EXPRESSION OF AKR MURINE LEUKEMIA VIRUS gp71-LIKE AND BALB(X) gp71-LIKE ANTIGENS IN NORMAL MOUSE TISSUES IN THE ABSENCE OF OVERT VIRUS EXPRESSION*

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Most murine C-type viruses are endogenous to a variety of inbred strains of mice and have been classified into two groups of viruses based on host range (1). By hybridization of viral cDNA to cell DNA, sequences homologous to the xenotropic virus isolates have been detected in all the strains examined (2). In contrast, comparable experiments using cDNA from the ecotropic AKR Gross-type of virus have demonstrated that some strains of mice lack a portion (<50%) of the viral genome (3). Consistent with these data, strains lacking the complete AKR MuLV genome have never been shown to produce a complete infectious Gross- or AKR-type virus. These results therefore suggest that most strains of mice could be expected to express all the antigens associated with a complete xenotropic virus and at least a portion of the AKR or Gross viral antigens.

In several recent studies, the expression of viral antigens in the absence of overt virus expression has been suggested (4, 5). Such observations have led to the hypothesis that there exists "noncoordinate" regulation of the expression of viral antigens, and that such expression supports the notion that oncornaviruses play a role in development and differentiation (6, 7). Unfortunately, such conclusions have not been warranted for two reasons. First, the assays used to date to detect the expression of viral antigens have been either immune precipitation or competition assays using antisera with broad reactivity. Thus, no clear conclusions can be made as to the homology of the cellular antigens with viral antigens. Second, the results could not distinguish between expression of a cellular gene with close homology to viral antigens and expression of sequences physically associated with the complete viral genome. Thus, neither the structural homology of the antigens to viral proteins nor the relationship of the genes for these antigens to viral sequences is known.

During the last 2 yr we have extensively analyzed the role of endogenous ecotropic, AKR-type viruses in radiation leukemia of mice. The results show no seroepidemiological relationship between these viruses and leukemia, and no overt virus expression in most of the thymomas (8, 9). Nevertheless, to further examine virus expression during the course of thymoma development, we extensively examined the major lymphoid populations and other tissues by competition radioimmune assays for viral antigens. The results from some of these experiments are reported here and demonstrate that an antigen indistinguishable from the AKR viral gp71 by homologous competition assays is constitutively expressed in bone marrow cells of all mice examined; an antigen

indistinguishable from the xenotropic gp71 is expressed in the sera of most mice examined; and, the expression of either of these antigens is independent of any apparent overt virus expression.

Materials and Methods

Experimental Animals. With the exception of 129, SWR, and A strains, all of the mouse strains and the Fischer 344/N rats were bred at the Central Animal Facility of the Frederick Cancer Research Center. These animals were maintained under specific pathogen-free conditions until used. The 129, SWR, and A strains were obtained from The Jackson Laboratory, Bar Harbor, Maine. The wild mice were kindly provided by Dr. Donald Fine of the Frederick Cancer Research Center.

Preparation of Tissues and Homogenates. Bone marrow was expressed from femurs and tibiae with Ham's F-12 medium, and washed once with Ham's F-12 by centrifugation at 600 g for 10 min. Thymocytes, splenocytes, and lymph node cells were prepared by teasing the tissues in Ham's F-12, followed by lysing erythrocytes in 0.83% (wt/vol) NH_4Cl. The cells were washed as above. All cell pellets were kept at $-20°C$ until used.

The cells were resuspended in 100 μl of 0.01 M sodium phosphate buffer, pH 7.0, and allowed to swell for 5-10 min. The suspensions wree homogenized in 1-ml capacity Kontes tissue grinders (Kontes Co., Vineland, N. J.). Two additional homogenization steps followed the additions of 100 μl of 0.01 M Tris-HCl, 1 M KCl, and 1% (wt/vol) Triton-X-100, pH 7.5 (TKT buffer)[1] and 500 μl of 0.01 M Tris-HCl, 0.1 M NaCl, 0.001 M EDTA, and 0.01% (wt/vol) Triton-X-100, pH 7.5 (TNET). The resulting homogenates were centrifuged at 25,000 g for 20 min, after which the supernates were removed and stored at $-20°C$ until used. Protein concentrations in the homogenates were assayed by the method of Lowry et al. (10).

