IDENTIFICATION OF A UNIQUE ERYTHROLEUKEMIA-ASSOCIATED RETROVIRAL gp70 EXPRESSED DURING EARLY STAGES OF NORMAL ERYTHROID DIFFERENTIATION

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During normal hematopoietic differentiation, lineage-specific cell surface antigens have been identified in both avian (1) and mammalian (2) species. These differentiation antigens can be found in most cellular compartments of the hematopoietic system and have added a new dimension to the classical morphologic identification of individual cell types during normal differentiation and leukemia. In addition, expression of these antigens at the cell surface has made it possible to purify live cells in a functionally intact state.

In the mouse, the most extensively studied antigen systems have been those of the various functionally distinct lymphocyte subpopulations (e.g., LyT (3) and LyB (4)). In addition, the expression of a number of endogenous retroviruses or products thereof have been observed in the mouse within lymphoid organs (5, 6), reproductive organs (7), and in the embryo (8), and have been likened to differentiation antigens because of their apparent lineage-specific expression. These retroviral antigens are apparently encoded by multiple retroviral gene sequences integrated throughout normal mouse genomic DNA (9). However, many more sequences than antigenic gene products have been identified, and little is known of the possible function of these endogenous viral sequences.

A panel of monoclonal antibodies has been developed in our laboratory which has allowed the serologic identification and distinction of a wide variety of murine ecotropic, xenotropic, and dualtropic viruses (10–12). The high degree of specificity of these reagents has made possible the distinction of qualitatively different endogenous retroviral products expressed in tissues of normal and leukemic mice, something that has not been possible with recombinant DNA technology.

In the current study, we have identified a unique retroviral envelope gp70 protein, the expression of which is linked to normal erythroid differentiation in both the fetus and adult mouse. This viral protein is expressed at the cell surface and appears to be serologically and structurally similar to a gp70 previously found to be expressed during the late malignant, but not the early proliferative

phase of Friend erythroleukemia (13). The presence of this gp70 may identify the target cell of malignant transformation induced by the Friend virus (FV)[1] complex.

Materials and Methods

Animals.   C57BL/10Sn, B10.A, A.BY, and A/WySn mice were purchased from The Jackson Laboratory, Bar Harbor, ME. F_1 hybrids were bred at the Rocky Mountain Laboratories, Hamilton, MT. Adult mice were between 2 and 4 months of age.

Cells and Viruses.   The B-tropic strain of FV complex was obtained from Dr. F. Lilly, Albert Einstein, School of Medicine, Bronx, NY. The virus stocks used for animal inoculation were prepared in (C57BL/10Sn X A.BY)F_1 mice and assayed as previously reported (14). SC-1 cells (15) were maintained and infected with virus as previously described (16). The mink lung fibroblast cell line CCL64 was obtained from Dr. M. W. Cloyd, Rocky Mountain Laboratories and maintained in RPMI 1640 media supplemented with 2% fetal calf serum (FCS). The Friend-mink cell focus-inducing virus (FMCF-1) and Friend–murine leukemia virus (F-MuLV) strain 57 were obtained from Dr. L. Evans, Rocky Mountain Laboratories. Xenotropic-MuLV including AKR-6 were obtained from Dr. M. W. Cloyd, Rocky Mountain Laboratories.

The clonal cell lines AA60, AA62, and L2 were obtained from spleen cells of grossly leukemic (B10.A X A/WySn)F_1 mice 60 d after FV inoculation. The cell lines were cloned by limiting dilution immediately after in vitro growth was detected. The cells caused transplantable tumors in syngeneic mice. These lines released no infectious virus nor could reverse transcriptase be detected in the supernatants. Growth in 1% dimethylsulf-oxide caused all of the cell lines to synthesize hemoglobin (17).

The other cell lines used in this study have been described previously. They include AA41, YA97, and 2C (18); AA25 (19); 7303, 7229, 7201, 7320, and 2800 (20); P815 (21); MBL-2 (22). P3-NS1/1-Ag4-1 was obtained from the Salk Cell Distribution Center, La Jolla, CA (23).

Early and Late Leukemic Spleen Cells and Embryonic Liver Cells.   Leukemic spleen cells were obtained from (B10.A X A/WySn)F_1 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) (13). Fetal liver cells were prepared from (B10.A X A/WySn)F_1 12–18 d fetuses. Gestational ages were determined in the following manner. Each cage was partitioned with a removable wire screen that separated one male from two females. After 3 d, the screens were removed and 24 h later the males were removed. This 24-h period was considered day 0. Pregnancy generally occurred in less than half of the females. Livers from the fetuses of an individual female were pooled and single cell suspensions prepared.

