ANTIBODY-INDUCED LOSS OF FRIEND VIRUS LEUKEMIA CELL SURFACE ANTIGENS OCCURS DURING PROGRESSION OF ERYTHROLEUKEMIA IN F_1 MICE

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In both young and adult mice Friend leukemia virus (FV)[1] causes an extremely rapid erythroleukemia in which the spleen is the primary site of growth of neoplastic cells (1–4). Splenomegaly is apparent 8–9 days after virus inoculation, at which time the majority of cells are infected with the virus and have virus-induced cell surface antigens (5, 6). Mouse strains such as DBA/2, BALB/c, and (BALB/c × A/J)F_1 have FV viremia and exhibit FV-induced cell surface antigens on leukemic spleen cells throughout the course of the disease (5, 6). However, certain F_1 hybrids e.g., (B10.A × A/WySn)F_1, (B10.A × A.BY)F_1, (C57BL/6 × DBA/2)F_1, and (C57BL/10 × BALB/c)F_1 recover from FV viremia 30–60 days after virus inoculation in spite of persistent leukemia (5). At this time these F_1 hybrids have virus neutralizing antibodies and cytotoxic antibodies specific for FV leukemia cells (5). In the present work, it was found that 30–60 days after virus inoculation persistent leukemic spleen cells from these F_1 mice were resistant to cytolysis by anti-leukemia cell antibody and complement, and FV-induced cell-surface antigens were reduced by about 80%. This loss of FV-induced cell surface antigens could also by induced by transfer of high-antigen early FV spleen cells into irradiated immune recipients, and was reversed when low-antigen cells were transferred to irradiated nonimmune recipients. The data suggested that the loss of FV-induced cell surface antigens was due to antigenic modulation caused by circulating anti-FV antibodies. This antigen loss might be responsible for the persistence of the leukemic spleen cells in the F_1 mice studied.

Materials and Methods

Animals. B10.A, A.BY, A/WySn, and B10.D2 mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. B10. HTG mice were a gift from Dr. Frank Lilly, Department of Genetics, Albert Einstein School of Medicine, Bronx, N. Y. F_1 hybrids were bred at the Rocky Mountain Laboratory.

Virus. The B- and N-tropic strains of Friend virus (FV-B and FV-N) were obtained from Dr. Frank Lilly. The virus stocks were made in (B10.A × A.BY)F_1, (B10.A × A/WySn)F_1 or DBA/2 mice as previously described (7).

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[1] *Abbreviations used in this paper*: C′, complement; FFU, focus-forming units; FV, Friend leukemia virus; FV-B, B-tropic Friend leukemia virus; FV-N, N-tropic Friend leukemia virus; G, Gross; anti-MLV, anti-Moloney leukemia virus; MuLV, murine leukemia virus; PBS, phosphate-buffered saline; TL, thymus-leukemia.

Anti-FV Sera. (B10.A × A.BY)F_1 or (B10.A × A/WySn)F_1 mice which had recovered from FV-induced splenomegaly 30–60 days after FV-B inoculation (15–150 focus-forming units [FFU]) or 14–21 days after FV-N inoculation (1,500 FFU) were injected two to five times with 20–25 × 10^6 leukemic spleen cells obtained from syngeneic mice 8–9 days after intravenous inoculation with 1,500 FFU of FV-B. Mice were bled from the tail 7–14 days after the last injection to obtain anti-FV sera or were used as immune recipients in cell transfer experiments.

Anti-H-2 Sera. (C57BL/10 × BALB.B)F_1 anti-(B10.D2 × BALB/c)F_1 (anti-H-2^d) and (B10.D2 × BALB/c)F_1 anti-B10.A(2R) (anti-H-$2K^k$) sera were prepared as previously described (8). In addition, NIH contract serum D-13 (C57BL/10 × LP.R III) anti-B10.A(5R) (anti-H-$2D^d$) was used.

Cell Culture. AA41 (H-2^a) and Y57 (H-2^b) FV-induced leukemia cell lines were grown in tissue culture as described (9). Primary cultures of leukemic spleen cells were incubated in RPMI-1640 medium with 10% fetal calf serum and 10^{-5} M 2-mercaptoethanol at 0.1–7 × 10^6 viable cells per ml.

Antibody Plus Complement (C')-Mediated Cytotoxicity. A two stage cytotoxicity assay in microtiter trays was carried out using mouse leukemia-cell absorbed rabbit C' and ^{51}Cr-labeled target cells as previously described (5). Target cells were normal or leukemic spleen cells, or AA41 or Y57 cell lines.

Quantitative Absorption. Anti-H-2 or anti-FV serum was diluted to the end of the plateau of the titration curve in the direct cytotoxicity assay. The serum was then absorbed with serial twofold dilutions of nucleated leukemic or normal spleen cells. 1–80 × 10^6 cells were placed in separate centrifuge tubes and spun for 10 min at 2,000 rpm. The supernate was removed, and the cell pellet was subjected to a second (short) spin and residual supernate again removed. Then 85 μl of diluted serum was added to each tube, and the cell pellet was suspended. The serum plus cells were incubated for 1 h at 4°C, and shaken once during this time. The cells were spun for 10 min at 2,000 rpm in the cold and 25-μl triplicate samples of supernate from each tube were dispensed to microtiter trays for assay of cytotoxicity.