Preparation of Viral Reagents. AKR, Friend, and BALB(X) viruses and viral proteins were isolated and purified, and specific antisera were made in rabbits as previously described (11, 12). Viral proteins were iodinated using $Na^{125}I$ and chloramine-T according to the method of Greenwood et al. (13).

Competition Radioimmune Assays. Tissue extracts were prepared by homogenizing either washed single cell suspensions or intact tissue. Single cell suspensions were obtained by pushing organs through a wire mesh screen while constantly washing with Eagle's minimum essential medium, filtering the suspension through gauze, and then pelleting the cells by centrifugation. Erythrocytes were subsequently removed by resuspending the cells in 0.85% ammonium chloride for 10 min at 20°C, and intact cells were removed by centrifugation. Comparable results were obtained with either cell suspensions or tissue homogenates, although extracts of single cell suspensions interfered less in the assays at high protein concentrations. In either case, an equal volume of 1% Triton, 1 M KCl, and 0.1 M sodium phosphate buffer at pH 7.0 was added, and the cells or tissues were homogenized with 10 strokes in a ground glass homogenizer. One volume of H_2O was added and the tissue rehomogenized. The extract was then centrifuged at 15,000 rpm for 20 min, and the supernate aliquoted and frozen at $-20°C$ for assays.

Competition radioimmune precipitation assays were comparable to those previously reported (11). Tissue extracts were diluted twofold in 0.2 ml of 0.01% Triton, 0.1 M NaCl, and 0.05 M Tris-HCl, pH 7.5. The necessary concentrations of antisera required to precipitate approximately 50% of the labeled antigen of monospecific rabbit antisera to either AKR murine leukemia virus (MuLV) gp71, p30, or p12 or a goat antiserum against the endogenous virus of C57L mice (provided by Dr. Raymond Gilden of the Frederick Cancer Research Center) and approximately 0.01 ml of normal rabbit or goat gamma globulins were added. The mixtures were incubated 1 h at 37°C, iodinated antigen was added, and the mixtures incubated 3 h at 37°C and overnight at 4°C. Goat antisera to rabbit gamma globulins or rabbit antisera to goat gamma globulins were then added at the appropriate concentration to reach the equivalence point for precipitation of the added carrier rabbit gamma globulins and incubated 2 h at 37°C and 3 h at 4°C. Immune precipitates were then collected by centrifugation, washed once with the above buffer, and counted in a gamma counter.

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[1] *Abbreviations used in this paper:* MuLV, murine leukemia virus(es); TNET, 0.01 M Tris-HCl, 0.1 M NaCl, 0.001 M EDTA, and 0.01% (wt/vol) Triton-X-100, pH 7.5.
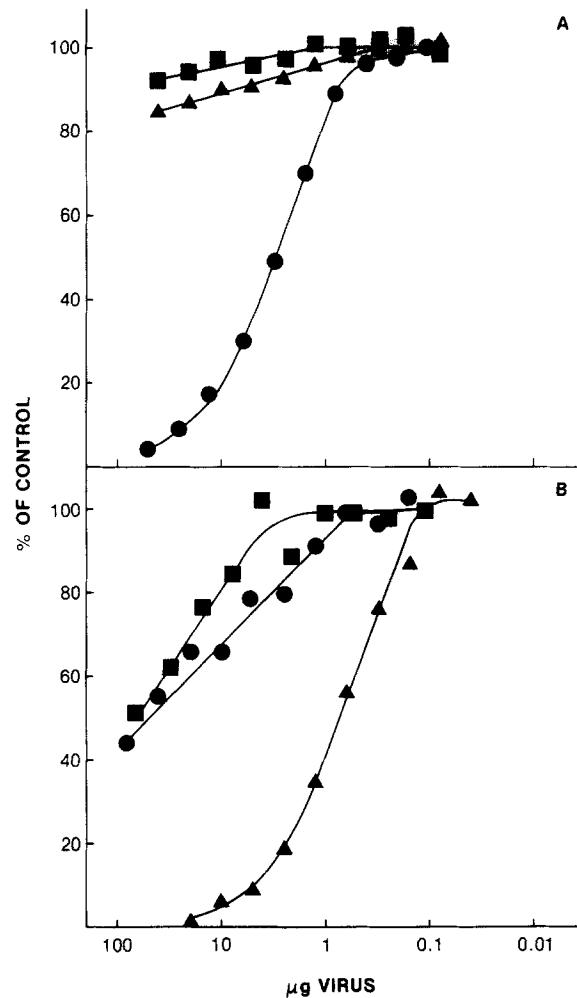
FIG. 1. Comparisons of the reactivities of AKR MuLV (●), BALB(X) MuLV (▲), and Friend MuLV (■) in radioimmune assays for the major envelope glycoprotein, gp71. Viruses disrupted in 0.5 M KCl, 0.5% (wt/vol) Triton-X-100, 0.01 M Tris-HCl, pH 7.5 and assayed at serial twofold dilutions in 0.2 ml of TNET for their ability to compete with the indicated ^{125}I-labeled gp71 at limiting antiserum dilution. (A) Rabbit anti-AKR gp71, ^{125}I-labeled AKR gp71. (B) Goat anti-C57L-MuLV, ^{125}I-labeled BALB(X) gp71.