Stimulation of Erythropoiesis.   An erythroproliferative state was induced in vivo by injection of mice with a hemolytic agent, phenylhydrazine (PHZ) (24). PHZ was dissolved in PBBS at 1 mg/ml. This solution was inoculated immediately intraperitoneally at a dose of 1 ml (1 mg) per day for 3 d. Mice were sacrificed on day 4. At this time, the hematocrit was generally <30% and the spleens were slightly enlarged.

Antisera and Monoclonal Antibodies.   Monoclonal antibodies 18-1, 24-6, 18-2, 618, and 18-6 were derived from mice undergoing graft-vs.-host disease. A detailed description of these antibodies and their viral specificities has been reported (11, 12). All are reactive with endogenous MuLV. Monoclonal antibodies 48, 273 (10), and 514 (25) were derived from mice inoculated with FV and F-MuLV, respectively. All of the monoclonals except

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[1] *Abbreviations used in this paper:*   CT, chymotrypsin; DPBS, Dulbecco's phosphate-buffered saline; FCS, fetal calf serum; FFU, focus-forming units; FMCF-1, Friend-mink cell focus-inducing virus; F-MuLV, Friend murine leukemia virus; FV, Friend virus; MCF, mink cell focus-inducing; α-NBE, α-naphyl-butyrate esterase; PBBS, phosphate-buffered balanced salt solution; PHZ, phenylhydrazine; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

18-2 have specificity for gp70 or the gp70-p15(E) disulfide-linked complex gp85 (10–12, 25). The polypeptide specificity of 18-2 is unknown.

The anti-spectrin antiserum was prepared in rabbits by injection of spectrin purified from mouse erythrocyte ghosts (26). The specificity of this reagent has been confirmed both by radioimmunoassay, immunoprecipitation, and immunofluorescence on acetone-fixed cells (data not shown). Using a panel of erythroid, myeloid, and lymphoid cell lines (20), this serum was found to bind only to those cells of the erythroid lineage.

Immunofluorescence Assays. Indirect membrane immunofluorescence on live cells was carried out in two ways. The standard technique utilizing monoclonal antibody and FITC-conjugated goat anti-mouse Ig has been described previously (13). This assay was used on late Friend erythroleukemic spleen cells, leukemia cell lines, thymus, and fetal liver cells because of the relative paucity of membrane Ig-positive B lymphocytes. Examination of adult spleen and bone marrow cells, however, required the use of arsenylated monoclonal antibodies prepared by the technique of Wallace and Wofsy (27) and detected by FITC-rabbit anti-ars prepared in our laboratory.

Staining for spectrin was carried out on acetone-fixed cells deposited on microscope slides by cytocentrifugation. Binding of the rabbit anti-spectrin antibodies was detected by fluorescein isothiocyanate guinea pig anti-rabbit IgG prepared in our laboratory.

Histochemistry. The α-naphyl-butyrate esterase (α-NBE) and Sudan black stains used in this study have been described previously (20). Cells to be stained were prepared by cytocentrifugation, air dried, fixed 4 min in formaldehyde vapor, and stored at $-70°C$.

Radiolabeling of Cells. This procedure has been published previously (13). Briefly, leukemic spleen cells, leukemia cell lines, and embryonic liver cells were washed three times with PBBS and resuspended in Dulbecco's phosphate-buffered saline (DPBS) at 20 $\times$ 10^6 cells/ml. 1 ml of this solution was added to a glass vial containing 50 μg of Iodogen (Pierce Chemical Co., Rockford, IL). 1 mCi of carrier-free ^{125}I (ICN, Riverside, CA) was added and the solution was incubated on ice for 10 min. The cells were washed with DPBS and lysed in 0.5% Nonidet P-40, 0.15 M sodium chloride, and 0.01 N Tris-HCl, pH 7.38. The lysates were stored at $-70°C$.

Immunoprecipitation and Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE). The methods of immunoprecipitation using formalin-fixed *Staphylococcus aureus* Cowan I strain and analysis of immunoprecipitates by SDS-PAGE using autoradiography have been described previously (13). For reasons of low affinity, immunoprecipitation of F-MuLV gp70 required a mixture of antibodies 48 and 273, each of which reacts with a different epitope of the gp70 molecule (10).