Membrane Immunofluorescence. Spleen cells were harvested and erythrocytes were lysed with Tris-buffered NH_4Cl for 10 min at room temperature (10). The cells were washed twice with phosphate-buffered balanced salt solution plus 2% fetal calf serum and 0.01 M NaN_3. 4 × 10^6 cells were dispensed into 10 × 75-mm tubes and spun at 1,000 rpm for 5 min. Indirect membrane immunofluorescence was assayed as previously described (8), using anti-FV sera and fluorescein-conjugated rabbit anti-mouse immunoglobulin. For the direct membrane immunofluorescence assay, fluorescein-conjugated goat anti-Moloney leukemia virus (anti-MLV) (obtained from the NCI Viral Oncology Program; Huntington Research Center, lot 5010101) was absorbed twice for 40 min at room temperature with 100 mg acetone precipitated mouse liver and spleen tissue powder per milliliter serum and spun at 30,000 rpm for 10 min. 5 μl of absorbed serum was added to the cells to be tested and the cells were resuspended and incubated for 20 min at 4°C, then washed twice and examined with a fluorescence microscope. Cells which had visible halos or patches of membrane fluorescence were scored as positive.

Intracellular Immunofluorescence. 50–100 μl of washed nucleated spleen cell suspension (2–10 × 10^6 cells/ml) was placed on marked areas of microscope slides coated with 5% bovine serum albumin. The slides were placed in a humidity chamber, and the cells were allowed to settle onto the slide for 30 min. Then the slides were placed in acetone at 4°C for 30 min or at −20°C for a minimum of 12 h. After removal from the acetone, slides were immediately washed in phosphate-buffered saline (PBS) at room temperature (10 min three times with constant shaking), drained, and a 1/40 dilution of absorbed fluorescein-conjugated goat anti-MLV (see above) was added. The slides were then incubated for 1 h at 37°C in a humidity chamber washed three times in PBS at room temperature, dried, mounted in buffered glycerol mounting medium, and examined with a fluorescence microscope.

Irradiation. Mice were given 900 rads whole-body X-irradiation with a Maximar type III 250 KV deep therapy X-ray unit. The machine delivered 19.1 rads/min at 24 inches from the target.

Results

Lysis of FV-Induced Leukemia Cells with Anti-FV Cytotoxic Antibody. Previous experiments indicated that FV-induced leukemic spleen cells persisted in (B10.A × A/

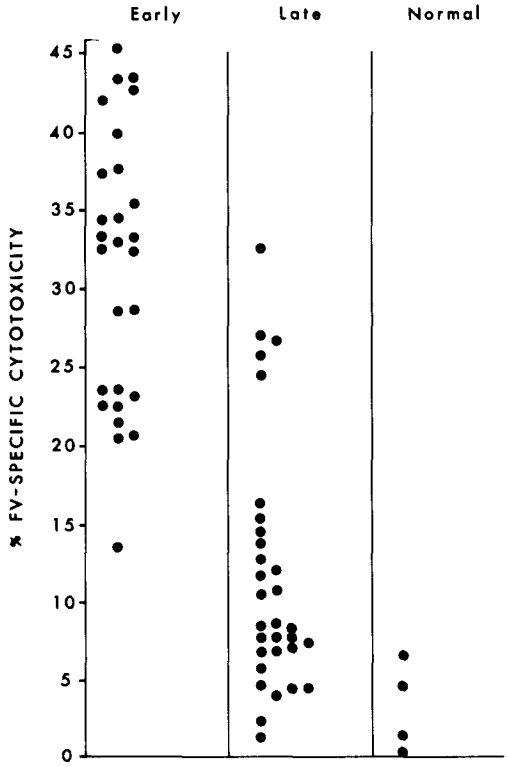

FIG. 1. Anti-FV antibody plus complement-mediated lysis of F_1 spleen cells. Early (8–9 days postinfection) (B10.D2 × A.BY)F_1, (B10.A × A.BY)F_1, and (B10.A × A/WySn)F_1 leukemic spleen cells; late (30–90 days postinfection) (B10.D2 × A.BY)F_1, (B6 × A/TL$^-$)F_1, (B6/TL$^+$ × A)F_1, (B10.HTG × A.BY)F_1, (B10.A × A/WySn)F_1 leukemic spleen cells; normal (B10 × A.BY)F_1 or (B10.A × A/WySn)F_1 spleen cells. All these F_1 hybrids, which differed only for genes in or near the H-2 complex, were found to give similar results, and therefore we have combined all our data on different strains.

WySn)F_1 and (B10.A × A.BY)F_1 mice in spite of the presence of anti-FV leukemia cell cytotoxic antibodies (5). Therefore, we wanted to determine whether leukemia cells from these mice could be lysed in vitro by anti-FV serum plus rabbit C′. High specific lysis (20–45%) was seen with leukemic spleen cells from 27 of 28 mice (96%) 8–9 days after FV-B inoculation (1,500 FFU) (Fig. 1). In contrast, leukemic spleen cells of only 5 of 31 mice 30–90 days after FV-B inoculation had greater than 20% specific lysis. These results suggested that in later stages of the disease (>30 days after FV inoculation) the leukemic spleen cells of these F_1 mice had reduced amounts of FV-induced cell surface antigens.

