

NEW CELL SURFACE gp70 RELATED TO FRIEND MINK
CELL FOCUS-INDUCING VIRUS IS EXPRESSED ON
FRIEND VIRUS-INDUCED ERYTHROLEUKEMIC SPLEEN
CELLS AFTER ELIMINATION OF ECOTROPIC
FRIEND VIRUS gp70 IN Rfv-3^{r/s} MICE

BY WILLIAM J. BRITT, JAMES K. COLLINS,* AND BRUCE CHESEBRO

*From the Department of Health and Human Services, Public Health Service, National Institutes of Health,
National Institute of Allergy and Infectious Diseases, Laboratory of Persistent Viral Diseases, Rocky
Mountain Laboratories, Hamilton, Montana 59840*

Multiple host genes have been shown to influence the course of erythroleukemia induced by Friend virus (FV)¹ (1). Two H-2-associated genes and a non-H-2-linked gene are required for recovery from FV leukemia (2, 3). The non-H-2-linked gene, Rfv-3, appears to influence the production of anti-FV antibody independent of the H-2 genotype (3). Although mice of the Rfv-3^{r/s} genotype produce high levels of anti-FV antibody, they fail to recover from FV-induced leukemia unless the appropriate H-2-associated genes are present (3). Thus, FV leukemia cells continue to proliferate even in the presence of anti-FV antibody, possibly because of their resistance to antibody-complement-mediated lysis (4). Furthermore, FV leukemic spleen cells late in the disease express decreased amounts of FV cell surface antigens and release less infectious virus than leukemic spleen cells early in the course of the disease (5). The low levels of FV cell surface antigens and infectious virus observed in mice with anti-FV antibody were found to be reversible after transfer of leukemic spleen cells into nonimmune animals (5). Therefore, anti-FV antibody appeared to play a role in altering the expression of viral antigens and infectious virus release in these cells. To examine further this phenomenon, we studied the expression of individual FV-encoded proteins in FV leukemic spleen cells at various times in the course of the disease. Our results indicated that the presence of anti-FV antibody late in the disease was associated with a decreased expression of Friend helper virus (F-MuLV)-encoded intracellular and cell surface proteins, whereas the expression of the replication-defective spleen focus-forming virus (SFFV)-encoded proteins appeared to be minimally altered. This selective loss of F-MuLV proteins appeared to correlate with the specificity of the mouse anti-FV antibody that was found to recognize F-MuLV but not SFFV-specific proteins. In addition, we have also demonstrated a new gp70

* Present address: Clinical Laboratory, University of Texas Medical Branch, Galveston, Tex. 77550.

¹ *Abbreviations used in this paper:* C, complement; CTL, cytotoxic T lymphocytes; FCS, fetal calf serum; FFU, focus-forming unit; FITC, fluorescein isothiocyanate; FMCF, Friend mink cell focus-inducing virus; F-MuLV, ecotropic Friend helper murine leukemia virus; FV, Friend virus complex; 2-ME, 2-mercaptoethanol; NP-40, Nonidet-P-40; PBBS, phosphate-buffered balanced salt solution; PBS, phosphate-buffered saline; RIA, radioimmunoassay; RLV, Rauscher murine leukemia virus; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SFFV, spleen focus-forming virus.

molecule on the surface of FV leukemic spleen cells late in the disease. This molecule differs from gp70 in the ecotropic Friend virus, which was inoculated into these mice, in that it shares strong cross-reactivity with Friend mink cell focus-inducing virus (FMCF). The potential importance of this recombinant viral protein to the maintenance of the malignant phenotype is discussed.

Materials and Methods

Animals. C57BL/10Sn, B10.A, A.BY, and A/WySn mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. F₁ hybrids were bred at the Rocky Mountain Laboratories, Hamilton, Mont. Mice used in all experiments were between 2 and 4 mo of age.

Cells and Virus. The B-tropic strain of Friend virus complex (FV) was obtained from Dr. F. Lilly, Albert Einstein School of Medicine, Bronx, N. Y. The virus stocks used for inoculation were prepared in (C57BL/10Sn × A.BY)F₁ mice and assayed as previously reported (2). A mink lung cell line producing xenotropic murine leukemia virus (xenotropic MuLV) from C57L mice was obtained from Dr. J. Levy, University of California, San Francisco, Calif. A mink lung cell line producing BALB IU-1 (6) xenotropic MuLV was obtained from Dr. M. Cloyd, Rocky Mountain Laboratories, Hamilton, Mont. FMCF-producing mink lung cell lines were provided by Dr. D. Troxler (FMCF-Troxler strain) (7), National Cancer Institute, Bethesda, Md., and Dr. M. Cloyd (FMCF-Ishimoto strain) (6). The mink lung cell lines were grown in RPMI 1640 media supplemented with 2% fetal calf serum (FCS). SC-1 cells (8) were maintained and infected with FV or the F-MuLV helper virus alone as previously reported (9). The erythroleukemia cell lines, Y57 (clone 2C) and AA41, have been described previously (4, 10).

Early and Late Leukemic Spleen Cells. Leukemic spleen cells were obtained from (B10.A × A/WySn)F₁ mice given 5,000 focus-forming units (FFU) of FV intravenously. Large (>1 g) leukemic spleens were removed, and single cell suspensions were prepared in phosphate-buffered balanced salt solution (PBBS) 8–10 d after virus inoculation (early leukemic spleen cells) and 30–60 d after virus inoculation (late leukemic spleen cells) (4).