Two-dimensional Peptide Mapping. Cell surface radiolabeled antigens were immunoprecipitated and analyzed by SDS-PAGE. The appropriate gp70 was visualized by autoradiography and excised from the gel. The gel slice was rehydrated with distilled water, minced with a scalpel, and ground in a Dounce-homogenizer. The finely dispersed gel was incubated in 0.05 M NH$_4$HCO$_3$, 0.1% SDS, 1% 2-mercaptoethanol, pH 8.1 buffer at 37°C for 4 h. The gel matrix was removed by centrifugation at 10,000 g for 30 min. Trichloroacetic acid was added to the supernatant to a final concentration of 20%. After incubation on ice for 1 h, the denatured protein was collected by centrifugation at 10,000 g for 45 min. The pellet was washed once with 70:30 ethanol/ether and then with ether. After evaporation of the ether, the protein was dissolved in 200 μl of 0.05 M NH$_4$HCO$_3$, pH 8.1. To this solution 12 μg of α-chymotrypsin (Worthington Biochemical Corp., Freehold, NJ) was added and the solution was incubated for 2 h. An additional 10 μg of α-chymotrypsin was added and the solution was incubated for 2 h. Distilled water was added and the sample was lyophilized. This was repeated three times to remove the NH$_4$HCO$_3$. The pellet was then resuspended in 5 μl of distilled water and applied to a thin layer plate (Polygram Cel 300; Brinkman Instruments, Westbury, NY). The sample was electrophoresed at 600 V for 45 min at 4°C in a buffer containing 0.05% acetic acid and 0.005% pyridine in water. Following drying, the thin layer plate was then chromatographed using a 43:57 pyridine, *n*-butanol solvent system. Following drying the sample was autoradiographed using a Dupont Lighting Plus intensifying screen.

Results

Monoclonal Antibodies Specific for Endogenous Murine Retroviruses React With the Cell Surface of FV-induced Erythroleukemia Cells. A panel of envelope-specific monoclonal antibodies reactive with various endogenous and exogenous retroviruses was used to characterize the non–F-MuLV gp70 expressed on the surface of FV-induced late leukemic spleen cells. Three antibodies, 18-1, 24-6, and 18-6, were found to react with approximately 50–60% of late leukemic spleen cells and with an FV-induced erythroleukemia cell line AA60 (Table I). In a previous report, we showed that antibody 18-1 reacts with all endogenous retroviruses tested, whereas antibody 24-6 detects an antigen common to most xenotropic viruses (11). In contrast to 24-6, which reacts with xenotropic viruses as a group and with a large number of MCF viruses, antibody 18-6 has been found to react with only one xenotropic (AKR-6) and only four out of several dozen MCF viruses studied (11). Neither AA60 nor the late leukemic spleen cells from which it was derived reacted with antibodies specific for F-MuLV and endogenous ecotropic viruses (48, 273, 18-2) (Table I).

Since antibody 18-6 also reacts with some MCF viruses (Table I), we used two monoclonal antibodies (618, 514) to further characterize this erythroleukemia-associated antigen. In our examination of 50–60 different MCF viral isolates of diverse origin, all have been reactive with one or both of these two antibodies. Since these antibodies do not react with ecotropic, xenotropic, or amphotropic virus, they are considered MCF specific (12, 25). Neither 618 nor 514 reacted with late FV spleen cells (Table I), suggesting that the 24-6+, 18-6+, 48/273-gp70 expressed by these cells resembled a xenotropic, not an MCF envelope protein. In contrast, a subpopulation of the cell line AA60 expressed a

TABLE I

Reactivity of Anti-MuLV Envelope Antibodies With FV-Induced Erythroleukemia Cells

Monoclonal antibody	Viral specificity*				Late FV spleen	AA60 cell line
	Friend‡	Akv	Xeno	MCF		
					%	%
18-1	−	+	+	+	55§	100
48	+	−	−	−	<5	0
273	+	−	−	−	<5	0
18-2	+	+	−	+	<5	0
24-6	−	−	+‖	+¶	60	100
18-6	−	−	+‖	+¶	58	100
618	−	−	−	+	<5	10–20
514	−	−	−	+	<5	10–20

* This information represents a summary of cumulative data reported previously in detail (10–12, 25).

‡ Friend, Friend ecotropic virus (F-MuLV); Akv, endognous ecotropic virus; Xeno, xenotropic virus; MCF, MCF virus.

§ Numbers represent the percent of total viable cells staining by indirect membrane immunofluorescence with the respective monoclonal antibodies.

‖ As described in the text, 24-6 reacts with a group-specific determinant expressed by most xenotropic viruses, whereas 18-6 reacts with only one minor subgroup of xenotropic viruses.