Quantitation of FV-Induced Cell Surface Antigens. To quantitate and confirm this finding, the relative amount of FV-induced cell surface antigen on early (8–9 day) and late (30–90 day) (B10.A × A/WySn)F_1 leukemic spleen cells was determined by quantitative absorption of cytotoxic anti-FV antisera. As seen in Fig. 2 early leukemic spleen cells had substantially more FV-induced cell surface antigen than late cells. Late leukemic spleen cells were also clearly distinguishable from normal spleen cells, and late cells were capable of clearing the serum of anti-FV antibodies. When 10 early and 10 late leukemic F_1 mice were tested in the absorption assay, it was found that an average of $4.0 \pm 1.8 \times 10^6$ early leukemic spleen cells were required to absorb

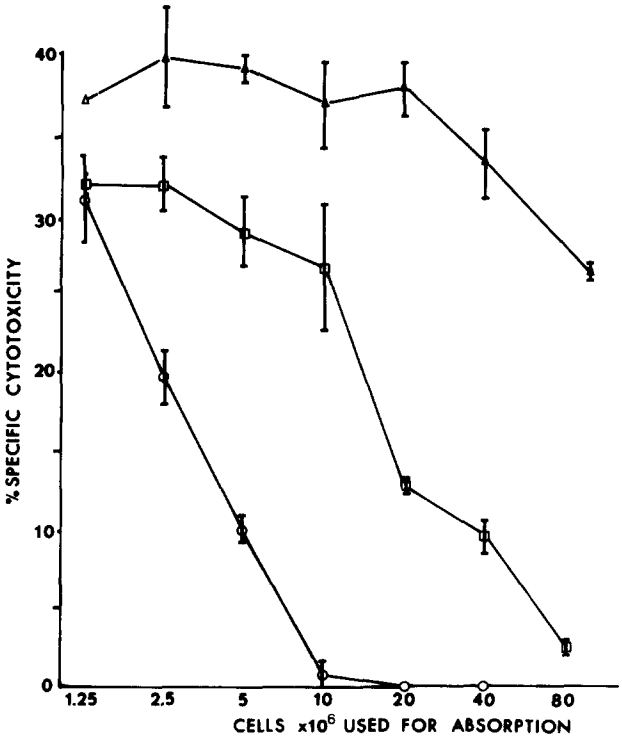

FIG. 2. Absorption of cytotoxic anti-FV serum by early and late (B10.A × A/WySn)F_1 leukemic spleen cells. AA41 cells were used as targets in the cytotoxicity assay. 85 μl of a 1/100 dilution of anti-FV serum was absorbed and the cytotoxicity assay was done in triplicate. Mean percent specific cytotoxicity values are shown ± standard error. □, late cells; ○, early cells; △, normal cells. Unabsorbed anti-FV serum gave 41% specific cytotoxicity.

85 μl of a 1/100 dilution of serum to 50% of maximum cytotoxicity, whereas 18.6 ± 2.2 × 10^6 late spleen cells were required to achieve a similar absorption. Thus, early spleen cells had 4.5 times more FV-induced cell surface antigen than late cells. The late spleen cells appeared to have lost 78% of their original FV-induced cell surface antigens.

Quantitative Absorption of H-2 Surface Antigens. We wanted to know whether FV-induced antigens were the only cell surface antigens decreased in late stages of FV leukemia. Therefore, we compared the amount of $H\text{-}2^d$ antigen on normal and leukemic spleen cells by quantitative absorption. We absorbed (C57BL/10 × BALB.B)F_1 anti-(B10.D2 × BALB/c)F_1 (anti-$H\text{-}2^d$) serum with (B10.A × A/WySn)F_1 FV leukemic spleen cells or normal spleen cells. No difference in absorption of anti-$H\text{-}2^d$ was seen when 8–9 day (early) leukemic spleen cells were compared with 30–90 day (late) leukemic spleen cells or with normal spleen cells (Fig. 3). The K and D region antigens of the H-2 complex were also examined separately in early, late and normal (B10.A × A/WySn)F_1 mice by quantitative absorption. No changes in either H-2K or H-2D antigens were observed (data not shown).

Fluorescent Antibody Studies. To confirm and extend the cytotoxic antibody data with an antibody binding assay, virus-induced cell surface antigens on 8–9 day and >30 day leukemic spleen cells from F_1 mice were also studied by membrane immunofluorescence. Cells were examined using both an indirect (mouse anti-FV serum