The results are presented as the percent of labeled antigen precipitated relative to that precipitated in the absence of competing antigen.

The specificities of the radioimmune competition assays used in these studies are shown in Fig. 1. As seen in Fig. 1 A, the assay for the AKR-type MuLV gp71 is type specific. AKR MuLV competes, whereas neither Friend MuLV nor BALB(X) MuLV competes. The specificity of the assay for the BALB(X) gp71 is shown in Fig. 1 B. In this assay both the NZB (not shown) and the BALB(X) viruses compete completely, whereas Friend MuLV and AKR MuLV compete only partially. The specificities of competition assays for AKR MuLV p30 and p12 have been described (8). Briefly, all murine C-type viruses examined compete completely in the p30 assay, whereas generally only the N-tropic Gross-type viruses compete completely in the p12 assay.

Preparation of RNA. AKR MuLV 70S RNA was isolated from purified virus by phenol extraction and sucrose density gradient centrifugation as previously described (9). Cellular and
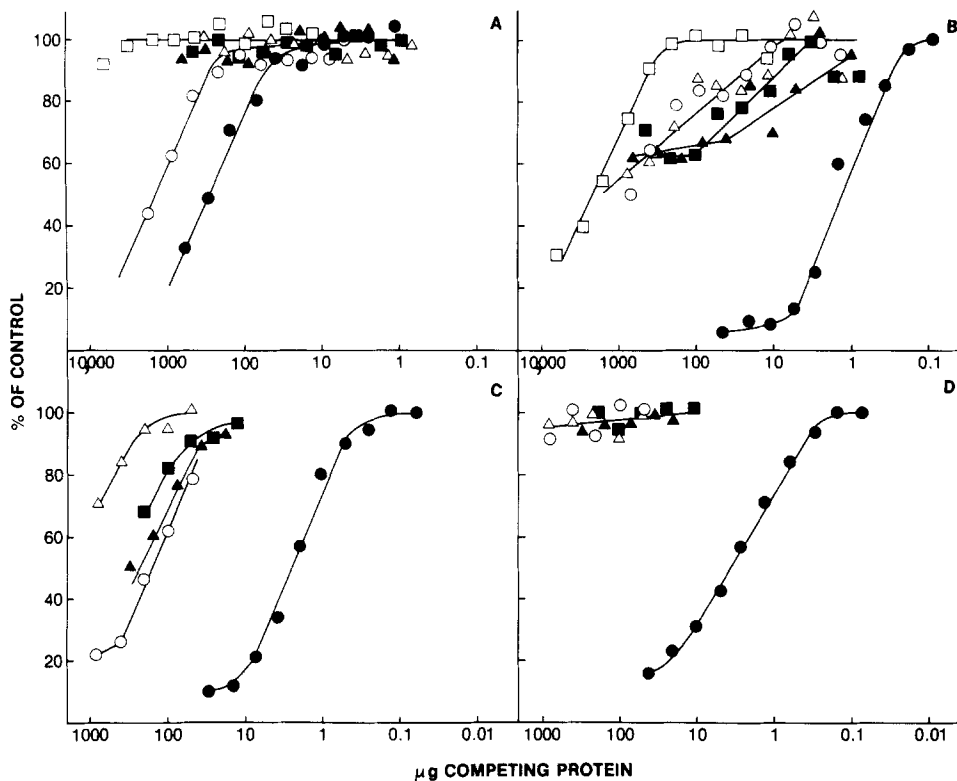
FIG. 2. Radioimmune assays of C57BL/6 tissues for the MuLV proteins. Homogenates were prepared from bone marrow (○), lymph node (△), spleen (▲), and thymus (■) cells as described in the Materials and Methods. Serum (□) was adjusted to 0.01 (wt/vol) Triton-X-100, and the appropriate purified virus or viral protein was used to generate a standard curve (●). These were serially diluted and reacted as described for Fig. 1. (A), AKR gp71; (B), BALB(X) gp71; (C), AKR p12; (D), AKR p30. For the AKR gp71, AKR p12, and AKR p30 standard curves the abscissas are nanograms of competing protein.

tissue RNAs, prepared from cytoplasmic extracts by phenol extraction, contained intact ribosomal RNA with less than 5% DNA contamination.