Antisera. Anti-FV antisera were prepared in C57BL/10Sn and (C57BL/10Sn × A.BY)F₁ mice repeatedly immunized with 30×10^6 viable early FV leukemic spleen cells from (C57BL/10Sn × A.BY)F₁ mice. Antisera prepared in both strains of mice gave identical results when used to immunoprecipitate radiolabeled spleen cell lysates. Furthermore, anti-FV antisera prepared in C57BL/10Sn mice exhibited no detectable cell surface reactivity against normal spleen cells from (C57BL/10Sn × A.BY)F₁ or (B10.A × A/WySn)F₁ mice. Antisera to the purified Rauscher leukemia virus (RLV) proteins were obtained from the Viral Oncology Program, National Cancer Institute. The following lots were used: anti-gp70, 5S-167, anti-p12, 5S-37, and anti-p30, 2S-658. Rabbit anti-FMCF antiserum was provided by Dr. M. Cloyd. This antiserum was prepared by injecting viable FMCF-infected SIRC cells intravenously into New Zealand white rabbits.

Antibody and Complement (C) Cytotoxicity Assay and Quantitative Absorption. Antibody and C-mediated ⁵¹Cr release cytotoxicity assay was performed and calculated as previously described (11). Briefly, 0.085 ml of a dilution of the antiserum at the plateau of cytotoxicity was absorbed with 0.5×10^6 – 40×10^6 washed cells at 0°C for 60 min. The absorbing cells were pelleted for 10 min at 800 g, and the supernate was tested in triplicate in the ⁵¹Cr release cytotoxicity assay.

Radioimmunoassay (RIA). This assay is described in detail in a previous report (12). Purified rabbit anti-RLV gp70 serum and rabbit anti-RLV-p12 serum, as well as the purified RLV proteins, p12 and gp70, were kindly provided by Dr. J. Ihle, Frederick Cancer Research Center, Frederick, Md. The purified proteins were iodinated with ¹²⁵I using chloramine T. Initially, 0.11 ml of antiserum, diluted to the point that it precipitated 50% of labeled antigen, was mixed with increasing numbers of leukemic or normal spleen cells. After a 1-h incubation at 4°C, the cells were removed by centrifugation, and the absorbed antiserum was tested for its ability to precipitate ¹²⁵I-labeled antigen. The antiserum was incubated with a known amount of ¹²⁵I antigen, followed by precipitation of the antibody-bound antigen with 10% (vol:vol) formalin-fixed *Staphylococcus aureus* Cowan I strain. The percentage of antigen bound was then calculated.

Membrane Immunofluorescence. We have described the details of this assay previously (13). To remove erythrocytes, spleen cells were treated with 0.83% NH_4Cl buffered with 0.017 M Tris-HCl, pH 7.3 (4). Washing buffer was PBBS with 0.01 M NaN_3 . Approximately 10^5 washed viable spleen cells were incubated with 0.05 ml of undiluted tissue culture supernate containing monoclonal antibody for 30 min at 4°C. Antibodies used were: 48, anti-F-MuLV gp70 and 34, anti-FMR p15 (13). After two washes with buffer, the cells were then incubated with 0.005 ml of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin (Ig) (lot 73S-000004; Biological Carcinogenesis Branch, National Cancer Institute). Cells were washed twice with buffer, resuspended in 2% formalin (vol:vol) in PBS, and viewed under a Lietz Orthoplan microscope (E. Leitz, Inc., Rockleigh, N. J.).

Radiolabeling of Cells. Leukemic spleen cells were washed with PBBS and then incubated for 30–45 min at 37°C in PBBS. Cells were washed with PBBS and then resuspended in methionine-free Eagle's minimal essential media (Grand Island Biological Co., Grand Island, N. Y.) at 50×10^6 cells/ml. [^{35}S]methionine (978.4 Ci/mM; New England Nuclear, Boston Mass.) was added at a concentration of 100 $\mu\text{Ci/ml}$, and the cells were incubated at 37°C for 2 or 4 h. The cells were then washed twice with PBBS and lysed with lysing buffer (0.5% Nonidet-P-40 [NP-40] in 0.15 M sodium chloride and 0.01 M Tris-HCl, pH 7.2). The lysates were stored at -70°C before use. Leukemic spleen cells were labeled with [^3H]glucosamine by incubating spleen cells at 50×10^6 cells/ml in 3 ml of RPMI 1640, 10% FCS, 5×10^{-5} M 2-mercaptoethanol (2-ME) plus 200 μCi [^3H]glucosamine (250–350 $\mu\text{Ci/mmol}$; New England Nuclear) for 16 h at 37°C. The cells were then washed twice with PBBS and lysed as above. Leukemic spleen cells were radioiodinated using carrier-free ^{125}I (ICN Nutritional Biochemicals, Cleveland, Ohio) and Iodogen (Pierce Chemical Co., Rockford, Ill.). Leukemic spleen cells were washed twice with PBBS, erythrocytes were lysed as described above, and the cells were then washed three times with Dulbecco's modified PBS, pH 7.3. 20×10^6 viable spleen cells in 1 ml of PBS were added to a liquid scintillation counting vial containing 25 μg of Iodogen. 2 mCi of ^{125}I was then added, and the vial was incubated for 10 min at 0°C. The iodinated cells were washed once with cold PBS and lysed as above.

Immunoprecipitation and Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The methods of immune precipitation using formalin-fixed *S. aureus* Cowan I strain and analysis of immune precipitates by SDS-PAGE using autoradiography have been described previously (12).

Preparation of Cell Extracts. The procedure for preparing NP-40-solubilized cell extracts has been described previously (12). The protein content of the individual extracts was determined by the method of Lowry et al. (14) and found to vary from 5 to 10 mg/ml. Productive virus infection of cell lines was monitored by measurement of reverse transcriptase in cell culture supernate as previously described (11).

