Proc. Natl. Acad. Sci. USA
Vol. 76, No. 11, pp. 5784–5788, November 1979
Genetics

Antibody-induced modulation of Friend virus cell surface antigens decreases virus production by persistent erythroleukemia cells: Influence of the *Rfv-3* gene

(infectious center assay/immune response gene/murine leukemia virus/immunofluorescence)

BRUCE CHESEBRO, KATHY WEHRLY, DON DOIG, AND JANE NISHIO

Laboratory of Persistent Viral Diseases, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratories, Hamilton, Montana 59840

Communicated by Lloyd J. Old, August 6, 1979

ABSTRACT **The *Rfv-3* gene was found to influence the level of Friend leukemia virus production in spleens of leukemic mice later than 30 days after virus inoculation. *Rfv-3*$^{r/s}$ mice [(B10.A × A)F_1 and (B10.A × A.BY)F_1] had decreased spleen virus levels 30–90 days after virus inoculation compared to *Rfv-3*$^{s/s}$ mice [A.BY, A, BALB.B, and (BALB/c × A)F_1)]. In (B10.A × A)F_1 × A backcross mice the spleen virus titer segregated with the level of viremia. The *Rfv-3* gene appeared to act by controlling anti-Friend virus antibody production. The interaction of antiviral antibody with infected cells led to a decrease in release of infectious virus by late leukemic spleen cells in *Rfv-3*$^{r/s}$ mice to 1/300th that in *Rfv-3*$^{s/s}$ mice. This decrease in virus release appeared to be due to interference with the virus budding process due to antibody-mediated modulation of virus-induced cell surface antigens.**

The Friend virus (FV) complex induces a rapid erythroleukemia in the spleen and bone marrow of adults and newborns of many mouse strains (1–4). Several different mouse genes have been found to affect the course of this leukemia (4). In particular, two genes within the major histocompatibility gene complex (*H-2*) influence the incidence of spontaneous recovery from leukemia (5–7). In addition, we have found that an autosomal non-*H-2*-associated gene, *Rfv-3*, acts in a complementary fashion with *H-2* to induce recovery from leukemia (8, 9). In the absence of the appropriate high-recovery *H-2* genotype, the resistant *Rfv-3*$^{r/s}$ genotype influences recovery from viremia at around 30 days after FV inoculation even in the presence of progressive fatal leukemic splenomegaly (9). The serum of leukemic nonviremic mice contains antiviral antibodies that can neutralize virus and lyse leukemia cells in the presence of complement (10). However, the leukemic spleen cells of these individuals are not lysed *in vivo* or *in vitro* because they appear to have lost 80% of their FV-induced cell surface antigens (11).

The mechanism of the *Rfv-3* gene influence on these events is not completely understood; however, we believe that one likely possibility is that *Rfv-3* may influence anti-FV antibody production. These antibodies appear to be able to modulate or disperse FV-induced cell surface antigens on leukemia cells (11–13). The present data indicate that modulation of FV antigens is associated with a decrease in virus production by infected cells, to 1/300th that in *Rfv-3*$^{s/s}$ mice. It appears likely that this is due to interference with viral budding as a result of dispersion of cell surface viral antigens by the antibody-induced modulation process. This means of reduction of virus production could occur with any virus that buds from the cell surface, and it might be an important host defense mechanism for control of virus infection *in vivo*. It also could explain some conditions of virus persistence in the presence of an apparently adequate humoral immune response.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Mice. A.BY, A/WySn (A), B10.A, and (BALB/c × A/J)F_1 mice were purchased from The Jackson Laboratory. Other F_1 hybrids, (B10.A × A)F_1 × A backcross mice, and BALB.B mice were bred at the Rocky Mountain Laboratory. All mice were 3–6 months of age when inoculated with FV, except that the A mice were 11–14 months old because age-matched individuals were not available at that time.

Virus. The B-tropic strain of FV was used (14), and stocks were grown as described (6). Mice were inoculated intravenously with 15, 150, or 1500 spleen focus-forming units (FFU) of FV to induce leukemia. Mice were followed for development of FV leukemia by spleen palpation under ether anesthesia (6). At 30–90 days after FV injection, mice were bled and killed for analysis of spleen weight and virus. Mice with spleen weight >0.5 g were considered to be leukemic.