¶ Antibody 24-6 reacts with a majority of MCF viruses, whereas 18-6 reacts with only 3–5 out of >50 MCF viruses tested to date.

618/514+ gp70, suggesting that an MCF-like gp70 was expressed in this cell population but was apparently distinct from the 18-6+ gp70 because of the disparity in frequency of positive cells.

Expression of an 18-6 Reactive Antigen on Erythroid Cell Lines. Table II presents the results of a survey of various hematopoietic cell lines examined by indirect membrane immunofluorescence for the binding of antibody 18-6. It can be seen that 18-6 reacted with seven out of eight of the erythroid lines but with none of the myeloid, mast cell, or lymphoid lines, suggesting the possibility that 18-6 may define an erythroid-specific determinant.

Cells of the Normal Hematopoietic System Express a Cell Surface Antigen Recognized by Antibody 18-6. If antibody 18-6 reacted with a determinant specific for erythroid cell lines, it might be expected that this antigen should be expressed by cells during normal erythroid differentiation. We therefore examined adult spleen and bone marrow cells with 18-6-ars and FITC-rabbit anti-ars (see Materials and Methods). As shown in Table IIIA, both of these hematopoietic organs contained a minor, but clearly recognizable, subpopulation of 18-6 reactive cells.

In contrast to the findings in adult animals, the liver of early fetuses contained a high frequency of 18-6 reactive cells (Table IIIA). As the liver is the primary site of erythropoiesis in the fetus (28), this result suggested that antibody 18-6 was indeed detecting an antigen expressed on cells of the erythroid lineage. In

TABLE II
Reactivity of Antibody 18-6 With Cell Lines of Differing Lineage

Cell line	Lineage	% 18-6 positive cells*
AA60	Erythroid[‡]	100
AA62	Erythroid	100
AA25	Erythroid	100
AA41	Erythroid	100
YA97	Erythroid	100
2C	Erythroid	100
L2	Erythroid	10–20
2800	Erythroid	0
7229	Myeloid[§]	0
7235	Myeloid	0
7301	Myeloid	0
7320	Myeloid	0
P815	Mast cell[∥]	0
7303	Lymphoid[¶]	0
MBL-2	Lymphoid	0
NS-1	Lymphoid	0

* Reactivity was determined by indirect membrane immunofluorescence.
‡ The erythroid cell type was determined by the capacity to be induced to hemoglobin production by dimethyl sulfoxide, the expression of spectrin, and/or positive staining for α-naphyl butyrate esterase.
§ The myeloid cell type was determined by morphology in the case of well differentiated cells and by positive staining with Sudan black.
∥ P815 is reported to be of mast cell origin and contains cytoplasmic granules that stain with neutral red.
¶ The lymphoid cell type was determined by morphology, the expression of Thy-1, and exclusion of the tests described above.

TABLE III

Frequency of 18-6 Reactive Cells in Hematopoietic Organs

Organ	18-6*	MIg+*	Erythroid‡	Myeloid§
	%	%	%	%
A. Adult spleen	3	39	3	8
Adult bone marrow	3	8	22	55
Adult thymus	<1	<1	<1	<1
Early fetal liver∥	58	<1	70	17
B. Spleen post-PHZ	23	4	61	16
Bone marrow post-PHZ	25	2	63	32
Thymus post-PHZ	<1	<1	NT	NT

* The frequency of membrane Ig (MIg) positive cells (B lymphocytes) was determined by direct immunofluorescence with FITC-goat anti-mouse Ig. Cells expressing the 18-6 reactive antigen were detected by indirect membrane immunofluorescence as described in Materials and Methods.

‡ Erythroid cells were identified by histochemical staining for α-naphyl butyrate esterase and staining with anti-spectrin antiserum.

§ Myeloid cells were identified by morphology in Giemsa-stained preparations and by demonstration of characteristic Sudan black staining cytoplasmic granules.

∥ Fetal liver cells were obtained (without attention to stage of gestation) at the earliest visible sign of pregnancy.

NT, not tested.

addition, this high frequency of 18-6 positive cells corresponded to the high frequency of cells expressing two erythroid markers in the mouse, α-naphyl-butyrate esterase (α-NBE) and spectrin (Table IIIA).

Further evidence of the erythroid nature of cells detected by antibody 18-6 was obtained by determining the frequency of 18-6 positive cells during fetal development. At various times after impregnation, fetuses were removed and the frequency of cells from the fetal liver reacting with 18-6 was quantified. The frequency of positive cells decreased from ~70% on day 12 of gestation to <5% immediately before birth, coincident with a similar decrease in frequency of spectrin-positive cells (Fig. 1). These results also correlated with the previously reported progressive decline of morphologically recognizable erythroid cells within the fetal liver during embryogenesis (28).

