocrit, 65%), splenomegaly, peripheral erythroblastosis, and BFU-EI by days 15 to 30. Rapid onset of disease was not due to SFFVp recruitment of *Fv-2^{ss}* hemopoietic cells (group 5, no graft, virus only).

^b Donor cells from primary viremic chimeras were monitored for dormant FV tumors by engraftment into *Fv-2^{ss}* mice (11).

^c *MyK* donors were inoculated intravenously 24 h prior to marrow cell harvest with 0.2 ml of anti-asialo GM1 serum (rabbit; 10 mg/ml; Wako Chemicals) to deplete NK cell activity (24). Engrafted +/+ recipients were given intravenous injections of anti-asialo GM1 serum 2 days before and 5, 18, and 30 days after lethal irradiation and cell transfer.

^d On day 40, infected and control mice were examined clinically and monitored for SFFVp-MuLV (8, 11) and the presence of marrow- and spleen-derived dormant FV tumors.

fourfold as compared with peak leukemic responses (hematocrit, 70 to 42%). However, approximately 50% of these progenitors maintained erythropoietin independence.

Adult +/+ mice engrafted with marrow from NK cell-deficient *bg/bg* donors were hemopoietically reconstituted and chronically infected (Table 1, group 2). Despite a reduced ability to replicate SFFVp (Table 1, *bg/bg*→+/+ normal), the overall pattern and frequency of clinical disease were similar to those in *MyK*→+/+ mice, with one exception. The frequency of individual *bg/bg*→+/+ mice harboring a persistent population of BFU-EI (when present, about one-third of all erythroid progenitors able to be cultured) was 88%, as compared with <20% for *MyK*→+/+ mice. We speculated that sustained BFU-EI in the range of 15 to 30% of all erythroid progenitors detected in individual disease-free animals predicts SFFVp-induced tumor dormancy (see below).

The low level of primary tumor induction in *bg/bg*→+/+ mice may have resulted from the action of radioresistant NK cells (8, 9) within +/+ recipients or from an intrinsic deficiency in SFFVp target cells in *bg/bg* marrow (Table 1). To address these issues, we determined the frequency of leukemia induction by SFFVp contained in an *Fv-2^{ss}*-adapted FV complex (clone BB6 [16]) in *bg/bg*→*bg/bg* marrow chimeras. The results (Table 1, study groups 3 and 4) indicate that NK cell-deficient *bg/bg*→*bg/bg* mice resist leukemia induction (20% leukemia) and standard SFFVp

replication (100-fold less than that in *MyK*→+/+ mice). However, *Fv-2*-adapted SFFVp efficiently replicated and induced erythroleukemia with polycythemia in *bg/bg*→*bg/bg* marrow chimeras (Table 1, group 4), indicating that *Fv-2* is the major resistance determinant.

SFFVp envelope (*env*) gene expression in chimeric mice. Since it was conceivable that SFFVp variants could be selected in +/+ hosts and since the SFFVp-MuLV strain BB6 contains a preformed recombinant (Friend mink cell focus-forming virus [FrMCF]), we sought to determine the stability of SFFVp-MuLV *env* gene expression in marrow chimeras and the potential for FrMCF participation in the leukemogenic process (1, 41). Primary cultures of marrow, spleen, and liver from leukemic *bg/bg*→+/+ and *MyK*→+/+ mice were metabolically labeled with [³⁵S]methionine. Immunoprecipitation followed by electrophoretic analysis (29) was done with polyclonal antiserum which detects ecotropic and recombinant xenotropic virus-related *env* gene products. The pattern of *env*-encoded glycoprotein expression in *bg/bg*→+/+ and *MyK*→+/+ (only beige shown here) marrow and spleen cells cultured from leukemic mice (Fig. 2, lanes 7, 10, and 16) was identical to that for the virus used to initially infect these mice (lanes 2, 4, and 5). In addition, a precursor form of the *gag* gene product (Pr65^{gag}) was easily detected (goat anti-MuLV *gag*, lanes 8, 11, and 17). Primary liver cultures containing circulating tumorous hemopoietic cells were also positive for these proteins (lanes 13 and 14).