Virus Assays. The Friend-MuLV helper virus (F-MuLV) was assayed on S^+L^- cells (clone D56 of 3T3FL) provided by R. Bassin (15). The details of this assay were as described (16). Titers were given as plaque-forming (PFU).

Preparation of Plasma and Spleen for Virus Assay. Mice were bled from the tail or axilla, and blood was collected in heparinized tubes. Plasma was removed and stored at −70°C in small portions either undiluted or diluted 1:110 in buffer plus 2% fetal calf serum. Spleens were removed aseptically, weighed, and dissociated in phosphate-buffered balanced salt solution (10) to give a 10% (wt/vol) suspension. Cells were sedimented at 500 × *g* for 5 min, and supernatant fluid was stored at −70°C in small portions for virus assays. The cells sometimes were washed three times and assayed by the S^+L^- infectious center assay as described (17). The minimal plasma F-MuLV titer detectable was 220 PFU/ml = 1 PFU/0.5 ml (volume assayed) × 110 (minimal dilution assayed). Similarly, the minimal spleen F-MuLV titer detectable was 200 PFU/g of spleen = 1 PFU/0.5 ml (volume assayed) × 10 (minimal dilution assayed) × 10 ml/g (i.e., 10% spleen suspension).

Immunofluorescence. Washed spleen cells were tested for leukemia virus-specific membrane or cytoplasmic immunofluorescence by using described techniques (11) with fluorescein-conjugated goat anti-Moloney leukemia virus serum

Abbreviations: FV, Friend virus complex; A, A/WySn; FFU, focus-forming units; F-MuLV, Friend murine leukemia helper virus; PFU, plaque-forming units.

provided by Jack Gruber (National Cancer Institute Viral Oncology Program).

Cell Transfer Experiments. Washed leukemic spleen cells (25×10^6) were injected intravenously into x-irradiated [900 rads] normal or hyperimmunized mice. Seven days later, the spleens of these mice were removed, and the cells were analyzed for virus-specific immunofluorescence and incidence of F-MuLV infectious centers. Hyperimmune recipients were produced by immunizing (B10.A × A.BY)F_1 mice, which had recovered from FV-induced leukemia, with two to five inoculations of syngeneic FV-induced leukemic spleen cells (11). Hyperimmune recipients were used for cell transfer experiments 7–14 days after the last booster inoculation and had high titers of cytotoxic anti-FV antibody in their plasmas.

RESULTS

Spleen F-MuLV Levels in Different Mouse Strains. In order to investigate the possible role of mouse genes in the control of virus production, spleens from leukemic mice of various strains were tested for F-MuLV 30–90 days after FV inoculation (Fig. 1). Although there was some overlap of individual mice from most strains in the range 10^5–10^6 PFU/g, (B10.A × A.BY)F_1 and (B10.A × A)F_1 mice had lower titers of spleen F-MuLV (10^3–10^6 PFU/g) than did A.BY and A parental strains and (BALB/c × A)F_1 and BALB.B mice (10^5–3×10^7 PFU/g). The A mice used in this experiment were unusually old (11–14 months) at the time of virus inoculation, and this factor may have been responsible for the generally lower spleen virus titers observed in these mice compared to the congeneic A.BY mice.

The mouse strain distribution of high and low levels of spleen F-MuLV was similar to that previously seen for levels of viremia in leukemic mice at 30 days or later after FV inoculation (9, 10). Because the differences in viremia were shown to be influenced by the *Rfv-3* gene in these mouse strains, it seemed likely that this gene was also responsible for the differences in spleen virus titer.

Association of Spleen and Plasma Virus Levels. When plasma and spleen F-MuLV titers were compared quantitatively in splenomegalic mice 30–90 days after FV inoculation, virus titers in plasma and spleen appeared to be associated in individual mice, regardless of the strain tested (Fig. 2). Mice with undetectable plasma virus (<220 PFU/ml) had low spleen virus levels (2×10^3–5×10^5 PFU/g) and mice with very high plasma virus ($\geq 4.4 \times 10^4$ PFU/ml) had high spleen virus titers (6×10^5–1.5×10^7 PFU/g). Mice with intermediate plasma virus titers (3×10^2–3×10^4 PFU/ml) usually had intermediate levels of spleen virus. Thus, it appeared likely that F-MuLV viremia was related to the level of virus production by cells in the spleen, which was the location of the majority of the leukemia cells in this disease. The correlation observed between viremia and spleen virus titer in these mouse strains provided additional evidence that the *Rfv-3* gene might influence viremia by control of spleen virus production.