Cell-mediated Cytotoxicity. The details of this ^{51}Cr release assay have been previously reported (11). Briefly, spleen cells from (C57BL/10Sn \times A.BY) F_1 (H-2 $^b/b$) mice 14 d after inoculation with 1,000 FFU of FV were used as attacker cells. 1×10^6 attacker cells and 1×10^4 ^{51}Cr -labeled Y57 cells were added to flat-bottomed microtiter wells in 0.125 ml of RPMI 1640 plus 15% FCS media. Cold target inhibitor cells were added at 3×10^6 , 1.5×10^6 , 0.75×10^6 , and 0.37×10^6 cells/well in 0.025 ml of RPMI 1640 plus 15% FCS. The trays were incubated at 34°C for 19 h and sampled. The effector cells in this assay were positive for cell surface Thy-1 antigen (15), as determined by sensitivity to anti-Thy-1 monoclonal antibody (16) and C.

Results

Analysis of Cell Surface FV Antigens on Early and Late Leukemic Spleen Cells Using Specific Antisera. To determine which FV cell surface antigens were decreased on late FV leukemic spleen cells, we compared the ability of late (30–60 d after virus inoculation) and early (8–10 d after virus FV inoculation) leukemic spleen cells to remove cytotoxic antibodies in goat antisera directed at *env*-encoded gp70 and *gag*-encoded p12. gp70 and p12 of RLV and FV share extensive antigenic reactivity; therefore, goat antisera raised against RLV proteins could be used to detect FV cell surface antigens (12).

Both early FV leukemic spleen cells and late FV leukemic spleen cells appeared to express equal amounts of cell surface gp70; however, early FV leukemic spleen cells expressed fourfold more cell surface p12 antigen than late leukemic spleen cells, as four times as many late leukemic cells were required to achieve a similar absorption of antibody (Fig. 1). These findings were confirmed with a competitive radioimmunoassay using ^{125}I -gp70 or p12 (Fig. 2).

Analysis of FV Cell Surface Antigens on Early and Late Leukemic Spleen Cells Using Monoclonal Antibodies. Mouse monoclonal antibodies reactive with F-MuLV proteins gp70 and gag-encoded p15 were used in indirect immunofluorescence to investigate further the cell surface antigens of early and late leukemic spleen cells. Previously we have shown that monoclonal 48 was highly specific for F-MuLV gp70 and that monoclonal 34 was likewise highly specific for Friend, Moloney, and Rauscher MuLV p15 (13). The majority of early FV leukemic spleen cells reacted both with anti-gp70 and anti-p15 monoclonal antibodies; however, late FV leukemic spleen cells were indistinguishable from background immunofluorescence (Table I). These results indicated that late leukemic spleen cells expressed a decreased amount of FV p15 cell surface antigens. Because p15 and p12 antigens are both encoded by the same viral gene and are expressed on the cell surface on the same polyprotein molecule (17, 18), these results agreed with those using goat anti-p12 serum, indicating that expression of gag-encoded proteins was reduced on late leukemic cells. However, in contrast to previous results with goat anti-gp70 serum, late leukemic spleen cells also expressed decreased F-MuLV gp70 as detected by monoclonal antibody. Because the mono-

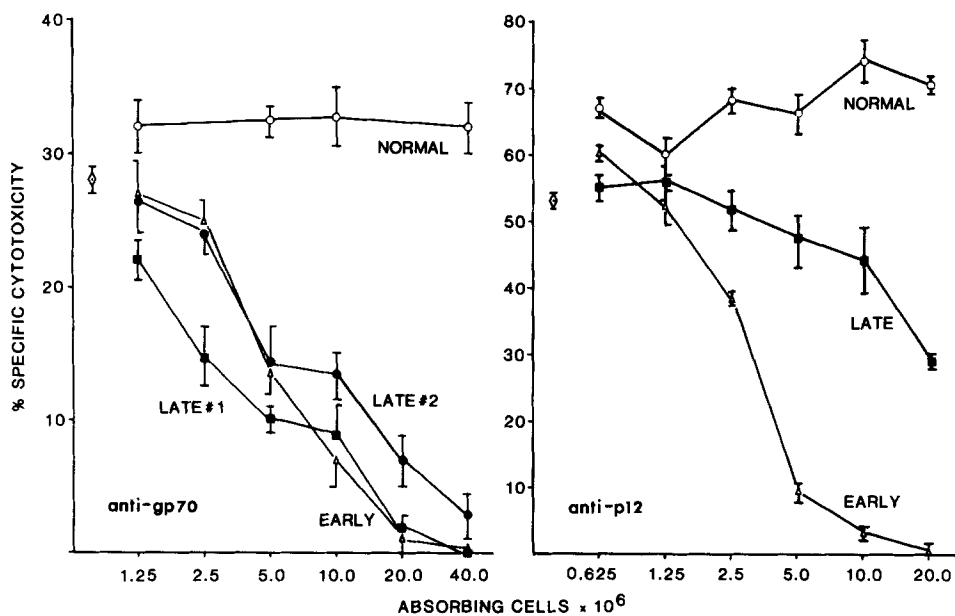


FIG. 1. Quantitative absorption of goat anti-RLV gp70 and goat anti-RLV p12 antisera by viable early and late leukemic spleen cells and normal spleen cells. The erythroleukemia cell line, AA41, was used as the ^{51}Cr -labeled target. $85\ \mu\text{l}$ of a 1:5,000 dilution of anti-gp70 or a 1:1,200 dilution of anti-p12 was absorbed by increasing numbers of cells. Mean percent specific cytotoxicity values are shown with percent standard error normal spleen cells (○), early leukemic spleen cells (△), late leukemic spleen cells (■, ●).