DNA-RNA Hybridization. [³H]cDNA was synthesized in the endogenous reverse transcription reaction with AKR MuLV and purified as previously described (9). The [³H]cDNA product had a specific radioactivity of $1-2 \times 10^7$ cpm/μg, and was greater than 99% digestible with nuclease S_1. At least 90% of the probe hybridized with an excess of AKR MuLV 70S RNA.

The [³H]cDNA and viral and cellular RNAs were hybridized in 0.45 M NaCl at 62°C for 48 h. The extent of hybridization was assayed with nuclease S_1 as described previously (8). The percent hybridization is defined as the counts per minute resistant to nuclease S_1 digestion divided by the total acid-precipitable counts per minute.

The thermal stabilities of the hybrids were measured by nuclease S_1 digestion after each increase in temperature in 0.15 M NaCl. The T_m is defined as the temperature at which 50% of the hybrid tested is rendered sensitive to nuclease S_1.

Results

The distribution of viral antigens in a variety of tissue extracts and sera from C57BL/6 mice was examined by competition radioimmune assays (Fig. 2). In the assay for AKR MuLV gp71 most tissues, including lymph node, spleen, thymus,

or serum, contained no competing antigen. In contrast, extracts from bone marrow cells competed in this assay and with a slope of competition identical to that seen with purified AKR MuLV gp71. Of the tissues examined, only sera competed in the assay for BALB(X) gp71 (Fig. 2 B) and gave curves with both extents and slopes of competition identical to those obtained with BALB(X) virus. In this assay, however, other tissues including lymph node, the presence of thymus, spleen, and bone marrow gave partial competition suggestive of the presence of partially cross-reactive antigens. The results obtained with these samples in competition assays for p30 and AKR MuLV p12 are shown in Figs. 2 C and 2 D. In assays for p30, all the tissues examined competed comparably and possessed low concentrations of p30, consistent with previous results (14, 15). In contrast, none of the tissues examined competed in the assay for AKR MuLV p12. These results demonstrate a unique expression of an AKR MuLV gp71-like antigen in bone marrow cells and a BALB(X) gp71-like antigen in sera without a comparable increase in the expression of p30.

Typical results of additional experiments using sera and tissue extracts from 129/J mice are shown in Fig. 3. Comparable to the results obtained with C57BL/ 6 mice, only homogenates from 129/J bone marrow cells competed completely in the assay for AKR MuLV gp71 (Fig. 3 A) although this particular lymph node extract competed to some extent at high protein concentrations. Similarly, only sera from 129/J mice competed completely in the assay for BALB(X) gp71 (Fig. 3 B), although the other tissues examined gave incomplete competition. None of the tissues competed in the assay for AKR MuLV p12 (Fig. 3 D), and all the tissues competed at high protein concentrations in the assay for p30 (Fig. 3 C). Therefore, as with C57BL/6 mice, these results suggest the unique expression of an AKR MuLV gp71-like antigen in bone marrow cells and a BALB(X) gp71-like antigen in sera of 129/J mice.

Typical results obtained with competition assays for AKR MuLV virion proteins and homogenates of bone marrow cells from several additional strains of mice are summarized in Table I. All the homogenates examined from the genus *Mus* competed strongly in the assay for AKR MuLV gp71. Among the inbred strains of mice examined, the levels of antigen were comparable with the exception of AKR mice which had increased levels due to active replication of the virus as demonstrated by the increases in p30 and p12. Of particular interest were the results obtained with NIH Swiss, C57L, and SWR/J mice. In bone marrow cells, these mice express an antigen serologically identical to AKR MuLV gp71, although as in 129/J mice an infectious AKR-type MuLV has never been found in these mice. In contrast no competition was seen in assays for the AKR MuLV p12, except in AKR mice, and only low levels of p30 were found in most of the extracts.