To confirm this possibility, viremia and spleen virus were tested in a backcross between (B10.A × A)F_1 mice (low viremia, low spleen virus) and A mice (high viremia, high spleen virus). The results indicated that plasma and spleen virus titers were associated in this backcross (Fig. 3). Thus, the genes controlling plasma virus levels and spleen virus titers segregated together, and spleen F-MuLV titer appeared to be influenced by the *Rfv-3* gene in this population.

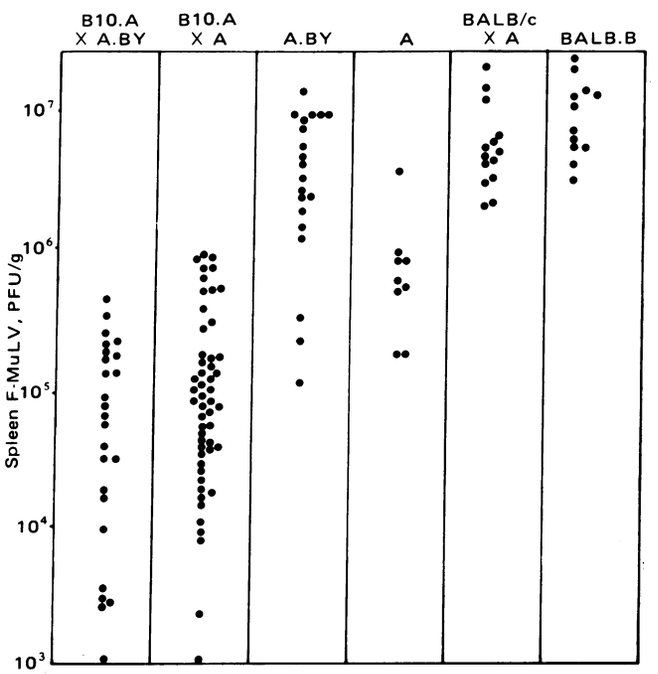

FIG. 1. Spleen F-MuLV in leukemic mice 30–90 days after FV inoculation.

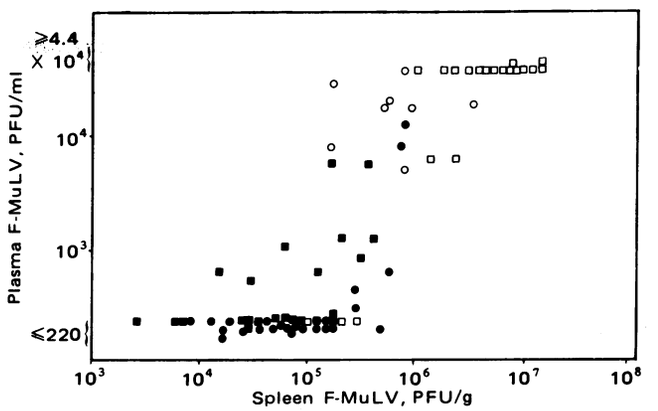

FIG. 2. Plasma and spleen F-MuLV titers in individual leukemic mice of various strains 30–90 days after inoculation with FV. ○, A; □, A.BY; ■, (B10.A × A.BY)F_1; ●, (B10.A × A)F_1.