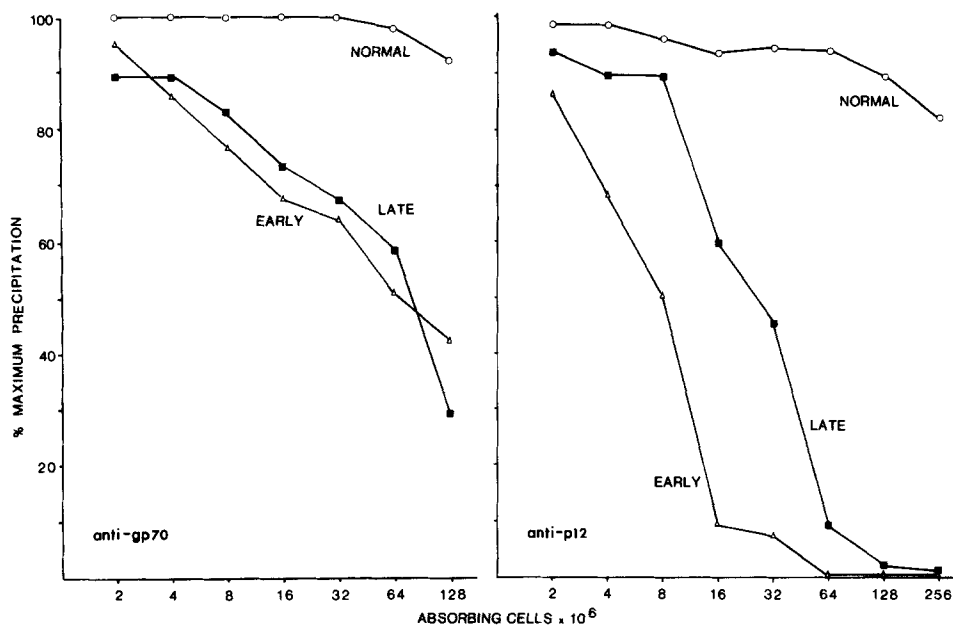


FIG. 2. The precipitation of ^{125}I -RLV gp70 or ^{125}I -RLV p12 by rabbit anti-RLV gp70 or rabbit anti-RLV p12 sera was determined after preabsorption of antisera with increasing numbers of viable early and late leukemic spleen cells and normal spleen cells as demonstrated in competitive radioimmunoassay. Normal spleen cells (○), early leukemic spleen cells (△), late leukemic spleen cells (■).

TABLE I

Use of Hybridoma Antibodies for Membrane Immunofluorescence Analysis of p15 and gp70 Antigens on Early and Late (B10.A × A/W_ySn)F₁ Leukemia Cells

First antibody	Second antibody	Percent membrane immunofluorescent positive spleen cells		
		9-d leukemic	30- to 60-d FV leukemic	Normal spleen cells
None	FITC goat anti-mouse Ig	17 ± 5 (10)*	11 ± 6 (17)	42 ± 7 (7)
34 (anti-p15)	FITC goat anti-mouse Ig	64 ± 12 (9)	16 ± 8 (12)	37 ± 7 (6)
48 (anti-gp70)	FITC goat anti-mouse Ig	56 ± 11 (11)	16 ± 9 (17)	43 ± 6 (7)

* Average percent membrane fluorescence ± standard deviation. Value in parenthesis is the number of spleens studied.

clonal anti-gp70 was specific for F-MuLV gp70, whereas the goat antisera cross-reacted with many MuLV, it appeared that late leukemic spleen cells might express an altered or new gp70 that was detected by the goat anti-gp70 serum and not by the monoclonal anti-F-MuLV gp70.

Characterization of the gp70 Present on Late FV Leukemic Spleen Cells. The cell surface proteins of early and late leukemic spleen cells were also studied by immunoprecipitation and analysis on SDS-PAGE. When radioiodinated leukemic spleen cells were precipitated with goat anti-RLV gp70, a 70,000 mol wt and a 55,000 mol wt protein were seen on both early and late leukemia cells (Fig. 3). In contrast, mouse anti-FV serum, with predominant reactivity for ecotropic F-MuLV, precipitated the gp70 on

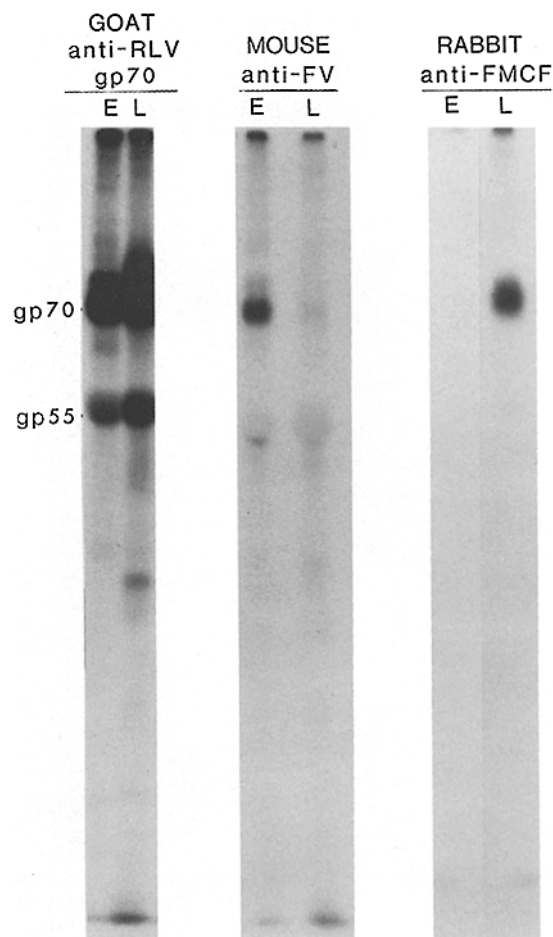


FIG. 3. SDS-PAGE of ^{125}I -labeled surface proteins of early (E) and late (L) leukemic spleen cells. Approximately 10^6 cell equivalents of lysate were immunoprecipitated with goat anti-RLV gp70, C57BL/10 anti-FV, or rabbit anti-FMCF sera. Immunoprecipitation of ^{125}I -normal spleen cells with these sera gave no detectable bands (data not shown).