Pertubation of Normal Erythropoiesis in Adults Caused an Increased Frequency of 18-6 Reactive Cells. The low frequency of 18-6 reactive cells in normal adult bone marrow and spleen as compared to their high incidence in early fetal liver suggested that during homeostatic conditions only a minor subpopulation of erythroid cells at a specific stage of differentiation expressed the antigen detected by antibody 18-6. Pertubation of normal hematopoiesis might then lead to an expansion of the population of 18-6 reactive cells. Erythropoiesis was stimulated by phenylhydrazine (PHZ), an agent that causes a profound hemolytic anemia and increased erythropoiesis. After PHZ treatment (see Materials and Methods), the frequency of 18-6 positive cells increased seven- to eightfold in both the spleen and bone marrow (Table IIIB). In the same cell populations the frequency of α-NBE staining cells increased ~30-fold in the spleen as compared to a 3- to 4-fold increase in the bone marrow (Table IIIB). The frequency of cells exhibiting morphological and histochemical characteristics (Sudan black staining) of
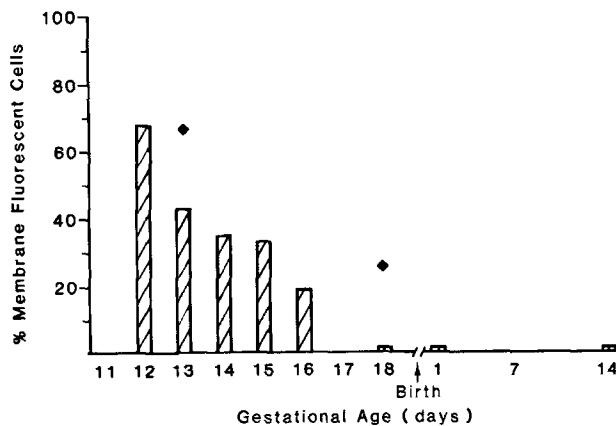
FIGURE 1.   Frequency of antibody 18-6 reactive cells in liver. Liver cells from gestationally timed fetuses were assayed by indirect membrane immunofluorescence with antibody 18-6 and FITC-goat anti-mouse Ig. Bars represent the percent of cells expressing the 18-6 reactive determinant at the cell surface. ◆ represents the frequency of spectrin-positive cells assayed after fixation with acetone.

myeloid cells decreased slightly in the bone marrow and increased slightly in the spleen (Table IIIB). The decrease in frequency of membrane Ig positive lymphoid cells in spleen and bone marrow after PHZ treatment probably reflects an increase in the number of erythroid cells, not a decrease in the absolute number of lymphoid cells (Table IIIB). These results strongly suggested that cells expressing the antigen detected by antibody 18-6 were of the erythroid lineage. In addition, the apparent discrepancy between the frequency of 18-6 positive cells and α-NBE staining in both the spleen and bone marrow (Table IIIA and B) further suggested that antibody 18-6 was detecting a subpopulation of erythroid cells presumably at a specific stage of differentiation.

