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Host Control of Endogenous Murine Leukemia Virus Gene Expression: Concentrations of Viral Proteins in High and Low Leukemia Mouse Strains

(murine leukemia virus proteins/mouse genetics)

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ABSTRACT **Two of the major molecular components of murine leukemia virus particles, the internal protein (p30) and the envelope glycoprotein (gp69/71) have been measured in the spleens of normal, 6- to 10-week-old mice of various inbred strains and crosses. Both proteins were detected in virtually all mice. Extracts of high leukemia, high murine leukemia virus strains (AKR, C58, PL) showed high levels of both proteins; extracts of other strains usually showed lower levels. Of particular interest, however, were the exceptions to these general observations: (1) Very little gp69/71 could be detected in spleens of BALB mice, and this trait was dominant in crosses with AKR and DBA/2, both of which express a high level of gp69/71. Thymus-deficient BALB/c-*nu/nu* (nude) mice, in contrast, showed a higher concentration of gp69/71 typical of other low leukemia strains, suggesting that the virtual absence of the protein in normal BALB/c mice may result from immunologic suppression. (2) With C57L, C57BL/10Sn, and C57BL/6 strains the concentration of p30 was lower, in some cases much lower, than would be expected from the concentration of gp69/71. (3) DBA/2 mice showed high levels of gp69/71, 10-fold greater than that of p30, whereas congenic DBA/2-*Fv-1*^{b} mice showed the opposite pattern. (4) Mice of 129-G$_{IX}^-$ strain showed no detectable levels of either p30 or gp69/71 proteins, although the congenic 129 (G$_{IX}^+$) showed appreciable levels of both. The absence of these proteins in 129-G$_{IX}^-$ mice is a recessive trait, as F$_1$ hybrids with AKR and DBA/2 showed appropriate levels of both proteins. It is concluded that expression of viral p30 and gp69/71 proteins in mice is not coordinately linked and that expression is complex, being influenced by several genes, including *Gv-1*, *H-2*, *Fv-1*, and probably still others.**

Evidence from many sources, culminating with the demonstration that clonal lines of virus-free mouse embryo cells can be induced to yield type-C RNA viruses, has suggested that the genetic determinants of murine leukemia viruses (MuLV) are present as heritable components in all mouse cells (1–5).

This viral gene information is under several forms of host control and may exist in unexpressed form for many generations. One extensively studied genetic factor is the host range restriction system governed by the *Fv-1* gene (6). In this system, N-tropic MuLV readily propagates in N-type (*Fv-1*^{n} homozygous) cells but grows poorly in B-type (*Fv-1*^{b} homozygous) cells; B-tropic viruses show the reciprocal pattern, and both types of virus grow poorly in heterozygous (*Fv-1*^{n}/*Fv-1*^{b}) cells (7, 8). A second major genetic system has been elucidated in studies of the inheritance of infectious MuLV or of MuLV proteins in crosses of AKR, a high leukemia strain with high levels of infectious MuLV and viral proteins, with strains showing little or no MuLV or viral protein (9–11). These studies showed that two unlinked, dominant chromosomal loci, *Akv-1* and *Akv-2*, are the determinants of MuLV in AKR mice, and both loci have the capacity of eliciting virus expression early in life.

The host control of viral gene expression is not complete. Type-C viral protein antigens have been detected in tissues of a large number of different strains of mice, including low leukemia strains (12–16). For most of these early studies, the specific proteins measured in assays of type-C virus group specific (gs) antigens is unknown. One would speculate that the main component is the viral p30 protein, as it is the major structural component of the virion. Recently Parks *et al.* have shown specifically that the p30 can be detected in tissues of C57L and BALB/c low leukemia mice (14) and Stephenson *et al.* have purified the p30 and p12 proteins from NIH Swiss liver and spleen and from NIH/3T3 and BALB/3T3 cultured cells (16). As yet, it has not been determined whether the proteins detected reflect low titer virus release below the level of virus detection by current assays, or independent synthesis of specific viral proteins. Thus, it is not known whether there is coordinate control of gene expression of all proteins of C-type viruses or if there is independent expression or control of different components of the virus.