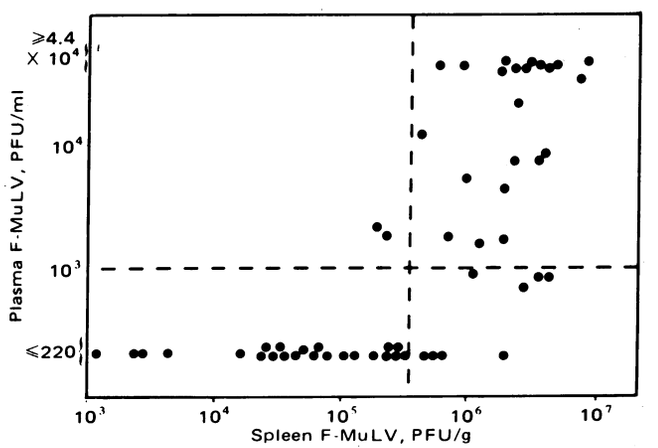

FIG. 3. Association of plasma F-MuLV titer and spleen F-MuLV titer in individual leukemic (B10.A × A)F_1 × A backcross mice 35 days after FV inoculation (1500 FFU). Results with the parents of this backcross at 30–90 days after FV inoculation (ref. 18; Figs. 1 and 2) indicated that most *Rfv-3*$^{r/s}$ mice had plasma F-MuLV titers $<10^3$ PFU/ml and spleen F-MuLV titers $<4 \times 10^5$ PFU/g, whereas most *Rfv-3*$^{s/s}$ mice had plasma and spleen F-MuLV titers greater than these values. Based on these titers to define the *Rfv-3* genotypes in the backcross population, there was a strong association between plasma and spleen F-MuLV titers ($\chi^2 = 23.67$; $P < 0.001$).

Table 1. Comparison of virus-specific immunofluorescence and incidence of F-MuLV-producing cells in leukemic spleens of three mouse strains early (9 days) and late (>30 days) after FV inoculation

Strain	Days after FV*	*Rfv-3* genotype	Mice, no.	% cells with virus-specific immunofluorescence†		% F-MuLV infectious centers‡
				Intracellular	Membrane	
(BALB/c × A)F_1	30–40	*s/s*	7	60 (53–70)	67 (58–76)	36 (16–73)
BALB.B	30–40	*s/s*	12	61 (52–66)	65 (52–76)	50 (26–90)
(B10.A × A)F_1	30–90	*r/s*	11	58 (51–72)	19 (9–34)	0.17 (0.015–2.0)
(B10.A × A)F_1	9	*r/s*	6	71 (52–78)	68 (57–77)	60 (25–90)

* Dose was 150 or 1500 FFU of FV inoculated intravenously.
† Values shown are the arithmetic mean, with range in parentheses.
‡ Values shown are the geometric mean, with range in parentheses.

Decreased Virus Production by Late (B10.A × A)F_1 Leukemic Spleen Cells. Previous experiments indicated that the percentage of leukemic spleen cells that had FV-induced cell surface antigens detectable by membrane immunofluorescence in *Rfv-3*$^{r/s}$ mice at 30–90 days after FV inoculation (late) was one-fifth to one-half that at 8–10 days after FV inoculation (early) (11). This decrease in FV antigens was influenced by the *Rfv-3* gene and did not occur in mice with the *Rfv-3*$^{s/s}$ genotype (18). To study the possible association between the decrease in FV antigens and the decrease in virus titers observed in *Rfv-s*$^{r/s}$ mice 30–90 days after FV inoculation, the percentage of cells containing membrane or cytoplasmic viral antigens and the percentage of cells scored as F-MuLV-producing infectious centers *in vitro* was determined on the same leukemic spleen cell populations. (B10.A × A)F_1 mice (*Rfv-3*$^{r/s}$) were compared to (BALB/c × A)F_1 and BALB.B mice (both *Rfv-3*$^{s/s}$) because A.BY and A mice were unavailable for this study.

At 30–40 days after FV inoculation, both (BALB/c × A)F_1 and BALB.B mice had a high incidence of spleen cells positive for virus-specific cytoplasmic and membrane immunofluorescence (52–76%), and most of these cells were scored as F-MuLV infectious centers (Table 1). Nine days after FV inoculation (B10.A × A)F_1 spleen cells were similar to the late (BALB/c × A)F_1 and BALB.B cells. In contrast, although late leukemic (B10.A × A)F_1 spleen cells were positive for cytoplasmic FV antigens (51–72%), these cell populations had a decreased incidence of cells expressing viral antigens on the cell surface (9–34%) and a very marked decrease in the incidence of F-MuLV infectious centers (0.015–2%). Thus, late (B10.A × A)F_1 leukemic spleen cells had 1/3rd the cells positive for membrane fluorescence and 1/300th to 1/200th the incidence of infectious centers of early (B10.A × A)F_1 cells or late (BALB/c × A)F_1 and BALB.B cells. These data provided direct evidence that there was a significant decrease in virus production by virus-infected late leukemia cells from (B10.A × A)F_1 mice.