FIG. 3. Dot hybridization of somatic cells from *MyK* transgenic mice (female C57BL/6 harboring the tandemly duplicated fusion gene *MK'* [38]). *MK'* fuses the promoter-regulatory region of the mouse metallothionein-1 (MT-1) gene to the structural gene of herpes simplex virus thymidine kinase, which is foreign to the mouse. This sequence provides an easy means of detecting by hybridization hemopoietic cells of donor origin following engraftment into lethally irradiated adult isologous recipients. The indicated numbers of cells were spotted onto Genescreen Plus (New England Nuclear Corp., Boston, Mass.) and hybridized with the *Bgl*III-*Pvu*II probe. 1°, Primary; 2°, secondary.

Cells nonproductively infected with *Fv-2^{tr}*-adapted SFFVp contained an altered SFFVp *env* gene product (lanes 1 and 3) which was clearly distinguished from gp52^{SV} by its apparent molecular weight of 42,000. Although SFFVp-infected *bg/bg*→+/+ or *MyK*→+/+ spleen cells cocultured with adherent NIH 3T3 cells easily recruited the established NIH 3T3 cell line (lanes 20 and 21), no FrMCF-related *env* gene products were observed, i.e., *env*-related gp80 and gp69. Thus, disease progression was not associated with the emergence of an SFFVp host-adapted variant or mutant or with FrMCF (33, 43) preexisting in the standard preparations of NB-tropic FV complex.

Tumor dormancy in NK cell-deficient SFFVp-infected

bg/bg→+/+ chimeras. We have previously shown that a small percentage of SFFVp-infected *MyK*→+/+ mice harbor transplantable populations of SFFVp-induced preleukemic stem cells which are environmentally dependent in that the full progression of leukemic CFUs can be achieved only after retransplantation into B6.C mice (11). To determine whether NK (YAC-1) effector cells play a role in restricting the progression of SFFVp-infected CFUs to a preleukemic state, we monitored all of the viremic, nonleukemic animals (Table 1, study groups 1, 2, and 3) for the presence of dormant SFFVp-induced tumor cells. Spleen cell suspensions from individual hemisplenectomized donors were engrafted into lethally irradiated B6.C mice. In some instances, these same cells were engrafted into +/+ mice (Table 2, group 2). While SFFVp-infected *MyK*→+/+ mice had a low frequency of SFFVp-induced preleukemia, SFFVp-infected *bg/bg*→+/+ mice contained a high frequency of dormant tumors (Table 2, groups 1 and 2). These cells were seen to be environmentally dependent and were suppressed in *Fv-2^{tr}* secondary recipients. Spleen cells of donor origin (i.e., containing *MK'*; Fig. 3) retrieved from B6.C secondary recipients cultured in vitro contained many BFU-EI and formed tumors when infused into lethally irradiated tertiary B6.C and +/+ mice. Thus, the frequency of BFU-EI was 140 ± 18 per 10^5 spleen cells, and the incidences of leukemia in tertiary B6.C and +/+ hosts were five of six and six of nine, respectively. These transplantable tumors were established as environmentally independent, since no restriction could now be imposed by *Fv-2^{tr}* environments. *bg/bg*→*bg/bg* chimeric mice were also seen to have an increased frequency of established dormant tumors (Table 2, group 3).

To further characterize the potential role of NK cells in protecting against the progression of SFFVp-infected cells to a dormant tumor state, we injected prospective *MyK* marrow donors whose cells were later exposed to SFFVp-MuLV with anti-asialo GM1 serum (24). In addition, a group of 18 +/+ recipients of these marrow cells were infused with anti-asialo GM1 serum before and after bone marrow transplantation (Table 2, footnote c). After this serum treatment, recipient +/+ mice were depleted of NK cell function (percent specific lysis of YAC-1 cells, <5% at a 100:1 effector cell/target cell ratio) and chronically infected with SFFVp-MuLV ($\geq 10^3$ focus-forming units per ml of plasma). Thirty days after engraftment, each animal was sacrificed (after the last injection of serum), and individual marrow preparations were retransplanted into a group of congenic susceptible B6.C recipients. Of 18 NK cell-depleted *MyK*→+/+ donors, 14 had dormant Friend leukemia (Table 2, group 4). This is the first time we have observed preleukemic CFUs this early. The speed of preleukemia development in these anti-asialo GM1 serum-treated mice further indicates that the presence of functioning mature NK effector cells in primary recipient mice may temporarily retard SFFVp-induced stem cell progression to preleukemia. The donor origin of leukemia cell outgrowth in lethally irradiated B6.C adult mice was established by using, when appropriate, the beige neutrophil granulation marker as well as the presence of the fusion gene *MK'* derived from the *MyK* donor (Fig. 3) (38). Finally, chronic administration of high-titer SFFVp-MuLV intravenously into lethally irradiated B6.C adult mice in the absence of a marrow graft failed to recruit endocolonies or establish viremia (Table 2, group 5) (40). These results show that there is a dramatic rise in the frequency of dormant FV tumors in NK cell-deficient mice under the conditions established and suggest that these