FV Erythroleukemia Cells and Fetal Liver Express a Structurally Homologous gp70.   The serologic cross reactivity of this "erythroid gp70" on both erythroleukemia and fetal liver cells does not necessarily prove structural homology. Several examples exist of non-correlation between serologic cross reactivity and structural identity (29). In order to document that the 18-6 reactive determinant was expressed by a viral gp70 on the surface of these cells, we immunoprecipitated the radiolabeled cell surface proteins of early (10 d) FV spleen cells, late (30–60 d) FV spleen cells, the AA60 erythroleukemia cell line, and fetal liver cells and analyzed these proteins by SDS-PAGE. Early and late FV spleen cells from (B10.A X A)F$_1$ mice were previously found to differ in expression of both F-MuLV–encoded gp70 and a second gp70 unrelated to F-MuLV (13). As shown in Fig. 2, antibodies 18-1, 24-6, and 18-6 all precipitated a gp70-like polypeptide from the surface of late FV spleen cells, the AA60 erythroid cell line and 12-d fetal liver cells. The expression of F-MuLV gp70 precipitable by antibodies 48 and 273 and an 18-6 negative xenotropic gp70 precipitable by antibody 24-6 indicated that early FV spleen cells were phenotypically distinct from the other three erythroid cell populations. Further evidence of the relatedness of the non–F-MuLV, 18-6 reactive gp70 was obtained by digesting the respective gp70's
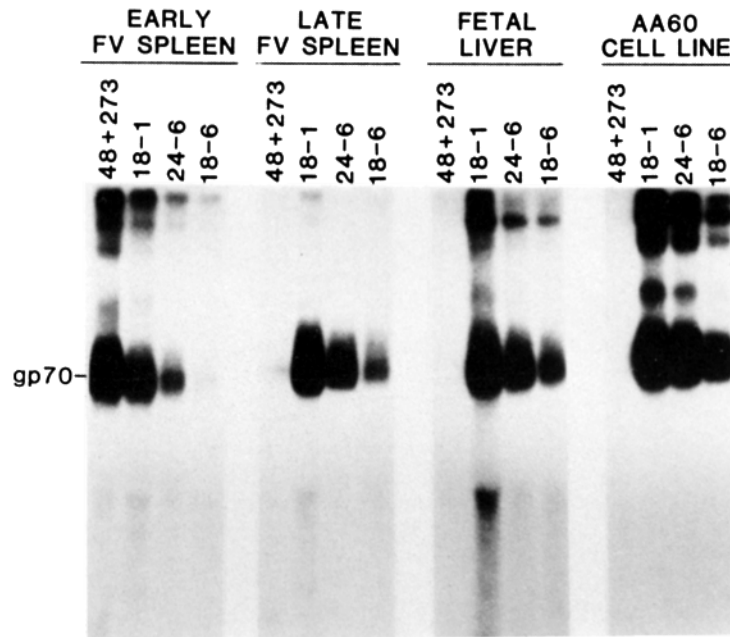
FIGURE 2.   Live cells were surface labeled with ^{125}I, immunoprecipitated with monoclonal antibodies 18-1, 24-6, 18-6, and a mixture of 48+273, and the precipitates resolved by SDS-PAGE. Note that 18-6 precipitated a xenotropic gp70 from fetal liver cells, the FV-induced erythroid cell line AA60, and late FV-leukemic spleen cells but not from early FV spleen cells. Conversely, 48+273 precipitated Friend MuLV gp70 from early FV spleen cells but not from the other three cell populations.

with α-chymotrypsin (CT) and resolving the fragments in two dimensions. The resulting peptide maps of the gp70 from late leukemic spleen cells, AA60 erythroleukemia cells, and fetal liver cells were strikingly similar and clearly distinguishable from the 48 and 273 reactive gp70 of F-MuLV (Fig. 3). These results indicated that the gp70 that was expressed on fetal liver cells, late FV erythroleukemia cells, and a cell line derived therefrom was not only antigenically identical but also structurally homologous.

Discussion

In this report, we have demonstrated the expression of an antigenically and structurally similar retroviral gp70 on the surface of both fetal liver cells and late FV erythroleukemia cells. Previously, we suggested that the gp70 on FV erythroleukemia cells was serologically closely related to FMCF gp70 (13). However, antigenic analysis with monoclonal antibodies specific for MCF viruses including FMCF-1 (12, 25) indicated that this gp70 did not express any MCF-specific antigens, nor did it react with any F-MuLV–specific antibodies (25) (Table I). Based on the pattern of reactivity with the panel of monoclonal antibodies used in this study, this late erythroleukemia-associated gp70 appeared to be xenotropic in nature and of endogenous origin.