The results obtained with homogenates of bone marrow cells from *Mus musculus domesticus* (Kent Island), *Mus musculus casteneus,* and *Mus cervicolor* are illustrated in Fig. 4 and summarized in Table I. Comparable to the results obtained with the inbred strains of mice, bone marrow extracts competed in the assay for AKR MuLV (Fig. 4 A) gp71 and gave slopes of competition comparable to that obtained with AKR MuLV gp71. There was only weak competition in the assay for p30 (Fig. 4 C) and no competition in the p12 assay (Fig. 4 B) with the exception of one *Mus musculus casteneus* which had high
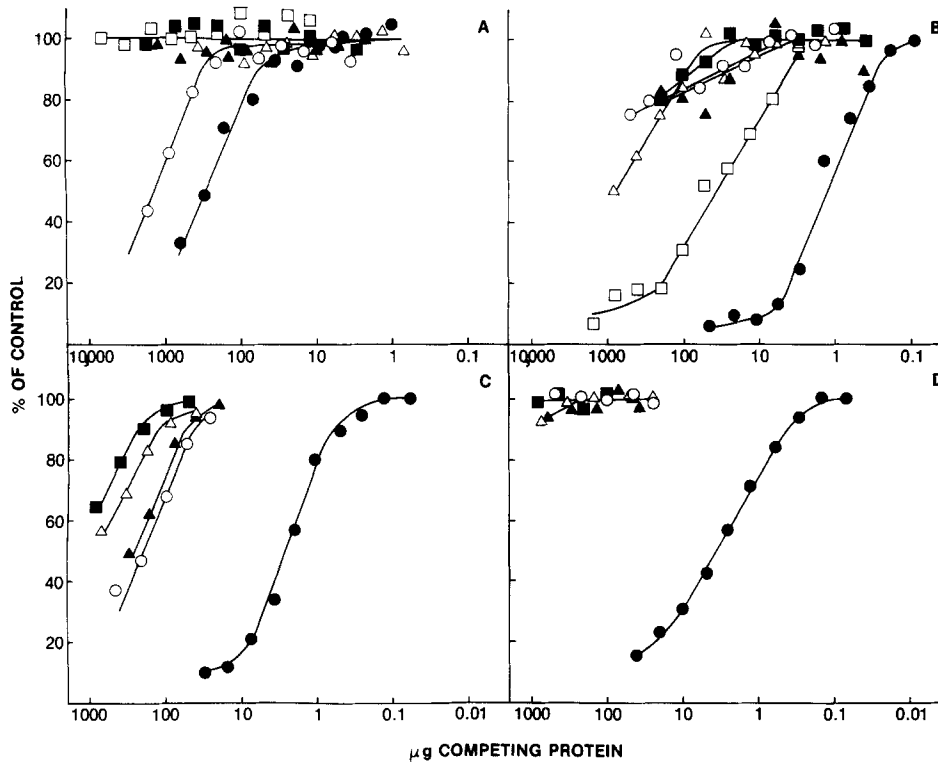
FIG. 3. Radioimmune assays of 129 tissues for the MuLV proteins. Homogenates were prepared from bone marrow (O), lymph node (△), spleen (▲), and thymus (■) cells as described in the Materials and Methods. Serum (□) was adjusted to 0.01% (wt/vol) Triton-X-100, and the appropriate virus or viral protein was used to generate a standard curve (●). These were serially diluted and reacted as described for Fig. 1. (A), AKR gp71; (B), BALB(X) gp71; (C), AKR p12; (D) AKR p30. For the AKR gp71, AKR p12, and AKR p30 standard curves, the abscissas are nanograms of competing protein.

levels of p30 in both lymph nodes (not shown) and bone marrow. Interestingly the lymph node extract of this mouse competed partially in the assay for AKR MuLV gp71, but with a slope and extent considerably different from AKR MuLV gp71 (not shown). Lastly, no competition was observed in any of the assays with extracts of bone marrow cells from rats.

The ability of the various tissues to compete in the BALB(X) gp71 assay is shown in Table II. In general, only sera competed significantly in this assay and not extracts of spleen, thymus, or bone marrow. Furthermore, although sera from *Mus musculus casteneus* competed partially in the assay, no competition was obtained with sera from *Mus cervicolor*. Also of interest was the observation that sera from either newborn BALB/c or NIH Swiss mice did not compete, although sera from 1- to 2-day-old mice have antigen levels comparable to sera from adults (data not shown).