early leukemic cells but not on the late leukemic cells (Fig. 3). Also, monoclonal antibody 48, which recognized only ecotropic F-MuLV gp70, immunoprecipitated the gp70 on early leukemic cells but not on late leukemic cells (data not shown). In contrast, the gp70 on late leukemic cells was precipitated by a rabbit anti-FMCF serum (Fig. 3). This reagent had predominant reactivity for FMCF-specific determinants, and it failed to precipitate ecotropic F-MuLV gp70 on early leukemic cells. These results suggested that the gp70 on late leukemic spleen cells was different from ecotropic F-MuLV gp70. To characterize further the gp70 molecules on early and late leukemic cells, we used extracts of nonradiolabeled F-MuLV-infected SC-1 cells, FMCF-infected mink cells, and xenotropic MuLV-infected mink cells to preabsorb the precipitating antisera. When goat anti-RLV gp70 serum was preabsorbed with FMCF-infected or xenotropic MuLV-infected mink cell extracts, the immunoprecipitation of the gp70 on early leukemic spleen cells was not inhibited (Fig. 4). Only F-

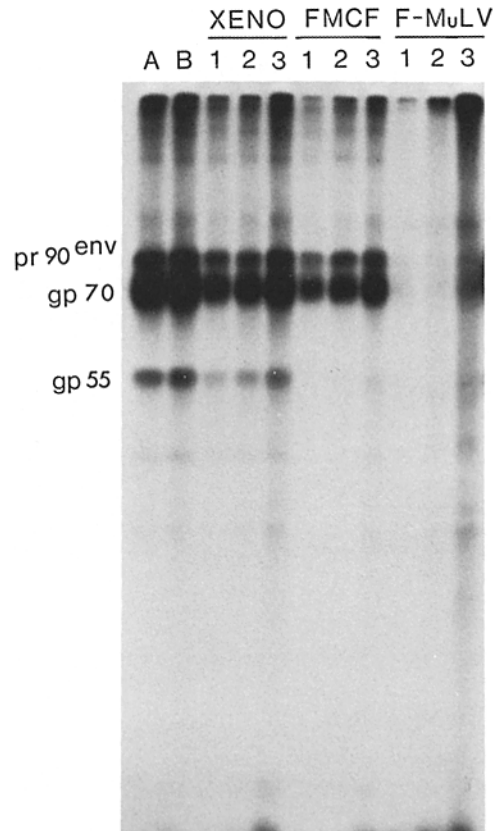


FIG. 4. SDS-PAGE of ^{125}I -labeled surface proteins of early leukemic spleen cells after immunoprecipitation with preabsorbed goat anti-RLV gp70 serum. 5 μl of a 1:100 dilution of goat anti-RLV gp70 serum was preabsorbed with (1) 200 μl , (2) 100 μl , and (3) 20 μl of extracts from cells infected with xenotropic MuLV, FMCF virus, and F-MuLV for 3 h at 0°C before addition of ^{125}I -labeled cell lysates. The goat anti-gp70 serum was also preabsorbed with extracts from uninfected SC-1 cells (track A) or uninfected mink lung cells (track B).

MuLV-infected SC-1 extracts inhibited the precipitation of the gp70 on early leukemic cells (Fig. 4). Therefore, as detected with goat anti-gp70 serum, early leukemic spleen cells expressed predominantly ecotropic F-MuLV gp70. When the gp70 on late leukemic cells was analyzed in a similar manner, all of the infected cell extracts inhibited the immune precipitation of gp70, indicating that the precipitation of the gp70 on late leukemic cells resulted from group-reacting antibodies present in the goat anti-gp70 serum (data not shown). In a further attempt to determine the antigenic nature of the gp70 on late leukemic cells, we absorbed the rabbit anti-FMCF serum with the nonradiolabeled virus-infected cell extracts. In this experiment, only FMCF-infected extracts prevented the immunoprecipitation of the gp70 on late leukemic spleen cells (Fig. 5). Thus, as detected with this rabbit anti-FMCF serum, the gp70 on late leukemic spleen cells shared antigenic cross-reactivity with FMCF only and not with F-MuLV or xenotropic MuLV.

Late FV Leukemic Spleen Cells Exhibit Decreased Intracellular FV Precursor Proteins. We have previously shown that late leukemic cells not only expressed decreased surface

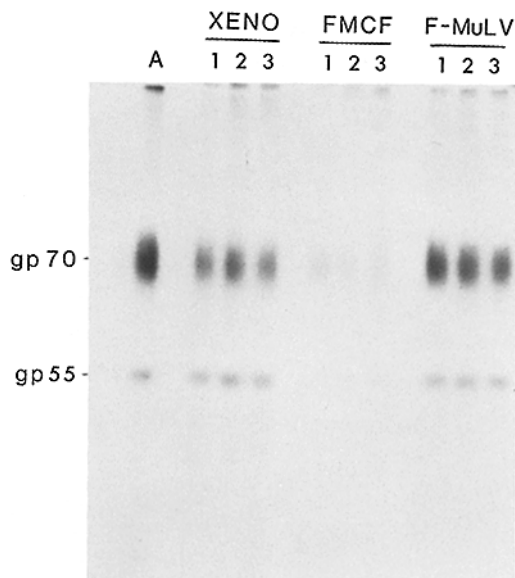


FIG. 5. SDS-PAGE of ^{125}I -labeled surface proteins of late leukemic spleen cells after immunoprecipitation with preabsorbed rabbit anti-FMCF serum. 5 μl of a 1:3 dilution of rabbit anti-FMCF serum was preabsorbed for 3 h at 0°C with (1) 200 μl , (2) 100 μl , or (3) 30 μl of the same virus-infected cells extracts used in Fig. 4. Track A represents unabsorbed antiserum at a 1:3 dilution.