Monoclonal antibody 18-6 reacted with this gp70 both in membrane fluorescence on live cells (Table I) and by immunoprecipitation (Fig. 2). It has been
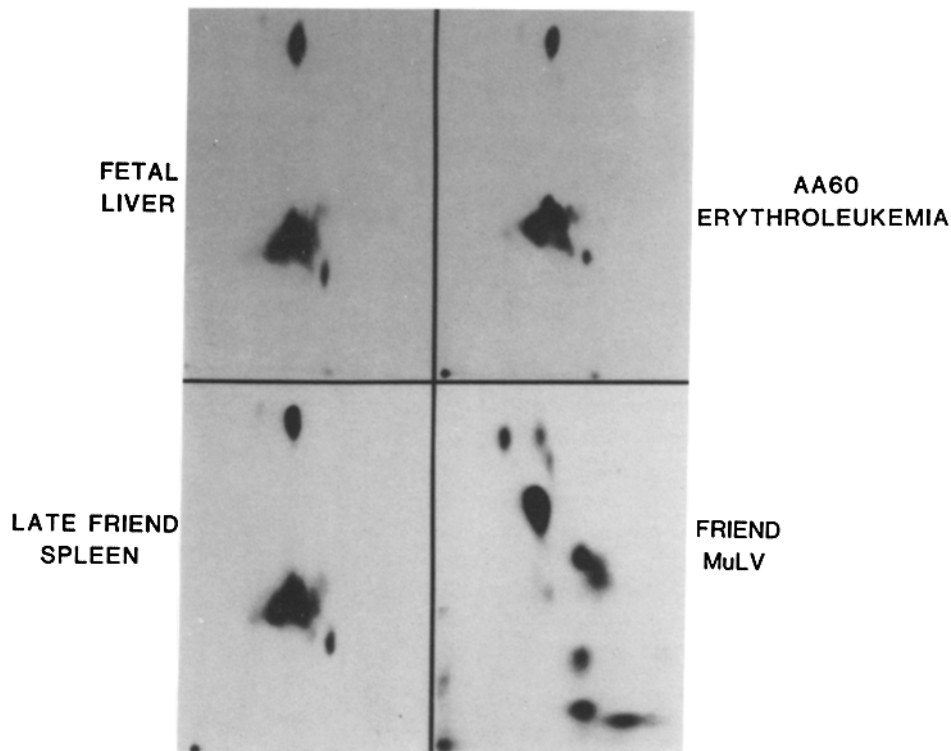
FIGURE 3. Chymotryptic peptide maps of the ^{125}I-labeled cell surface gp70 expressed by early fetal liver cells, late FV-leukemic spleen cells, the erythroid cell line AA60, and Friend MuLV virions. No unique spots were observed among the gp70 from fetal liver, late Friend spleen cells, or the AA60 cell line indicating a high degree of structural homology. The gp70 from Friend MuLV was clearly different. Electrophoresis is in the vertical and chromatography in the horizontal direction. Iodinated gp70 from surface-labeled cells and F-MuLV virions was isolated by immunoprecipitation and SDS-PAGE. The gp70 bands were excised from the gels, digested with chymotrypsin, and the peptide fragments resolved in two dimensions. Cell surface gp70 was immunoprecipitated with either antibody 18-1 or 18-6. The peptide maps of gp70 immunoprecipitated by these two antibodies were identical (not shown), indicating that the predominant gp70 species in these three cell populations was 18-6+. F-MuLV gp70 was immunoprecipitated with antibodies 48 + 273.

reported previously that this antibody reacts with only one xenotropic and three to five MCF viruses (11), out of more than 50 viral isolates of diverse origin that have been studied. Reactivity of 18-6 with the late erythroleukemia gp70 allowed the distinction of this polypeptide from other xenotropic gp70's commonly expressed by lymphoid cells (6). Thus, in Fig. 2, it can be seen that early FV spleen cells expressed a gp70 that was 24-6+ but 18-6-, indicating its antigenic dissimilarity to the 24-6+, 18-6+ gp70 expressed by late FV spleen and fetal liver cells. It is not possible to assess the expression of an 18-6- gp70 in late FV spleen or fetal liver cells using this combination of monoclonal antibodies, but these studies are in progress.

Several pieces of evidence suggested that the 18-6+ gp70 might be a marker of normal erythroid differentiation. FV erythroleukemia cell lines expressed this protein (Table II), whereas antibody 18-6 did not react with myeloid or lymphoid

cell lines (Table II) or with thymocytes (Table III). In addition, this gp70 was present on >60% of early fetal liver cells at the same time when 70% of cells from this organ were shown to be erythroid in nature (Fig. 1, Table IIIA). The progressive decrease in the frequency of 18-6 reactive cells in maturing fetal liver (Fig. 1) correlated with the declining frequency of erythroid cells in the fetal liver during embryogenesis (28). Additional evidence for the erythroid-associated expression of this gp70 was the seven- to eightfold increase in the frequency of 18-6 reactive cells in the spleen and bone marrow following treatment with PHZ (Table IIIB). PHZ has been shown to markedly increase erythropoietic activity by inducing a profound hemolytic anemia (24). This marked increase in erythropoiesis in the spleen and bone marrow was not accompanied by a significant increase in myelopoiesis or lymphopoiesis (Table IIIB); therefore the increased frequency of 18-6 reactive cells was directly correlated with heightened erythropoiesis.