Altered Virus Production after Adoptive Transfer of Leukemia Cells. We previously observed that FV-induced cells surface antigens, which were low on late leukemic (B10.A × A)F_1 spleen cells, were reexpressed when these cells were transferred to irradiated nonimmune recipients (11). To determine if the decreased virus production by these cells was also reversed in a nonimmune environment, late (B10.A × A)F_1 leukemic spleen cells were analyzed for F-MuLV infectious centers before and 1 week after transfer of 2.5×10^7 cells to lethally irradiated nonimmune and immune recipients. The results indicated that the low percentage of infectious centers seen in the original donor cell populations (0.01–2.7%) was increased 5-fold to 10,000-fold (4–100%) after transfer to irradiated nonimmune recipients (Table 2). In contrast, in irradiated immune recipients, percentage infectious centers was decreased (0.001–0.028%) compared to the donor cell populations. To determine if exposure of leukemia cells to an immune environment was also capable of decreasing the incidence of infectious centers from the high values seen in spleens 8–10 days after FV inoculation, early leukemic spleen cells from A.BY mice were transferred to irradiated immune and normal recipients. The percentage of F-MuLV infectious centers in the immune recipients was decreased to 1/10,000th to 1/2000th that in the nonimmune recipients and the original donor population (Table 2). The majority (51–70%) of spleen cells from all donors and recipients in these experiments contained cytoplasmic viral antigens detectable by immunofluorescence. Thus, most cells continued to be virus-infected in spite of the marked differences seen in the percentage of infectious centers.

These results demonstrated that the decreased levels of virus production seen in late leukemic *Rfv-3*$^{r/s}$ mice were a function of the immune environment of these mice because virus release

Table 2. Changes in percentage of F-MuLV infectious centers after transfer of leukemic spleen cells to irradiated immune and nonimmune recipients

Donor strain	*Rfv-3* genotype	Days after FV	% F-MuLV infectious centers		
			Donor	Normal recipients	Immune recipients
(B10.A × A)F_1	*r/s*	60	2.7	24; 40	0.028; 0.001; 0.001
		35	0.8	4; 4.7	0.001; 0.001; 0.001
		40	0.05	9	ND
		55	0.01	100	ND
A.BY	*s/s*	9	51	37	0.004; 0.003
		8	65	40; 62	0.020; 0.015

Leukemic spleen cells (2.5×10^7) were injected intravenously into normal or hyperimmunized (B10.A × A.BY)F_1 mice that first had been exposed to x-irradiation (900 rads). Seven days later, the recipients' spleen cells were tested for virus-specific immunofluorescence and F-MuLV infectious centers. Spleen cells from donors and from normal and immune irradiated recipients were 51–70% positive for virus-specific cytoplasmic immunofluorescence. ND, not determined.

was increased when leukemia cells were placed in nonimmune recipients and decreased when cells were transferred to immune recipients. These results parallel those observed for expression of FV-induced cell surface antigens (11, 18) and support the conclusion that antibody-induced modulation of FV cell surface antigens is associated with a decrease in virus release by infected cells.

DISCUSSION

A single non-*H-2* gene, *Rfv-3*, has been shown to influence FV viremia, expression of FV-induced cell surface antigens on leukemic spleen cells, and production of anti-FV leukemia cell antibody (18). The present results indicate that the *Rfv-3* gene also influences virus production by leukemia cells. At 30–90 days after virus inoculation, leukemic *Rfv-3*$^{r/s}$ mice had decreased titers of spleen F-MuLV compared to leukemic *Rfv-3*$^{s/s}$ mice. At this time, the percentage of leukemic spleen cells from *Rfv-3*$^{r/s}$ mice scoring as F-MuLV infectious centers was 1/300th to 1/200th that in early leukemic spleen cells from these mice and in late leukemic spleen cells from *Rfv-3*$^{s/s}$ mice. This striking decrease far exceeded the decrease (to one-third) in virus-specific membrane fluorescence seen in these same cell populations. Decreased virus release by infected cells appeared to account for the elimination of FV viremia seen in *Rfv-3*$^{r/s}$ mice (9, 18). Because neutralizing antibody and infectious virus–antibody complexes have been observed in viremic mice (unpublished observations), interaction of cell-free virus and anti-FV antibody did not seem to be significant in control of viremia.