antigens but also released 300-fold less infectious ecotropic F-MuLV (5). To determine whether this decrease in release of infectious virus was associated with alteration in viral protein synthesis, early and late leukemic spleen cells were labeled with [^{35}S]methionine, and immunoprecipitates were analyzed by SDS-PAGE. The *env*-encoded (pr90, pr80, gp70) and *gag*-encoded (pr65, p50, p42) intracellular proteins were markedly decreased in late leukemic spleen cells as compared with early leukemic spleen cells (Fig. 6). The SFFV-encoded protein, gp55, although decreased on late leukemic spleen cells, was readily detectable upon exposing the fluorograms for longer periods of time. In contrast, H-2 molecules were found in equal amounts in early and late leukemic spleens and in normal spleens (Fig. 6). Furthermore, an equal number of acid precipitable counts per cell equivalent was present in the lysates of radiolabeled normal, early leukemic, and late leukemic spleen cells. Thus, the apparent decrease of viral proteins in late leukemic spleen cells was not secondary to a general decrease in [^{35}S]methionine incorporation because H-2 and other proteins were similarly labeled in early and late leukemic cells. Because the gp70 of other MuLV have been associated with a larger (80,000–90,000 mol wt) precursor protein (12), it was unclear why we initially could not demonstrate a larger precursor protein in late leukemic spleen cells. However, when a shorter labeling period was used and fluorograms were exposed for an extended time, both a 70,000 and 90,000 mol wt protein were observed after precipitation with anti-gp70 serum (Fig. 7). Therefore, it appeared the gp70 antigens in late leukemic cells were also associated with a probable precursor protein. In addition, when radioiodinated late leukemic spleen cells were immunoprecipitated with goat anti-gp70 serum and eluted in the absence of 2-ME, a 90,000 mol wt protein as well as the gp70 was present (Fig. 8). Thus, in nonreducing conditions, much of the gp70 in lysates of late cells appeared disulfide linked with a smaller protein to form

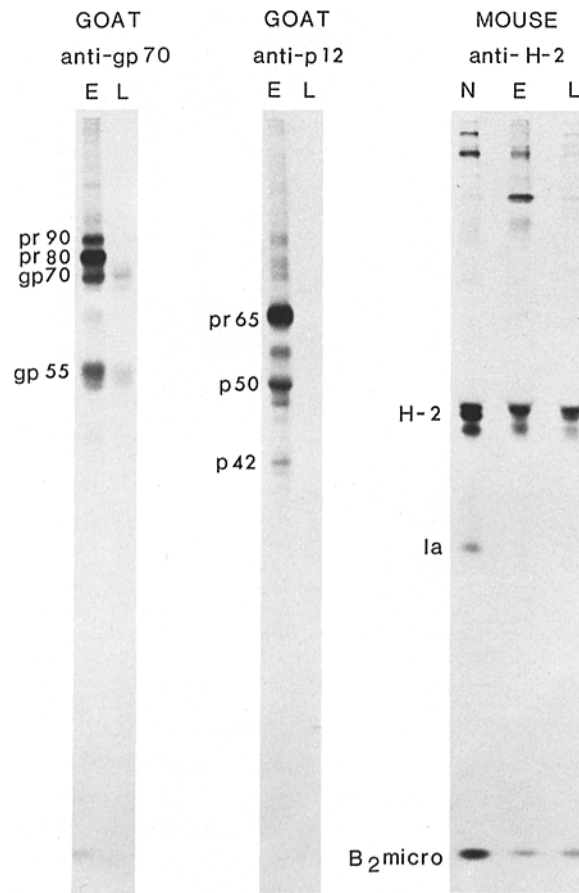


FIG. 6. SDS-PAGE of [35 S]methionine-labeled proteins from early (E) and late (L) leukemic spleen cells and normal (N) spleen cells. Early leukemic, late leukemic, or normal spleen cells were radiolabeled with [35 S]methionine for 4 h and lysed as described in Materials and Methods. Equal numbers of cell equivalents and acid-precipitable counts per minute were immunoprecipitated with goat anti-RLV gp70, goat anti-RLV p12, or mouse anti-H-2^a antiserum. Fluorograms were exposed for 2 d.

90,000 mol wt complexes similar to the disulfide linkage of the gp70 and p15E seen in MuLV virions after lysis in NP-40 (19).

Anti-FV Cytotoxic T Lymphocytes (CTL) Recognize Late FV Leukemic Spleen Cells. Previously we reported that late FV leukemic spleen cells continued to proliferate even in the presence of high levels of anti-FV cytotoxic antibody (4). This persistence of leukemia cells was correlated with the loss of cell surface virus-encoded antigens and the apparent resistance of late leukemia cells to anti-FV antibody and complement-mediated destruction (4). In addition, we have reported that anti-FV CTL apparently recognized cell surface F-MuLV-encoded gp70 (13). To determine whether late leukemia cells also escaped recognition by anti-FV CTL because of their loss of cell surface F-MuLV gp70, we examined the ability of late leukemic spleen cells to competitively inhibit anti-FV CTL. As shown in Fig. 9, late leukemic spleen cells, although expressing markedly decreased amounts of F-MuLV gp70, were