In order to examine the extent of possible virus replication in bone marrow cells, hybridization experiments were done. As seen in Table III, when AKR MuLV cDNA was used to detect AKR MuLV sequences in C57BL/6 cellular RNA, all of the tissues examined had concentrations of RNA complementary to

TABLE I

Strain Distribution of AKR MuLV Proteins* in Bone Marrow

Strain	Age	gp71	p30	p12
	wk			
C57BL/6	7	200	7	<1
C3H	11	240	9	<1
B6C3F₁	8	220	16	<1
BALB/c	8	180	17	<1
NIH Swiss	8	140	19	<1
SWR/J	6	240	17	<1
AKR	8	1400	240	34
129/J	6	170	5	<1
C57L	16	200	4	<1
A/J	8	200	19	<1
M.m. casteneus-1	26	270	16	ND§
M.m casteneus-2	26	100	960‖	ND
M. cervicolor-1	26	280	17	<1
M. cervicolor-2	26	740	25	<1
M.m. (Kent Isl.)	26	260	35	<1
Rat	6	5	10	<1

* The data are expressed as nanograms of the viral protein per milligram of total protein in the homogenates.

‡ Except for the wild mice, which were assayed individually, pools of bone marrow cells from five or more animals were used.

§ ND, not done.

‖ The lymph node cells from this animal competed in the AKR gp71 assay; however, the slope and extent of the reaction were considerably different from those seen with the AKR gp71 and bone marrow extracts.

AKR MuLV cDNA at 100-fold lower concentrations than in AKR fibroblasts or leukemic spleens actively replicating the virus. Also, the thermal stability of the hybrids detected with these tissue RNAs was approximately 7°C lower than with the homologous hybrids. This lower T_m indicates a significant lack of homology and is comparable to previously reported data (2). Table III shows that the hybrids between AKR MuLV cDNA and AT-124 or BALB(X) viral RNA have a lower T_m and extent of hybridization when compared to the homologous hybrid with AKR MuLV 70S RNA or tissues actively replicating AKR-type viruses. These results are compatible with the presence of low levels of xeno-tropic-like RNA sequences in most C57BL/6 tissues, but more importantly fail to detect any overt expression of AKR MuLV-type RNA in bone marrow cells.

Discussion

Several previous reports (4, 5, 16) have suggested the widespread expression in mice of glycoproteins of viral origin in the absence of overt virus expression. Most of these studies however used broadly reactive assays which detected antigens serologically related to the viral glycoprotein, but which did not have the specificity to distinguish among the viral glycoproteins of the known endogenous murine C-type viruses. In more recent experiments the molecular heterogeneity of this expression was indicated by differences in the tryptic maps of
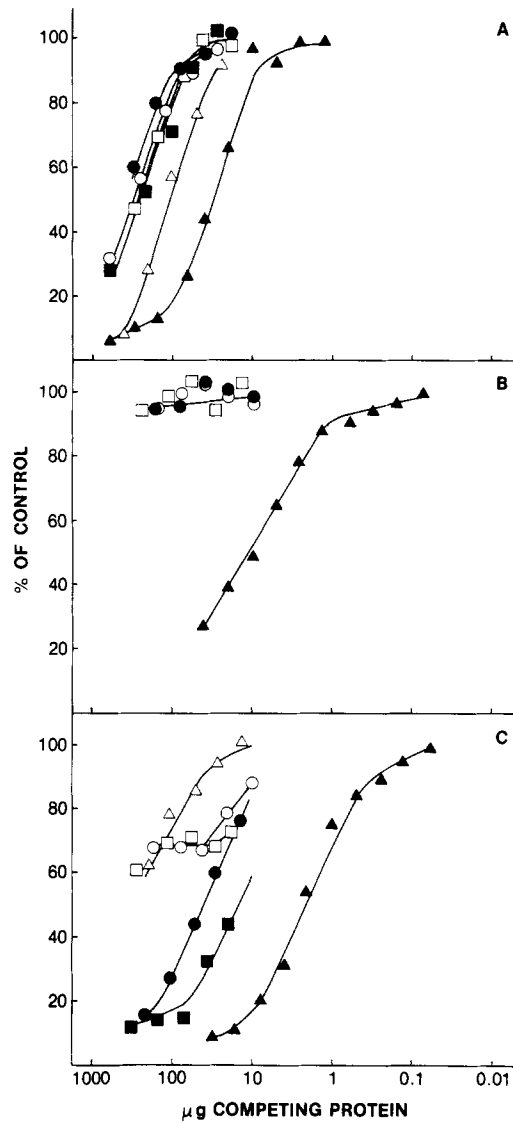
Fig. 4.   Radioimmune assays of feral mouse bone marrow for (A), AKR MuLV gp71; (B), AKR MuLV p12; and (C), AKR MuLV p30. Homogenates were prepared from bone marrow cells as described in the Materials and Methods. The homogenates were serially diluted and reacted as described for Fig. 1. (□), *Mus cervicolor*-1; (△), *Mus cervicolor*-2; (■), *Mus musculus casteneus*-1; (○), *Mus musculus casteneus*-2; (●), *Mus musculus domesticus* (Kent Island, Md.). (▲), purified protein standard. The abscissas for the standard curves are nanograms of competing protein.