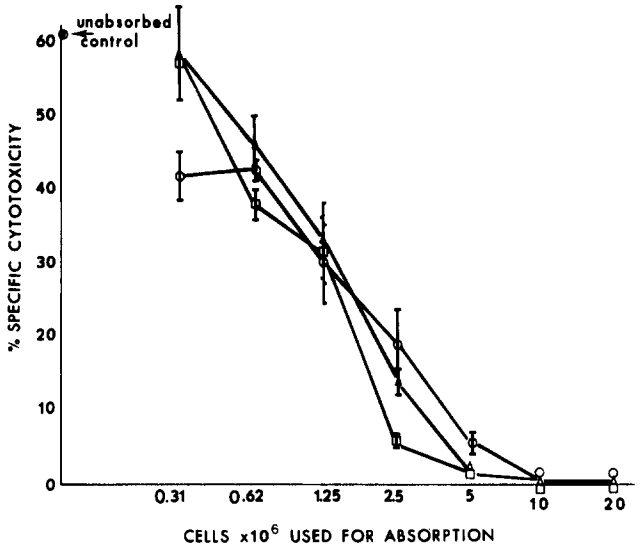

FIG. 3. Quantitative absorption of anti-H-2^d antiserum by early and late leukemic spleen cells and normal spleen cells from (B10.A × A/WySn)F_1 mice. AA41 cells were used as targets in the cytotoxicity assay. 85 μl of a 1/100 dilution of anti-H-2^d serum was absorbed and the cytotoxicity assay was done in triplicate. Mean percent specific cytotoxicity values are shown ± standard error. □, late (30–90 days after FV) leukemic spleen cells; ○, early (8 days after FV) leukemic spleen cells; △, normal spleen cells.

followed by fluorescein-conjugated rabbit anti-mouse immunoglobulin) (Fig. 4) and a direct (fluorescein-conjugated goat anti-Moloney leukemia virus) membrane fluorescence technique (Fig. 5). Good agreement was found between the mouse and goat antisera in the membrane fluorescence assay. Early (8–9 day) FV leukemic spleen cells had a high incidence of fluorescent cells (65% median) in the direct assay, however, late (>30 day) FV leukemia spleen cell populations had lower levels of fluorescent cells (median 19%) (Fig. 5). Little overlap was seen between the early and late percent positive fluorescent ranges. The results of both indirect and direct membrane immunofluorescence tests were consistent with the cytotoxic antibody and quantitative absorption data found using mouse anti-FV serum, and indicated a loss of FV-induced cell surface antigens in later stages of leukemia. Blocking of FV antigens by circulating anti-FV antibodies in the late plasma (11) was ruled out as a possible explanation for these results because the cells did not react with anti-mouse immunoglobulin in the indirect membrane fluorescence assay unless mouse anti-FV antibody was added first (data not shown). When acetone-fixed cells were tested for the presence of virus-induced cytoplasmic antigens using fluorescein conjugated goat anti-Moloney LV (Fig. 6), early and late F_1 leukemic spleen cells were indistinguishable. Early leukemic spleen cells were 50–80% positive while late leukemic spleen cells were 40–80% positive. Thus, it appeared that the loss of virus-induced cell membrane antigens from late FV leukemic spleen cells was a cell surface phenomenon since viral antigens were present in the cytoplasm of the late leukemic spleen cells.

In Vivo Reconstitution Experiments. The decrease in FV-induced cell surface antigens on late (B10.A × A/WySn)F_1 leukemic spleen cells was found to be reversible after adoptive transfer of these cells into lethally irradiated unimmunized mice. Late (B10.A × A/WySn)F_1 hybrid leukemic spleen cells (H-$2^{a/a}$ were injected into lethally

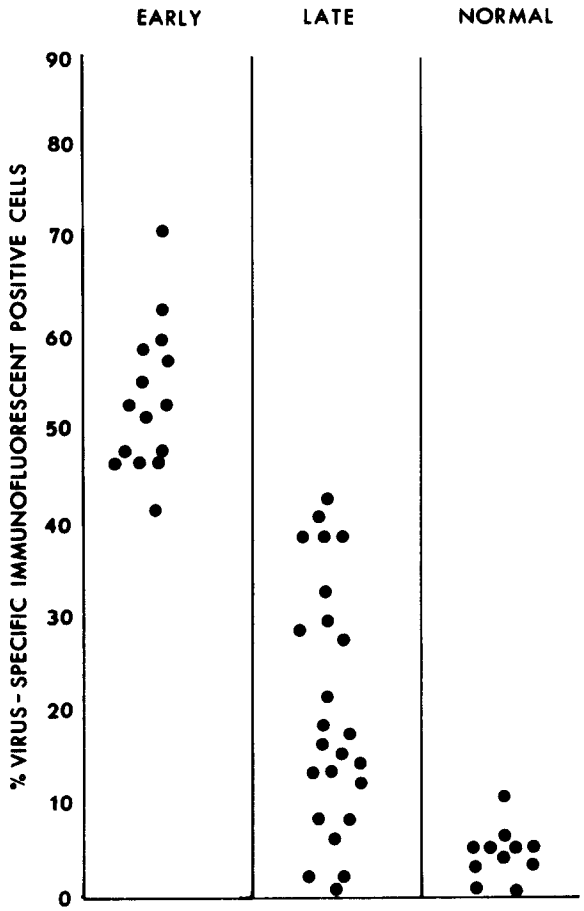

FIG. 4. Percentage of cells showing virus-specific membrane fluorescence in the indirect membrane fluorescence assay. Leukemic or normal nucleated spleen cells were reacted with either anti-FV serum or normal mouse serum, then washed and reacted with fluorescein-conjugated rabbit anti-mouse immunoglobulin. Percent virus-specific membrane fluorescence was calculated by subtracting the value obtained when the cells were reacted first with normal mouse serum from the value obtained with anti-FV serum. Each point represents one mouse spleen. 100–200 cells were counted per sample. Early leukemic spleen cells were 8–9 days post-FV infection from (B10.A × A)F_1 mice. Late leukemic spleen cells were 30–90 days post-FV infection from (B10.A × A)F_1, (B6/TL$^+$ × A)F_1 and (B6 × A/TL$^-$)F_1 mice. Normal (B10.A × A)F_1 spleen cells were used as controls.