Previous experiments indicated that the *Rfv-3* gene probably acted by influencing anti-FV antibody production (18). Anti-FV antibody appeared to decrease the expression of FV-induced cell surface antigens on leukemic spleen cells by the process of antigenic modulation (11–13, 18–23). The present data suggested that anti-FV antibodies were also responsible for the decrease in release of infectious virus by leukemia cells, because transfer of high-virus-producer cells to irradiated immune recipients caused a significant decrease in the percentage of cells scored as virus producers (Table 2). Although only *infectious* virus release was detected in the infectious center assay used in the present experiments, several facts suggested that *all* virus release by late *Rfv-3*$^{r/s}$ leukemia cells was markedly decreased. First, in cell-free spleen homogenates there was a close association of F-MuLV titers assayed by plaque assay and by reverse transcriptase assay (data not shown). Second, leukemic spleen cells did not have cell surface immunoglobulin detectable by immunofluorescence (11). Third, spleen cells tested in the infectious center assay were washed and plated at such low concentrations that it was highly unlikely that they could subsequently have picked up enough antiviral antibody from residual B lymphocytes to interfere with detection of infectious centers. Thus, release of antibody-coated virus did not appear to be the explanation for the decreased percentage of infectious centers seen.

Although antibody-induced modulation of cell surface antigens has been demonstrated in several systems (12, 13, 19–23) and appears also to operate in this model (11, 18), it remains unclear exactly how antigenic modulation could lead to a decrease in virus production without killing virus producer cells. The most likely explanation appears to be that the antibody-induced decrease in FV cell surface antigens involves dispersion or aggregation of viral antigens on the cell surface which in turn interferes with the final step in virus production, virus budding from the cell surface. Both the major envelope glycoprotein, gp70, and the glycosylated viral gag precursor polyproteins, gp95 and gp85 (24–27), are present on the cell surface of murine leukemia cells. It is conceivable that antibody-induced rearrangement of one of these cell surface viral protein molecules could interfere with the budding process and markedly decrease virus release. One might expect that this mechanism of decrease of virus release could operate even when viral cell surface antigens remained detectable by membrane immunofluorescence because the critical interfering event would be the rearrangement of viral proteins in the membrane rather than their removal.

This interpretation is supported by our observation that many (B10.A × A)F_1 leukemia cells still positive for FV-induced cell surface antigens did not score as F-MuLV producing infectious centers (Table 1). These cells did exhibit patching or capping of FV cell membrane antigens (data not shown), and therefore these antigens were probably "modulated" even though some molecules remained detectable on the cell surface (28). Furthermore, virus release need not have been totally eliminated in cells that did not score as infectious centers. We previously showed that the percentage of cells scoring as infectious centers in this assay was a function of the rate of virus production by cloned leukemia cells (17). Therefore, our present results could be due to a significant decrease, rather than a total cessation, of virus release by modulated cells.

Antibody-induced modulation of viral cell surface antigens has been observed in several virus systems (19–23) and could lead to a decrease in release of any viruses that bud through the plasma membrane of infected cells. Decreased virus release after treatment of cells with antibody has been documented for influenza, herpes simplex, and bovine leukemia viruses (29–31). In all three cases the effect was clearly due to antibody interference with virus release by the cell rather than simple neutralization of normally released virus. Although antibody-induced modulation of viral cell surface antigens was not postulated directly by the authors of those reports, it would appear to be a highly likely explanation for the results they observed. This possibility was strengthened by the requirement in the influenza system for bivalent rather than univalent antibody fragments (32), because antigenic modulation usually also requires bivalent antibodies (28, 33, 34). Furthermore, in the thymus-leukemia (TL) antigen system, antibody-induced modulation was reversible when modulated cells were cultured in the absence of antibody (13). Similarly in the bovine leukosis virus system, the antibody-induced decrease in virus release was reversible when cells were recultured in the absence of antibody (31). We have also observed reversibility of modulation *in vivo* with FV leukemia cells (Table 2).