gp71-like molecules from various tissues (17, 18). Our results confirm and extend these observations by demonstrating that by using type-specific competition assays both virus and tissue specificity can be detected. Thus, the results demonstrate the presence of an antigen serologically indistinguishable from the AKR MuLV gp71 in bone marrow cells of all mice examined and the presence of an antigen serologically indistinguishable from the BALB(X) MuLV gp71 in the

TABLE II

*Distribution of BALB(X) gp71 in Murine Tissues**

Strain	Age	Spleen	Thymus	Serum	Bone mar-row
	wk				
C57BL/6	7	<10	<10	60	<10
C3H	11	<10	<10	100	<10
B6C3F₁	8	<10	<10	80	<10
BALB/c	8	<10	<10	160	<10
BALB/c	Newborn	ND‡	ND	<10	ND
NIH Swiss	8	<10	<10	420	<10
NIH Swiss	Newborn	ND	ND	<10	ND
129/J	6	<10	<10	500	<10
AKR	8	<10	<10	90	<10
M.m. casteneus-1	26	ND	ND	260	<10
M. cervicolor-1	26	ND	ND	<10	<10

* The data are expressed as nanograms of the viral protein per milligram of total protein in the homogenates or serum. Sera from three to five mice at the indicated age were pooled and used in the assay. Newborn sera were obtained by pooling the sera obtained from two litters.

‡ ND, not done.

TABLE III

Hybridization of C57BL/6 Tissue RNA with AKR MuLV cDNA

Source of RNA	CO₁/₂*	T$_m$‡	Hybridization§
	mg/ml	°*C*	*%*
C57BL/6 liver	8.0	74	>38
C57BL/6 thymus	11	71	>35
C57BL/6 spleen	<20	72	>30
C57BL/6 fetal liver	<20	74	>30
C57BL/6 bone marrow	<20	71	>30
AKR spleen (leukemic)	0.01	79	92
AKR fibroblasts (MuLV)‖	0.08	80	90
AKR MuLV 70S RNA	0.03 (μg/ml)	80	90
AT 124 MuLV 70S RNA¶	0.10 (μg/ml)	73	50
BALB/c xenotropic MuLV**	0.06	76	42

* CO₁/₂ is the concentration of RNA required for 50% of maximum hybridization of AKR MuLV cDNA. If the maximum hybridization is not known, CO₁/₂ is based on total counts per minute.

‡ T$_m$ is the temperature at which 50% of the hybird is dissociated in 0.15 M NaCl. The deviation for T$_m$ values is approximately 1°C.

§ Hybridization is the extent of hybridization at saturation of AKR MuLV cDNA. (>) indicates that saturation was not obtained, and the values given are the maximum observed percent hybridization.

‖ AKR fibroblasts were from a tissue culture cell line that spontaneously initiated replication of MuLV.

¶ AT 124 is a xenotropic virus isolated from NIH Swiss mice.

** The cellular RNA obtained from a mink lung cell line infected with a xenotropic virus from BALB/c mice.

sera of most mice. Interestingly however, neither of these antigens were detectable in the thymus of 129/J mice, a site of expression suggested in previous studies (5, 19). The latter result might best be reconciled by the presence of additional glycoproteins serologically related to the viral glycoproteins which are not detected in our type-specific assays.