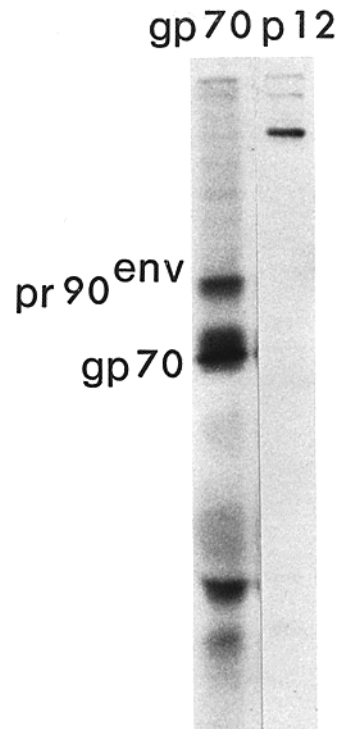


FIG. 7. SDS-PAGE of [^{35}S]methionine-labeled late leukemic spleen cells. After 2 h pulse labeling with [^{35}S]methionine, late leukemic spleen cells were lysed as described in Materials and Methods, and lysates were immunoprecipitated with either goat anti-RLV gp70 or goat anti-RLV p12 serum. Fluorograms were exposed for 10 d.

comparable with early FV leukemic spleen cells in their capacity to inhibit anti-FV CTL. A total of 8 of 11 late leukemic spleens were found to be comparable with early leukemic spleens in their ability to inhibit anti-FV CTL. This finding suggested that late leukemic spleen cells were recognized by anti-FV CTL even though they were apparently resistant to antibody-complement-mediated lysis.

Discussion

The results presented in this report demonstrated that both FV *env*- and *gag*-encoded antigens were decreased on FV leukemic spleen cells late in the course of leukemia as compared with FV leukemic spleen cells early in the disease. This loss of cell surface antigens appeared to account for the previously reported resistance of late leukemic spleen cells to antibody-complement-mediated lysis (4). Initially, when early and late leukemic spleen cells were studied with heterologous anti-gp70 antisera, either in quantitative absorption of cytotoxic antibody or in an RIA, we detected no difference in the expression of FV *env* cell surface antigens. However, mouse monoclonal antibody, which detected only ecotropic F-MuLV, revealed the loss of ecotropic F-MuLV gp70 from late leukemic spleen cells as compared with early leukemic spleen cells. Thus, it appeared that late leukemic spleen cells expressed a new gp70 that was cross-reactive with the anti-gp70 antibodies present in the heterologous antisera. This finding was consistent with previous reports that have shown that heterologous goat

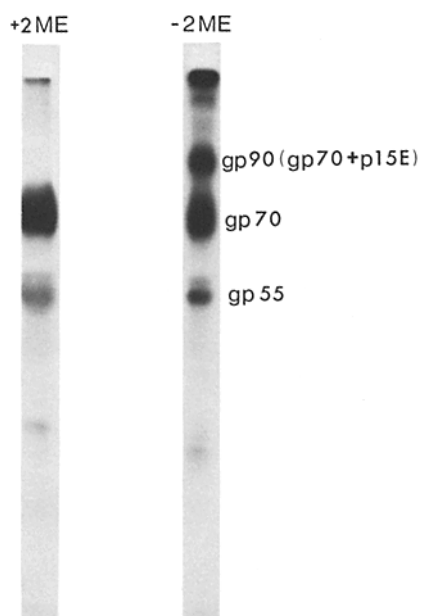


FIG. 8. ^{125}I -labeled surface proteins of late leukemic spleen cells were immunoprecipitated with goat anti-RLV gp70 serum. Immunoprecipitates were eluted with (+ 2 ME) or without (- 2 ME) 2-ME and analyzed in SDS-PAGE.

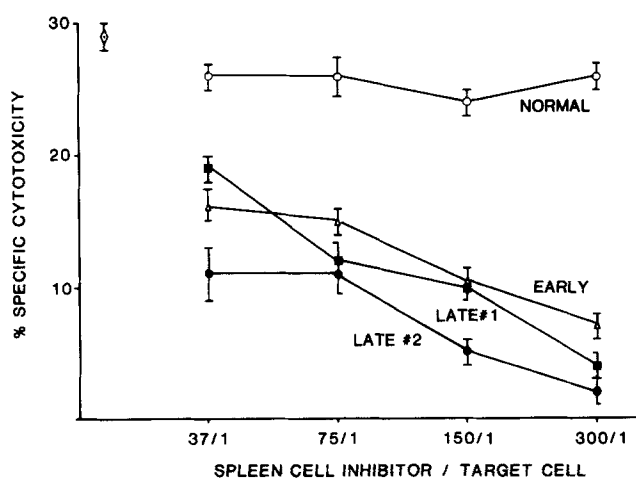


FIG. 9. Competitive inhibition of anti-FV CTL by early and late leukemic spleen cells and normal spleen cells. The erythroleukemia cell line, Y57 (clone 2C), was used as the ^{51}Cr -labeled target. Anti-FV CTL were inhibited by increasing numbers of normal spleen cells (○), early leukemic spleen cells (△), and late leukemic spleen cells (■, ●).

anti-gp70 sera often contained antibodies reactive not only with ecotropic F-MuLV gp70 but also with xenotropic MuLV gp70 and the SFFV-encoded gp55 (12, 20).

The finding of a cell surface gp70 on late leukemic cells, which shared antigenic reactivity with FMCF, was unexpected. The origin of this new gp70 on late leukemic cells is uncertain. Although SFFV contains defective *env* genomic sequences, it does not express a gp70 on either the virion or infected cells (20). Possibly, the gp70 on late

cells could have arisen from a recombination between the helper F-MuLV and as yet unidentified virus(es). This possibility was supported by the finding that infection of baby mice with the helper F-MuLV alone resulted in the generation of recombinant MCF viruses (M. Cloyd, personal communication).

The gp70 on late leukemic cells has not been associated with the release of infectious virus or RNA-dependent DNA-polymerase-containing particles (data not shown). Also, we have been unable to detect infectious MCF or xenotropic viruses in the virus stocks used for in vivo leukemia induction or in early spleen cells. The lack of release of infectious virus from late leukemic spleen cells could be the result of the lack of *gag* precursor polyproteins in late leukemic spleen cells. Thus, although an *env* gene product is available for virion assembly, the necessary *gag*-encoded structural proteins are not available for complete virion assembly.