Antibody-induced modulation of viral cell surface antigens may be a frequent finding in many infections with budding viruses and could be a major mechanism of host elimination of such infections. Antibody induction of a decrease in virus release could easily interfere with virus spread to uninfected cells. After modulation, the critical factor in determining the outcome of a virus infection would probably be the fate of the modulated cells. If the cells died, the infection would be eliminated, but if the cells survived, the infection would persist. The fate of the modulated cells might depend on many factors including cell type and location, virus type, and ability to escape other immune elimination mechanisms such as cell-mediated cytotoxicity or antibody-plus-complement-mediated lysis. For example, a short-lived bronchial or intestinal epithelial cell would probably die within a few days, leading to elimination of infection. However, a neuronal cell might survive indefinitely; a hematopoetic stem cell or leukemia cell might continue to divide, passing the infection onto daughter cells. If a modulated infected cell survived, this might allow some additional mechanisms of virus persistence to develop. Virus mutants with

altered envelope antigens could be expressed, leading to a renewed phase of active virus release and spread. Cyclical virus release associated with antigenic shifts have been observed with two retroviruses, visna and infectious equine anemia (35, 36). Virus mutants with defective virulence or interfering properties could also be selected. This has been observed with measles virus-infected cells after culture in the presence of antibody *in vitro* (37) and could be involved in development of the latent measles infection seen in patients with subacute sclerosing panencephalitis (38, 39).

The reason for the persistence of modulated FV leukemia cells in (B10.A × A)F_1 mice is not known. Because FV antigens are modulated, these cells are resistant to lysis by anti-FV antibody and complement (11). These mice have the *Rfv-3*$^{r/s}$ genotype which is associated with elimination of viremia and decreased virus release and is necessary for spontaneous recovery from leukemia (8, 9). However, they do not have the appropriate *H-2D* (*Rfv-1*) genotype, which is also necessary for recovery from leukemia (6, 7). Thus, they may lack a second genetically controlled mechanism essential for elimination of leukemic spleen cells. This missing mechanism appears to be the rapid generation of virus-specific cytotoxic T lymphocytes (40). However, even if late leukemic (B10.A × A)F_1 mice were finally able to generate appropriate cytotoxic lymphocytes, it is unclear whether or not modulated leukemia cells could be recognized and killed by these effector cells. In (C57BL/10 × A.BY)F_1 mice there is a high incidence of appearance of virus-specific cytotoxic lymphocytes associated with recovery from leukemic splenomegaly (40). However, in these mice we have recently discovered that a low level of active FV infection persists even after regression of splenomegaly (41). Therefore, a small number of FV-infected cells escape immune elimination, perhaps because viral cell surface antigens have been modulated. The relative kinetics of the development of cell-mediated and humoral virus-specific immunity in early stages of leukemia may determine the proportion of leukemia cells that are modulated before they can be lysed by cytotoxic T lymphocytes. If so, this could be critical in determining whether or not recovery from leukemia ultimately occurs.