irradiated (900 rads) (B10.A × A.BY)F_1 congenic hybrids (H-$2^{a/b}$). The spleens were harvested 6–7 days after injection, and the cells were analyzed by immunofluorescence for FV-induced cell-surface antigens and for FV-induced cytoplasmic antigens. As seen in Table I late F_1 leukemic spleen cells transferred into unimmunized lethally irradiated recipients showed an increased percent (51–66%) of cells positive for FV-specific membrane immunofluorescence. These values were similar to those seen previously in early (8–9 day) spleen cell populations (Fig. 5). Late F_1 cells transferred to immune lethally irradiated recipients failed to show this increase. Recovered leukemic cells from both immune and nonimmune recipients were found to have high levels of cytoplasmic virus-specific fluorescence (63–92%) when acetone-fixed cells were examined. This indicated that virus-infected leukemia cells were not eliminated after transfer to either type of recipient.

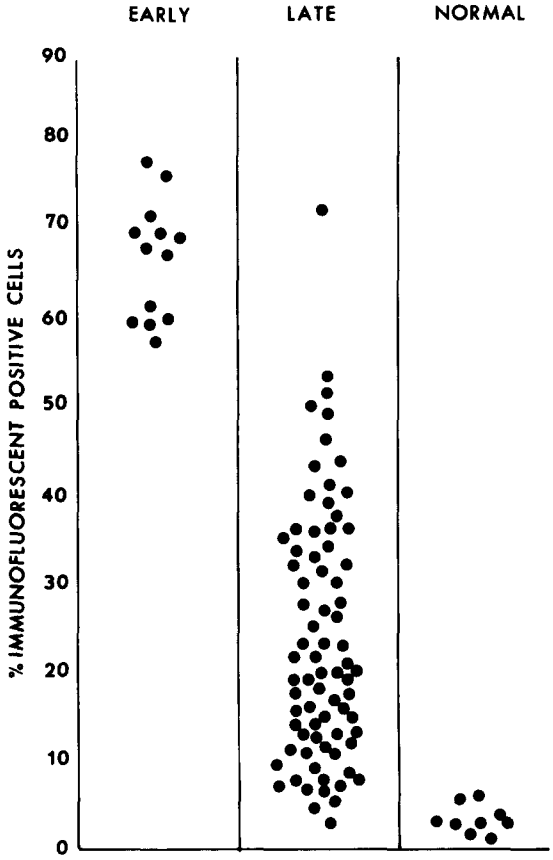

FIG. 5. Percent virus-specific membrane fluorescence in the direct assay. Leukemic or normal (B10.A × A)F_1 spleen cells were reacted directly with fluorescein-conjugated goat anti-Moloney LV serum. Early leukemic cells were 8–9 days post-FV infection. Late leukemic cells were 30–90 days post-FV infection. Each point represents one mouse spleen.

Early (8–9 day) leukemic spleen cells (66–76% positive membrane fluorescence) were similarly transferred to nonimmune and immune lethally irradiated recipients. 6–7 days after transfer into immune lethally irradiated recipients, 9–28% of recovered spleen cells were positive for membrane fluorescence (Table II). The percent of cells positive for FV-induced cell membrane antigens remained high when the early spleen cells were transferred to unimmunized lethally irradiated recipients. In both types of recipient, cytoplasmic virus-specific fluorescence was seen in 53–62% of the spleen cells recovered 6–7 days after transfer.

In Vitro Studies. Experiments were carried out to attempt to increase the expression of FV antigen on late F_1 leukemic spleen cells by incubating the cells in vitro in the absence of antibody. Washed late FV leukemic spleen cells were placed in culture at 37°C for various times from 1 to 72 h. The late FV spleen cells failed to show an increase in virus-induced cell surface antigens beyond initial levels, as assayed by direct membrane immunofluorescence (data not shown). In converse experiments, 8–9 day (early) FV leukemic spleen cells were placed in tissue culture for 1–48 h at 37°C with high concentrations of cytotoxic anti-FV antisera (1:4 or 1:5). However, no decrease in cell surface FV-induced antigen concentration was observed (data not