The significance of this MCF-like gp70 on late leukemic spleen cells is unclear. Recently MCF viruses have been implicated in spontaneous leukemogenesis (21, 22). The *env* region of some MCF viruses appears to contain the necessary genetic information required for leukemia induction (23). The MCF-like gp70 on late leukemia cells suggested the possibility that neoplastic transformation of erythroid cells in this disease may have been initiated by a recombinant MCF virus rather than by SFFV. This possibility was supported by reports of erythroleukemia induction in baby mice by F-MuLV in the absence of SFFV (24). Although the continued malignant phenotype of late leukemic spleen cells and the appearance of a new noncotropic gp70 seemed a more than coincidental finding, the possibility that this gp70 may be present on late leukemic cells as the result of an arrest at a particular stage of differentiation must also be considered. Other workers have identified MuLV gp70 on normal cells (25). The G_{IX} antigen, originally defined as an alloantigen on normal thymocytes of certain mouse strains, was subsequently shown to be located on a MuLV gp70 molecule (26).

Cell surface protein labeling with ¹²⁵I as well as metabolic labeling with [³⁵S]-methionine revealed the presence of the SFFV protein, gp55, on both early and late leukemic spleen cells. Further evidence of continued SFFV expression in late leukemic spleen cells was obtained by our finding that reinfection of late leukemic spleen cells with competent helper F-MuLV in vitro resulted in the quantitative rescue of the defective SFFV (W. Britt and B. Chesebro, manuscript in preparation). Thus, the expression of SFFV on late leukemic spleen cells appeared to be under separate control from the expression of the helper F-MuLV. It is possible that the continued expression of gp55 may result from the lack of a humoral immune response to this protein in these mouse strains (13).

We have reported that high levels of cytotoxic anti-FV antibody not only reduced the expression of cell surface FV antigens on leukemic spleen cells, but also dramatically decreased the release of infectious virus (5). [³⁵S]Methionine metabolic labeling followed by immune precipitation and analysis on SDS-PAGE revealed that late leukemic spleen cells expressed drastically reduced amounts of intracellular F-MuLV viral polyprotein precursors as compared with early leukemic spleen cells. Exposure of leukemia cells to cytotoxic antiviral antibody appeared to mediate these changes in both intracellular and cell surface proteins because transfer of late leukemia cells into mice lacking antibodies resulted in reexpression of helper virus proteins and release of infectious virus (5). Similarly, Fujamini and Oldstone (27) have reported that anti-

measles antibody not only reduced cell surface expression of measles antigens but also markedly reduced the production of intracellular measles proteins. Also, Yagi et al. (28) described the loss of infectious virion release and the marked alteration of virion glycoproteins in a mouse mammary tumor virus-induced cell line exposed to cytotoxic anti-MMTV antibody and immune spleen cells. The mechanism of cell surface antibody-induced alteration of intracellular viral precursor protein production remains unknown. Potential mechanisms might include antibody-induced cell surface viral protein redistribution with the subsequent disruption of virion assembly. Intracellular virion components could then accumulate, leading to the feedback inhibition of viral protein synthesis. Although such a speculative mechanism could explain our observations, another equally likely explanation could be the selection and overgrowth of a population of virus nonproducer cells as a result of the immune pressure exerted by cytotoxic anti-FV antibody. Our preliminary experiments with passive transfer of monoclonal anti-FV antibodies suggest that both mechanisms may be operating simultaneously in this system (W. Britt and B. Chesebro, manuscript in preparation).

Other investigators have suggested that cell surface antigen loss induced by antiviral antibody might protect infected cells from virus-specific cell-mediated lysis (29). This did not appear to be the case in the FV system. Our findings indicated that anti-FV CTL could recognize late leukemic spleen cells even though these cells expressed much decreased amounts of F-MuLV cell surface antigens. This result could be interpreted to suggest that anti-FV CTL may recognize antigens other than F-MuLV cell surface proteins, such as SFFV-encoded antigens (30). However, our previous results with cloned mutant erythroleukemia cells suggested that F-MuLV gp70 was the main target for anti-FV CTL (11). Therefore, it appeared more likely that these CTL were able to recognize residual low amounts of F-MuLV gp70 on late leukemic cells (31, 32). Alternatively, it was also possible that anti-FV CTL could detect cross-reactivity between the original F-MuLV gp70 and the new MCF-like gp70 on these leukemic cells. Because late leukemic cells with antibody-induced decreased cell surface antigens were recognized by anti-FV CTL, the lack of recovery from leukemia in $H-2^{s/a}$ Rfv-3^{r/s} mice appeared to be a result of lack of sufficient anti-FV CTL rather than to modulation or removal of CTL target antigens by anti-FV antibody.

Summary

Spleen cells from Rfv-3^{r/s} mice with Friend virus-induced erythroleukemia were analyzed for expression of virus-induced proteins with monoclonal antiviral antibodies and conventional antisera. Leukemic spleen cells, 30–60 d after virus inoculation, expressed decreased amounts of ecotropic Friend murine leukemia helper virus *gag*- and *env*-encoded cell surface and intracellular proteins compared with leukemic cells tested 8–10 d after virus inoculation. In contrast, the spleen focus-forming virus-induced protein, gp55, was present on both leukemia cell populations. This difference appeared to be mediated by the humoral antibody response in Rfv-3^{r/s} mice, which could recognize only ecotropic *gag* and *env* proteins, and not gp55. A new gp70 molecule cross-reactive with a recombinant Friend mink cell focus-inducing virus was found in large quantities on late leukemic cells. This protein appeared to be derived from a recombinant virus produced during the course of Friend virus infection. The appearance of this new gp70 suggests that recombinant viruses other than spleen focus-forming virus may play a role in Friend virus-induced erythroleukemia.

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