1. Friend, C. (1957) *J. Exp. Med.* **105,** 307–318.
2. Metcalf, D., Furth, J. & Buffett, R. F. (1959) *Cancer Res.* **19,** 52–58.
3. Friend, C., Patuleia, M. C. & de Harven, E. (1966) *Natl. Cancer Inst. Monogr.* **22,** 505–522.
4. Lilly, F. & Pincus, T. (1973) *Adv. Cancer Res.* **17,** 231–277.
5. Lilly, F. (1968) *J. Exp. Med.* **127,** 465–473.
6. Chesebro, B., Wehrly, K. & Stimpfling, J. (1974) *J. Exp. Med.* **140,** 1457–1467.
7. Chesebro, B. & Wehrly, K. (1978) *J. Immunol.* **120,** 1081–1085.
8. Chesebro, B. & Wehrly, K. (1978) in *Advances in Comparative Leukemia Research,* eds. Bentvelzen, P., Hilgers, J. & Yohn, D. S. (Elsevier/North-Holland, Amsterdam), pp. 69–73.
9. Chesebro, B. & Wehrly, K. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 425–429.
10. Chesebro, B. & Wehrly, K. (1976) *J. Exp. Med.* **143,** 73–84.
11. Doig, D. & Chesebro, B. (1978) *J. Exp. Med.* **148,** 1109–1121.
12. Boyse, E. A., Stockert, E. & Old, L. J. (1967) *Proc. Natl. Acad. Sci. USA* **58,** 954–957.
13. Old, L. J., Stockert, E., Boyse, E. A. & Kim, J. H. (1968) *J. Exp. Med.* **127,** 523–539.
14. Lilly, F. & Steeves, R. A. (1973) *Virology* **55,** 363–370.
15. Bassin, R. H., Tuttle, N. & Fischinger, P. J. (1971) *Nature (London)* **229,** 564–566.
16. Chesebro, B., Wehrly, K., Chesebro, K. & Portis, J. (1976) *J. Immunol.* **117,** 1267–1274.
17. Chesebro, B., Wehrly, K., Watson, K. & Chesebro, K. (1978) *Virology* **84,** 222–226.
18. Doig, D. & Chesebro, B. (1979) *J. Exp. Med.,* **150,** 10–19.
19. Aoki, T. & Johnson, P. A. (1972) *J. Natl. Cancer Inst.* **49,** 183–192.
20. Joseph, B. & Oldstone, M. (1975) *J. Exp. Med.* **142,** 864–876.
21. Calafat, J., Hilgers, J., Van Blitterswijk, W. J., Verbeet, M. & Hageman, P. C. (1976) *J. Natl. Cancer Inst.* **56,** 1019–1029.
22. Genovesi, E. V., Marx, P. A. & Wheelock, E. F. (1977) *J. Exp. Med.* **146,** 520–534.
23. Ioachim, H. L. & Sabbath, M. (1979) *J. Natl. Cancer Inst.* **62,** 169–180.
24. Witte, O. N. & Weissman, I. L. (1976) *Virology* **69,** 464–473.
25. Witte, O. N., Tsukamoto-Adey, A. & Weissman, I. L. (1977) *Virology* **76,** 539–553.
26. Ledbetter, J., Nowinski, R. C. & Emery, S. (1977) *J. Virol.* **22,** 65–73.
27. Kabat, D., Ruta, M., Murray, M. & Polonoff, E. (1979) *Proc. Natl. Acad. Sci. USA* **76,** in press.
28. Stackpole, C. W., Johnson, J. B. & Lardis, M. P. (1974) *J. Exp. Med.* **140,** 939–953.
29. Dowdle, W. R., Downie, J. C. & Laver, W. G. (1974) *J. Virol.* **13,** 269–275.
30. Skinner, G. R. B., Mushi, E. Z. & Whitney, J. E. (1976) *Intervirology* **6,** 296–308.
31. Driscoll, D. M., Onuma, M. & Olson, C. (1977) *Arch. Virol.* **55,** 139–144.
32. Becht, H., Hammerling, U. & Rott, R. (1971) *Virology* **46,** 337–343.
33. Loor, F., Forni, L. & Pernis, B. (1972) *Eur. J. Immunol.* **2,** 203–212.
34. Joseph, B. S. & Oldstone, M. B. A. (1974) *J. Immunol.* **113,** 1205–1209.
35. Narayan, O., Griffin, D. E. & Chase, J. (1977) *Science* **197,** 376–378.
36. Kono, Y., Kobayashi, K. & Fukunaga, Y. (1973) *Arch. Gesamte Virusforsch.* **41,** 1–10.
37. Rustigen, R. (1966) *J. Bacteriol.* **92,** 1805–1811.
38. Wechsler, S. L. & Fields, B. N. (1978) *Nature (London)* **272,** 458–460.
39. Hall, W. W., Kiessling, W. & Termeulen, V. (1978) *Nature (London)* **272,** 460–462.
40. Chesebro, B. & Wehrly, K. (1976) *J. Exp. Med.* **143,** 85–99.
41. Chesebro, B., Bloom, M., Wehrly, K. & Nishio, J. (1979) *J. Virol.,* in press.