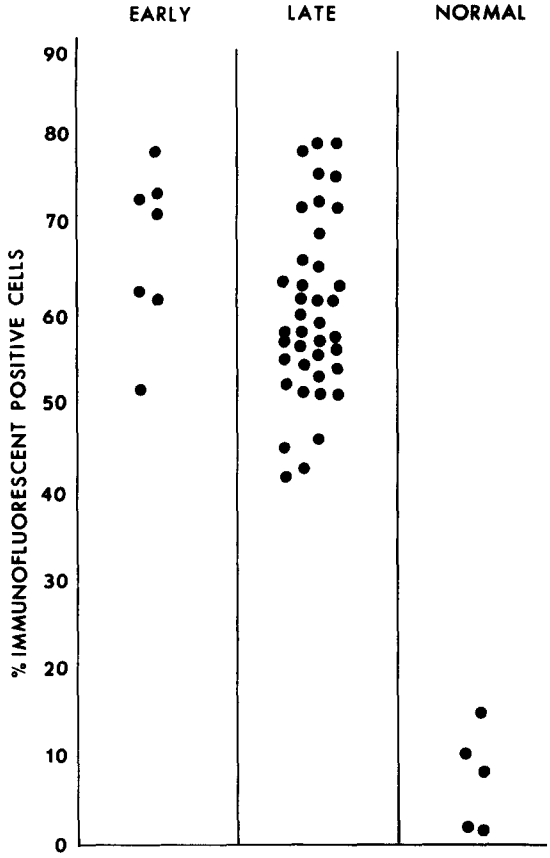

FIG. 6. Percentage of virus-specific intracellular fluorescence in acetone-fixed spleen cells from (B10.A × A/WySn)F_1 mice reacted with fluorescein-conjugated goat anti-Moloney LV. Early leukemic cells were 8–9 days post-FV infection. Late leukemic cells were 30–90 days post-FV infection. Each pont represents one mouse spleen.

shown). By indirect immunofluorescence and by cytotoxicity, the antibodies appeared to bind to the cell surface and remained there for the duration of the incubation (up to 48 h). No evidence for antigenic modulation was seen. Thus, we were unable to induce increased expression of FV-induced antigens on the leukemic spleen cells nor were we able to cause an antibody-mediated decrease or modulation of FV-induced cell membrane antigens in vitro.

Discussion

Leukemic spleen cells from (B10.A × A.BY)F_1 and (B10.A × A/WySn)F_1 mice had reduced amounts of FV-induced cell surface antigen 30–90 days after FV inoculation as compared to cells taken 8–9 days after inoculation. This was shown with fluorescent antibody, quantitative absorption and antibody plus C′ cytotoxicity. In vivo blocking of FV antigens by anti-FV antibody was ruled out as an explanation for these results by the indirect membrane fluorescence assay, which indicated that cells did not react with anti-immunoglobulin unless anti-FV antibody was added first. When FV-induced cell surface antigens were lost intracellular FV-induced antigens

TABLE I

In Vivo Reappearance of FV-Induced Cell Surface Antigens on Late F_1 Leukemic Spleen Cells

Experiment	Membrane fluorescence*		
	Donor	900 rad Nonimmune recipients	900 rad Immune recipients‡
		%	
1	36	57	15
2	37	61	30
3	6	51, 54, 51	17, 18
4	19	59, 54	18, 25
5	38	66	NT§
6	8	51	NT

* Late (30–90 days) (B10.A × A/WySn)F_1 leukemic spleen cells ($1–3 \times 10^7$) were transferred into lethally irradiated (900 rads) congenic (B10.A × A.BY)F_1 hybrids which were either normal or previously immunized against FV-induced cell surface antigens. Recipient spleen cells were examined for viral antigens by immunofluorescence 6–7 days after cell transfer. Percent of cells showing direct membrane immune fluorescence with fluoresceinated goat anti-Moloney LV serum. 100–200 cells were counted per population. All spleen cells recovered from donor mice or from recipient mice 6–7 days after transfer had high percentages of cells (63–92%) which were positive for cytoplasmic viral antigens by immunofluorescence analysis of acetone-fixed cells. In both types of recipients the spleen cells were of donor H-2 type as determined by direct cytotoxicity typing.

‡ (B10.A × A.BY)F_1 mice which had recovered from FV leukemia were repeatedly boosted with 8-day syngeneic leukemic spleen cells, and had high titers of circulating anti-FV cytotoxic antibodies at the time of irradiation.

§ NT, not tested.

TABLE II

FV-Induced Cell Surface Antigen Loss on Early (8–9 Day) Leukemic Spleen Cells Transferred to Lethally Irradiated Immune Recipients

Experiment	Membrane fluorescence*		
	Donor	900 rad Nonimmune recipients	900 rad Immune recipients‡
		%	
1	70	60, 63	9, 20
2	66	66	10, 17
3	76	75, 70	26, 18
4	68	66, 64	13, 28

* 8–9 day (early) (B10.A × A/WySn)F_1 leukemic spleen cells ($1–3 \times 10^7$) were transferred into lethally irradiated (900 rads) congenic (B10.A × A.BY)F_1 hybrids which were either normal or previously immunized against FV-induced cell surface antigens. Recipient spleen cells were examined for viral antigens by immunofluorescence 6–7 days after cell transfer. Percent of cells showing direct membrane immune fluorescence with fluoresceinated goat anti-Moloney LV serum. All spleen cells recovered from donor mice or from recipient mice 6–7 days after transfer had high percentages of cells (53–62%) which were positive for cytoplasmic viral antigens by immunofluorescence analysis of acetone-fixed cells.

‡ (B10.A × A.BY)F_1 mice which had recovered from FV leukemia were repeatedly boosted with 8-day syngeneic leukemic spleen cells, and had high titers of circulating anti-FV cytotoxic antibodies at the time of irradiation.

were still present. The membrane antigen loss observed appeared to be specific for FV antigens since there was no concomitant change in the expression of H-2 antigens at 8–9 and >30 days, as determined by quantitative absorption. The loss of FV-induced cell surface antigens on late leukemic spleen cells was seen only with F_1 hybrids which had B10.A or other B10 congenic mice as one parent. The A.BY and A/WySn parents did not lose FV-induced cell surface antigens in late stages of the disease.[2]