Several findings suggested that antibody 18-6 recognized a subpopulation of erythroid cells, presumably at a discrete stage of differentiation. At 12–13 d of gestation, the percent of spectrin-positive and 18-6 positive cells in fetal liver was nearly identical, whereas at 18 days gestation the frequency of spectrin-positive cells was ~10-fold higher than 18-6 positive cells. Similarly, after treatment with PHZ, >60% of the cells of the spleen and bone marrow were erythroid as determined by histochemical staining, yet only 25% of this same cell population was reactive with antibody 18-6 (Table IIIB). Furthermore, in normal adult animals only 3% of bone marrow cells expressed this gp70, whereas 22% were histochemically and morphologically identified as erythroid (Table IIIA). One erythroid cell line (2800) was found to be negative for the 18-6+ gp70 (Table II) and may represent a less mature stage of differentiation than the other erythroid lines studied. The erythroid nature of 2800 has been confirmed by enzyme histochemistry (20), the expression of erythroid spectrin (John L. Portis, unpublished observations), and hemoglobin induction by culture in hemin (Steven Palmieri, unpublished observations). Further analysis of cells expressing this gp70 with in vitro functional assays will be required to define accurately the stage of differentiation of the hematopoietic cells expressing this protein.

The expression of this gp70 on a subpopulation of erythroid cells indicated that it should be classified as a differentiation antigen. Similar to other more well-studied retrovirus-related differentiation antigens such as G_{IX}, G_{rada}, G_{erld}, and G_{aksL2} (30), this protein was expressed on both normal and neoplastic cells. However, in contrast to the expression of these differentiation antigens on infectious virions recovered from normal and neoplastic lymphoid cells, we have been unable to recover by co-cultivation from fetal liver cells, spleen, or bone marrow cells of PHZ-treated mice, or late FV erythroleukemia cells, infectious virus expressing an 18-6+ gp70. Furthermore, we have observed that the gp70 detected by antibody 18-6 was expressed on fetal liver cells of a variety of mouse strains, including AKR/J, C57BL/10Sn, C57BL/6J, BALB/c, A/WySn, DBA/2, and NFS, whereas the lymphoid differentiation antigens, G_{IX}, G_{rada}, G_{erld}, and G_{aksL2} exhibit extensive genetically restricted expression (30).

Although products of endogenous retroviruses can be detected in tissues actively undergoing differentiation (7), their function in the process of differen-

tiation has never been established (31). A chicken strain has been produced that lacks endogenous viral sequences, suggesting that they have no role in the differentiation of avian hematopoietic cells (32). Our inability to find a mouse strain that lacks the "erythroid gp70" perhaps suggests a selective advantage for this protein in evolution. It has been suggested that endogenous gp70's may, in fact, protect cells from infection by pathogenic retroviruses through a mechanism of interference (33). Conversely, the sequences encoding such proteins may be under the same transcriptional control as functionally relevant, perhaps neighboring, sequences activated during the respective stage of differentiation (31).

Regardless of its function, the expression of the 18-6 reactive gp70 on FV erythroleukemia cells from the late phase of Friend disease should allow a more accurate investigation into the stage of differentiation of the erythroid progenitor cells that are transformed by the FV complex. Previous reports have suggested that FV-induced leukemia is a multistage disease with an early, hyperproliferative nonmalignant phase and a late malignant phase (34, 35). This would suggest that two different cellular phenotypes are present during the disease. Our preliminary results support this hypothesis. Late in the course of disease, a major population of spleen cells expressed the 18-6+ gp70 (Table I), whereas early in the disease these cells were found only in low frequency (data not shown). In addition, we could detect an 18-6+ gp70 by immunoprecipitation in late but not early FV spleen cells (Fig. 2). Whether this gp70 has a role in the malignant transition or is merely a useful phenotypic marker for malignant erythroleukemia cells is currently being investigated.

Summary

Late in the course of Friend virus (FV)–induced erythroleukemia, leukemic spleen cells express a cell surface retroviral gp70 envelope protein not detected during the early proliferative phase of the disease. Characterization of this gp70 revealed it was unrelated to the input Friend murine leukemia virus (F-MuLV), but antigenically similar to a unique subset of endogenous xenotropic viruses. This gp70 was expressed by murine erythroleukemia cell lines but has not been identified on cell lines of other lineages. A monoclonal antibody (18-6) specifically reactive with this polypeptide was used to examine hematopoietic organs of normal uninoculated mice. This antibody detected a gp70 expressed by a majority of erythroid cells in fetal liver and by a small but significant percentage of normal adult spleen and bone marrow cells. Increased erythropoietic activity induced by treatment of adult mice with phenylhydrazine (PHZ) resulted in a seven- to eightfold increase in the frequency of spleen and bone marrow cells expressing this gp70. Peptide map analysis indicated that the 18-6 reactive gp70 expressed by Friend erythroleukemia cells and by cells from normal fetal liver were structurally identical. These results suggested that this unique gp70 was an erythroid-specific differentiation antigen.

References

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