The expression of the AKR MuLV gp71-like antigen in mouse bone marrow occurs independent of virus expression by several criteria. First, in bone marrow cells there was a unique expression of the antigen without any change in the "normal" concentration of p30. Second, by RNA-DNA hybridization, there was no increase in AKR MuLV-specific RNA in bone marrow cells relative to spleen or thymus and no alteration of the T_m of the hybrids, which might suggest the expression of the complete AKR MuLV genome. Lastly, the bone marrow AKR MuLV gp71-like antigen is found in mice genetically lacking a portion of the AKR MuLV genome (3). Because of this independence of expression from complete virus replication we hesitate to conclude that the antigens we detect are coded for by viral genes. Thus, although the data clearly demonstrate a striking serological similarity, we have no data to suggest that the tissue antigens are coded for by a "viral" gene as opposed to cellular genes. Because of this caveat we have referred to the proteins we detected as viral-like antigens.

The expression of the serum BALB(X) gp71-like antigen also appears to be independent of virus expression. This expression is constitutive in all strains examined and is seen in sera containing no or low levels of p30. These results confirm and extend those reported by Hino et al. (20). The independent expression of this antigen, however, is hard to assess since we cannot detect its site of synthesis. In particular, we have no evidence that it is expressed normally in spleen, lymph node, or bone marrow. Since it is present in serum and is expressed constitutively in serum after birth, the logical conclusion might be that it is a serum antigen and consequently synthesized in the liver. However, the antigen was not detected in perfused liver, suggesting at least a lack of accumulation in hepatocytes. Thus, although the site of synthesis of the BALB(X) gp71-like antigen is not known, the available evidence suggests that its presence in serum is not due to high levels of endogenous virus expression.

In previous studies we have extensively described the normal immune response of mice to the endogenous AKR MuLV-type gp71 (11, 12). The question therefore arises whether the expression of the bone marrow AKR MuLV gp71-like antigen is immunogenic in this response and/or whether such mouse sera are reactive with this antigen. In terms of the latter question, we have no data yet concerning the ability of normal mouse sera to react with this antigen. In terms of the immunogenic properties of the bone marrow expression, the available evidence suggests that it is not responsible for the immune response against AKR MuLV gp71. In particular, the development of an immune response against AKR MuLV gp71 has been shown to be both strain and age dependent, the differences being related to the spontaneous expression of the endogenous virus (21). In contrast, the AKR MuLV gp71-like antigen associated with bone marrow cells is both strain and age independent in its expression (data not shown). Other studies from our laboratory have also demonstrated indirectly the expression of an AKR MuLV gp71-like antigen on spleen cells by the ability

of highly type-specific antisera against AKR MuLV gp71 to inhibit mitogenic responses (22). This expression, however, was completely correlated with an immune response against the virus and with the ability to express endogenous AKR MuLV. Thus, both virus-independent and virus-dependent, biologically-specific and site-specific expression of AKR MuLV gp71-like antigens have been detected.

It is particularly interesting that the bone marrow expression of the AKR MuLV gp71-like antigen is constitutive in all strains of mice examined and is highly conserved evolutionarily as demonstrated by the presence of a serologically identical antigen in *Mus cervicolor*. These properties taken together suggest that this expression may be required for either differentiation or normal cellular function. Although our experiments do not directly address this question, the availability of monospecific antisera highly specific for this antigen will provide the basis for future experiments to define the biological properties of the cells expressing this antigen. In particular, the cell types expressing the AKR MuLV gp71-like antigen in bone marrow need to be identified and the question of intracellular versus cell surface expression needs to be resolved. These types of studies should help to better define the relationship of endogenous virus expression to both pathogenesis and normal cellular function.

Summary

By competition radioimmune assays with antisera against AKR murine leukemia virus (MuLV) gp71 or antisera against xenotropic virus, and iodinated AKR MuLV gp71 or BALB(X) gp71, antigens serologically indistinguishable from the viral antigens can be detected in tissues of normal mice in the absence of overt virus expression. An antigen serologically indistinguishable from AKR MuLV gp71 can be readily detected in normal bone marrow cells of the common strains of mice including NIH Swiss, 129/J, and SWR/J, as well as in *Mus cervicolor* and *Mus musculus casteneus*. In contrast, this antigen is not detected in normal spleen, thymus, lymph nodes, or serum.

Similarly, an antigen serologically indistinguishable from BALB(X) gp71 was found in all normal mouse sera examined. This antigen was not present in fetal liver, perfused adult liver, thymus, spleen, lymph nodes, or bone marrow of the mice examined. An equivalent antigen was detected in sera from *Mus musculus casteneus* but not in sera from *Mus cervicolor*.

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