Evidence for the reversibility of the loss of FV-induced cell membrane antigens was obtained by transferring late leukemic spleen cells to lethally irradiated recipients. FV-induced cell surface antigens reappeared within 6–7 days after transfer of late leukemic spleen cells to nonimmune irradiated hosts, but failed to reappear if the irradiated host had been previously immunized against FV leukemic spleen cells. Conversely, 8–9 day leukemic spleen cells expressing high levels of FV-induced cell surface antigens lost antigen 6–7 days after transfer to previously immune irradiated recipients, but failed to lose antigen when transferred to normal irradiated recipients. These results suggested an involvement of the anti-viral immune response in the induction or maintenance of FV-induced cell-surface antigen loss in the irradiated immune recipients. Preliminary data suggest that similar immune mechansims may also be responsible for the loss of FV-induced antigens seen in late stages of FV leukemia in (B10.A × A/WySn)F_1 mice. The F_1 hybrid mice have demonstrable circulating cytotoxic anti-leukemia cell antibody 30 days or later after FV infection, and the time course of FV antigen loss corresponds with the rise in circulating cytotoxic antibody titer.[2] Furthermore, A.BY and A/WySn parents of these F_1 hybrids which do not develop circulating cytotoxic anti-leukemia cell antibodies, do not show a decrease in FV-induced cell surface antigen during the course of the disease.

The mechanism of the reappearance of FV antigen on the late leukemic spleen cells injected into irradiated recipients is not completely clear. Antigen may be re-expressed on the original low antigen donor cell population and their progeny, or residual antigen positive leukemic cells may grow out after transfer to irradiated recipients. However, preliminary data indicate that pretreatment of the late leukemic spleen cell population with anti-FV antisera plus C′ to kill residual antigen positive cells prior to injection into irradiated nonimmune recipients does not alter antigen re-expression (data not shown). This finding appears to exclude the possibility of the outgrowth of residual high antigen cells after adoptive transfer. Furthermore, since the same percent of cells was positive for virus-specific cytoplasmic fluorescence in the donor and the recipient spleens, it seems most likely that antigen was re-expressed on virus-infected cells after transfer to unimmunized recipients.

Since we had demonstrated the reversibility of the FV membrane antigen loss in vivo in a nonimmune environment, we attempted to induce a similar antigen increase in vitro. We were unable to observe an increase in FV-induced cell surface antigens on late F_1 leukemic spleen cells when these cells were placed in tissue culture in the absence of anti-FV antibody. Conversely, since it was likely that cytotoxic antibody was involved in the induction of FV surface antigen loss observed in vivo, we attempted to induce a decrease in FV-induced cell surface antigen on early leukemic spleen cells by placing these cells in cultures with high concentrations of cytotoxic anti-FV serum. We were unable to decrease FV surface antigen on early cells with

[2] D. Doig and B. Chesebro. 1978. Manuscript in preparation.

this technique, and were even unable to decrease antigen by using an anti-FV/anti-immunoglobulin sandwich technique (data not shown). Old et al. (12) were able to cause a selective loss of TL (thymus-leukemia) antigens on ascites leukemia cells cultured in vitro in the presence of anti-TL antisera, and in vivo in immunized recipients (13). TL antigenic modulation was quite rapid and was shown to be an active metabolic process. The reappearance of TL antigen when the cells were cultured in the absence of anti-TL antibody was less rapid, and may have required several cell divisions (12). Genovesi et al. (14) observed modulation of FV antigen from the cell surface of a FV tumor line when incubated in vitro in the presence of anti-FV antibody. Antigenic modulation was seen to a greater extent when the cells were in logarithmic growth as compared to cells in a resting state. Failure of FV leukemic spleen cells to divide in vitro may explain why we were unable to induce in vitro modulation of FV antigens on early leukemic spleen cells or to observe FV antigen reappearance on late cells cultured in vitro. On the other hand, it is also possible that the FV antigen loss observed in vivo with leukemic spleen cells is substantially different from the antigenic modulation described with the TL system and with a FV tumor cell line in vitro.

Late F_1 leukemic spleen cells retained intracellular or cytoplasmic FV-induced antigens while their surface antigens were lost. Ioachim et al. (15), found a similar disjunction between membrane and cytoplasmic Gross murine leukemia virus (MuLV) antigen expression when G-MuLV lymphoma cells were injected into partially tolerized rats. Thus in both FV and G-MuLV systems, leukemia cells sometimes exhibit surface antigenic adaptations to immune pressures without altering intracellular antigen expression. This may be a means by which tumor cells escape immune destruction (16). It is clear that the presence of cytotoxic anti-leukemia cell antibodies is not by itself sufficient to cause a recovery from leukemia (5). This may be because the leukemic cells become insensitive to antibody-mediated lysis as a result of a loss of FV-induced cell surface antigens. Similar findings have been reported for measles-virus infected cells which can also grow normally in the presence of cytotoxic anti-measles antibody (11). In vivo studies of suppression of tumor-specific cell surface antigen expression (17, 18) have involved transfer of tumor cells to immune recipients in order to induce antigen loss. Ioachim et al. (15, 19) reported that transfer of Gross MuLV lymphoma cells to immune recipients induced a decreased tumor antigen expression, and this antigen loss was associated with increased tumorigenicity on subsequent transfer. In the present study, adoptive transfer of tumor cells was not required to induce antigen loss, and the observed decreased antigen expression may represent a tumor cell adaptation to host immune pressures generated during the course of FV leukemia.