FIG. 4. Concept of tumor progression in C57BL/6 *Fv-2^{rr}* mice. NK cell control of hemopoietic reconstitution may down-regulate totipotent hemopoietic stem cell (toti-HSC on figure) commitment to pluripotentiality, resulting in the maintenance of stem cell reconstituting activity and the restriction of the size of the SFFVp-infected CFUs (sfv-CFUs) pool. It is not known whether totipotent hemopoietic stem cells are recruited by SFFVp. RBC, Erythrocytes; sfv-HSC, SFFVp-infected hemopoietic stem cells. See the legend to Fig. 1 for definitions.

effectors are able to reject or suppress SFFVp-altered preleukemic CFUs in primary *Fv-2^{rr}*-infected hosts.

We have previously shown SFFVp-induced alterations of hemopoietic stem cell frequency and function (10–12). Mice reconstituted with serially transplanted stem cells can survive for ≥ 8 months free of disease. Thus, the high frequency of SFFVp-infected CFUs (12) directly predicts the presence of reconstituting totipotent hemopoietic stem cells (13) which have yet to be directly isolated and identified. Using this same marrow reconstitution model, we showed that a small percentage of primary SFFVp-infected *MyK*→+/+ chimeras harbor BFU-EI (21) and ultimately express a fatal or regressing form of Friend disease. We confirmed the difference in NK cell function between *MyK*→+/+ and *bg/bg*→+/+ chimeras and determined that NK progenitor or effector cells can be reconstituted within *MyK*→+/+ marrow chimeras in the presence of replicating SFFVp-MuLV. Injection of polyinosinic acid · polycytidylic acid was associated with a significant boosting of this activity. A retrospective study revealed that individually tested SFFVp-infected +/+ mice unresponsive to NK cell boosting in vivo could be classified as a group of viremic *MyK*→+/+ mice with an acquired defect in NK cell activity. The majority of these animals developed leukemia. The data presented here implicate NK cells as antileukemogenic effectors able to restrict the establishment of tumor dormancy by SFFVp. Fully progressed tumorous CFUs rapidly proliferate in the spleen and marrow cavities of B6.C recipients, are of donor origin, and have no reconstituting activity. Within 30 days postengraftment, tumorous CFUs are responsible for clinical disease which terminates in typical malignant Friend erythroleukemia. These immortalized CFUs are thus environment independent (11, 34), since retransplantation of cells from secondary B6.C mice into either +/+ or B6.C recipients is no longer associated with leukemia suppression. Our concept of tumor progression is depicted in Fig. 4. The beige defect in NK cell function is severe but not complete, since lysis of YAC-1 target cells does occur if one incubates the cells for 18 h rather than the usual 4 h (23, 39). Moreover, the ability of beige mice to reject marrow allografts is diminished but not absent. The more profound suppression of NK cell function by anti-asialo GM1 serum (37) is associated with a much higher incidence of preleukemia in

chimeric mice (Table 2). One cautionary note is advisable: neither the beige defect nor the effects of anti-asialo GM1 serum are absolutely specific for NK cells (42, 44). Treatment of mice with a potent source of monoclonal anti-NK-1.1 antibodies (26), when available, would strengthen the argument that NK cells are the effectors involved if the effects resemble those of anti-asialo GM1 serum.

The observation that *Fv-2^{rr}* mice previously treated with ^{89}Sr became susceptible to the leukemogenic and immunosuppressive effects of SFFVp-MuLV (28) indicates that both the NK cell barrier and the *Fv-2* barrier were broken. The effects of ^{89}Sr may extend beyond the effects on NK cells (28), unless the *Fv-2^{rr}* function is mediated by precursor NK cells not eliminated by treatment with anti-asialo GM1 serum or seriously affected by the *bg/bg* defect. Cloned cells with potent NK cell function infused into mice with defective NK cell function were able to restore marrow allograft reactivity, resistance to metastatic growth of tumor cells, and resistance to the induction of leukemia by fractionated doses of irradiation (47). It would therefore be interesting to determine whether such cells can protect against the induction of preleukemia and/or leukemia under the conditions described in this communication. In any event, the data reported here provide support for the contention that NK cells protect animals against the early stages of tumor progression.

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