In this study we have examined the expression of the C-type virus genome by measuring the concentration of two viral proteins in spleens of different strains of mice. The chief purpose of the study was to determine if there is coordinate or independent regulation of the cellular concentration of these viral gene products. One is the p30, the major internal protein of the virions with a molecular weight of approximately 30,000 (17), and the other the gp69/71, two glycopeptides which comprise the major glycoprotein of the external viral envelope (18, 19). As both of these proteins retain virus specific antigenic determinants when MuLV is propagated in tissue culture cells as divergent as murine and human (20), there is strong evidence that they are virus coded. In purified mature virions they exist in a weight ratio of approximately 2:1, 10–20% of the viral protein being p30 and 5–10%, gp69/71. In contrast, we have found marked differences in the relative concentrations of these proteins in different strains of mice, which suggests independent mechanisms for their synthesis or degradation. Moreover, the results also indicate that the ex-

Abbreviations: MuLV, murine leukemia virus; R-MuLV, Rauscher strain of murine leukemia virus; FeLV, feline leukemia virus.

pression in mice of MuLV components is complex, being influenced by several genes, including *Gv-1*, *H-2*, *Fv-1*, and probably still others.

MATERIALS AND METHODS

Purification of Virus Proteins. The major structural protein (p30) and the membrane glycopeptides (gp69/71) of Rauscher MuLV were purified by phosphocellulose column chromatography and Sephadex gel filtration, as previously described (18).

Antisera. Anti-Theilen feline leukemia virus (FeLV) serum was obtained from rabbits injected with 500 μg of protein of purified virus particles that had been disrupted by Triton X-100 at a final concentration of 0.4% and mixed with an equal volume of Freund's complete adjuvant for the primary injection. The antigen was injected into two footpads and multiple intradermal sites. Booster injections of approximately 200 μg of virus protein were given intra-muscularly in Freund's incomplete adjuvant. Horse antiserum to rabbit IgG was a gift from R. Porter, Oxford University, and goat antiserum to rabbit IgG was generously provided by R. Wilsnack, Huntingdon Research Center, Baltimore, Md.

Viruses. The Rauscher MuLV was provided by S. Mayyasi and D. Larson of the John L. Smith Memorial for Cancer Research. The virus was propagated in a BALB/c mouse bone marrow culture (JLS-V9) productively infected with Rauscher virus as described by Wright *et al.* (21), harvested, and purified as described previously (18).

Mice. Most of the animals supplying tissues for assay were from the colony of inbred strains and crosses maintained by F. Lilly. Some animals were obtained from the Jackson Laboratory, Bar Harbor, Maine (identified by the notation /J). Mice of strains 129, 129.G_{Ix}^-, B6.G_{Ix}^+, and hybrids of strains 129.G_{Ix}^- were supplied through the courtesy of Dr. E. Stockert, Sloan-Kettering Institute. Mice of the BALB/c-*nu/nu* strain were a gift from Dr. Seung-il Shin, Albert Einstein College of Medicine.

Radioimmunoassay of viral protein was carried out as described by Hunter (22). The reaction mixtures contained the following: 5 μl normal rabbit serum, 10 μl ^{125}I-labeled virus protein (1 or 2 ng of protein containing 10^4–10^5 cpm/ng), either 150 μl of cell extract or 10 μl of purified virus or viral proteins as competing proteins, and 10 μl of rabbit antiserum to Theilen FeLV at a concentration that precipitated approximately 50% of the ^{125}I-labeled antigen. The total volume of the assay was 0.2 ml, adjusted with TEN buffer (20 mM Tris hydrochloride, pH 7.6, 1 mM ethylenediaminetetraacetate (EDTA), 100 mM NaCl) containing 0.2% Triton X-100 and 2 mg/ml of crystalline bovine-serum albumin. Dilutions were made with TEN buffer containing 0.2% Triton X-100 and 20 mg/ml of carrier crystalline serum albumin for antiserum, purified virus, or viral proteins and 2 mg/ml for crude tissue extracts. The reaction mixture was incubated at 37° for 15–18 hr, after which 0.04 ml of horse or goat antiserum to rabbit IgG was added. Incubation was continued at 37° for an additional 2 hr followed by overnight incubation at 2–4°. Cold TEN buffer (0.5 ml) was added and the precipitate was collected by centrifugation at 4°. The pellet was washed twice with 0.5 ml of TEN buffer and the ^{125}I-labeled antigen present in the precipitate was measured in a gamma counter.