Summary

Friend virus (FV)-induced leukemic spleen cells from (B10.A × A)F_1 mice were found to lose sensitivity to antibody-mediated lysis during progression of erythroleukemia. This was correlated with a 78% loss of FV-induced cell surface antigens as determined by quantitative absorption of cytotoxic antibodies and with a decreased percentage of leukemic spleen cells showing membrane immunofluorescence with anti-FV antibody. Antigen loss was observed only with virus-induced antigens, and was limited to antigens expressed on the cell surface. FV-induced antigens were

regained when low-antigen leukemia cells from late stages of the leukemia were transferred to lethally irradiated nonimmune recipients, but not when these cells were transferred to hyperimmune lethally irradiated recipients. Conversely, when high-antigen leukemic spleen cells from early stages of the erythroleukemia were transferred to hyperimmune irradiated recipients, antigen loss was induced. The immune response to virus-induced antigens appeared to be involved in causing the antigenic changes observed on leukemia cells in this system.

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References

1. Friend, C. 1957. Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. *J. Exp. Med.* **105**:307.
2. Metcalf, D., J. Furth, and R. F. Buffett. 1959. Pathogenesis of mouse leukemia caused by Friend virus. *Cancer Res.* **19**:52.
3. Rossi, G. B., G. Cudkowicz, and C. Friend. 1973. Transformation of spleen cells three hours after infection in vivo with Friend leukemia virus. *J. Natl. Cancer Inst.* **50**:249.
4. Axelrad, A. A., and R. A. Steeves. 1964. Assay for Friend leukemia virus: rapid quantitative method based on enumeration of macroscopic spleen foci in mice. *Virology.* **24**:513.
5. Chesebro, B., and K. Wehrly. 1976. Studies on the role of the host immune response in recovery from Friend virus leukemia. I. Antiviral and anti-leukemia cell antibodies. *J. Exp. Med.* **143**:73.
6. Lilly, F. 1972. Antigen expression on spleen cells of Friend virus-infected mice. *In* RNA Viruses and Host Genomes in Oncogenesis. P. Emmelot and P. Bentvelzen, editors. North-Holland Publishing Co., Amsterdam. 229.
7. Chesebro, B., K. Wehrly, and J. Stempfling. 1974. Host genetic control of recovery from Friend leukemia virus-induced splenomegaly. Mapping of a gene within the major histocompatibility complex. *J. Exp. Med.* **140**:1457.
8. Chesebro, B., K. Wehrly, K. Chesebro, and J. Portis. 1976. Characterization of Ia8 antigen, thy-1.2 antigen, complement receptors, and virus production in a group of murine virus-induced leukemia cell lines. *J. Immunol.* **117**:1267.
9. Chesebro, B., and K. Wehrly. 1976. Studies on the role of the host immune response in recovery from Friend virus leukemia. II. Cell-mediated immunity. *J. Exp. Med.* **143**:73.
10. Shortman, K., N. Williams, and P. Adams. 1972. The separation of different cell classes from lymphoid organs. V. Simple procedures for the removal of cell debris, damaged cells and erythroid cells from lymphoid cell suspensions. *J. Immunol. Methods.* **1**:273.
11. Joseph, B. S., and M. B. A. Oldstone. 1975. Immunologic injury in measles virus infection. II. Suppression of immune injury through antigenic modulation. *J. Exp. Med.* **142**:864.
12. Old, L. J., E. Stockert, E. A. Boyse, and J. H. Kim. 1968. Antigenic modulation. Loss of TL antigen from cells exposed to TL antibody. Study of the phenomenon in vitro. *J. Exp. Med.* **127**:523.
13. Boyse, E. A., L. J. Old, and S. Luell. 1963. Antigenic properties of experimental leukemias. II. Immunological studies *in vivo* with C57Bl/6 radiation-induced leukemias. *J. Natl. Cancer Inst.* **31**:987.
14. Genovesi, E. V., P. A. Marx, and E. F. Wheelock. 1977. Antigenic modulation of Friend virus erythroleukemic cells in vitro by serum from mice with dormant erythroleukemia. *J. Exp. Med.* **146**:520.
15. Ioachim, H. L., S. E. Keller, B. H. Dorsett, and A. Pearse. 1974. Induction of partial

immunological tolerance in rats and progressive loss of cellular antigenicity in Gross virus lymphoma. *J. Exp. Med.* **139:**1382.

16. Jacobs, B. B., and D. E. Uphoff. 1974. Immunologic modification: a basic survival mechanism. Cellular adaptation contributes to tumor growth, allograft survival, and mammalian embryogenesis. *Science (Wash. D. C.).* **185:**582.
17. Ortaldo, J. R., C. C. Ting, and R. B. Herberman. 1974. Modulation of fetal antigen(s) in mouse leukemia cells. *Cancer Res.* **34:**1366.
18. Aoki, T., and P. A. Johnson. 1972. Suppression of Gross leukemia cell-surface antigens: a kind of antigenic modulation. *J. Natl. Cancer Inst.* **49:**183.
19. Ioachim, H. L., A. Pearse, and S. E. Keller. 1977. Antigenic deletion and malignant enhancement induced in lymphoma cells by passage through x-irradiated hosts. *Nature (Lond.).* **265:**55.