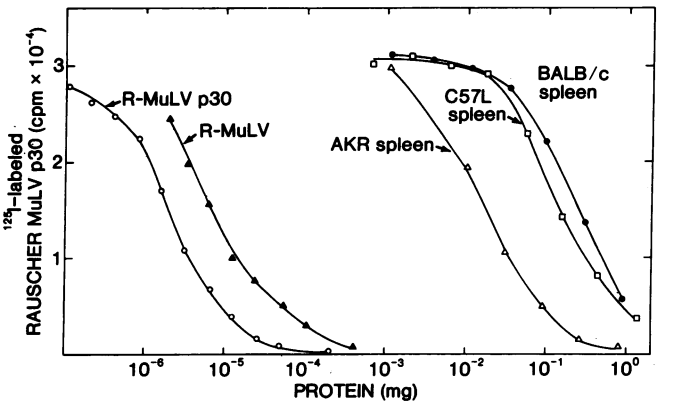

FIG. 1. Radioimmunoassay of mammalian C-type virus p30 protein in mouse spleen extracts. The competition radioimmunoassay was performed as described in Methods with 1 ng of ^{125}I-labeled Rauscher MuLV (R-MuLV) p30 (6.4×10^4 cpm/ng), rabbit antiserum to FeLV (1:5000 final dilution), and competing purified R-MuLV p30, disrupted R-MuLV virus, or extracts of mouse spleens as indicated, shown as total protein added.

RESULTS

Assay of Viral Proteins. Radioimmunoassay measurement of the viral p30 and gp69/71 proteins was carried out by a heterologous assay system with anti-feline virus serum and murine virus protein so as to utilize antibodies reacting specifically with the common interspecies antigenic determinants. This choice of a heterologous system was essential to a quantitative assay, as previous experiments have shown that the concentration and antibody affinity of the interspecies antigens were indistinguishable among the several different murine viruses tested, Rauscher, Friend, Moloney, and Gross and AKR. This was in contrast to the results obtained by use of a homologous assay system of antiserum to murine virus and the murine virus proteins, in which case the p30 or gp69/71 proteins of other viruses would not compete for binding of type specific antigens of the labeled protein (23).

The results of such an assay show that interspecies antigens cross-reactive with those of the p30 and gp69/71 proteins can readily be detected in both the high incidence of leukemia AKR strain and the low leukemia C57L strain (Figs. 1 and 2). The slopes of the competition curves closely resemble those of the purified standard protein and protein of disrupted virus, indicating that the competing antigens share the same specificities as the interspecies determinants measured in the assay. Moreover, from the extent of competition, it appears that the competing proteins contain most, if not all, of the interspecies antigenic determinants of the Rauscher virus p30 and gp69/71 proteins. With proteins of disrupted purified Rauscher MuLV the competition curve showed the appropriate values, with the p30 and gp69/71 proteins comprising 10–20% and 5–10% of the total viral protein, respectively. The concentrations of the viral proteins were estimated by comparison with competition curves of pure unlabeled p30 and gp69/71 proteins. For example, in these experiments the AKR spleen contained approximately 135 ng of p30 per mg of tissue protein and 55 ng of gp69/71 per mg, and the C57L, 15 ng of p30 per mg and 8 ng of gp69/71 per mg. The p30 protein, 7 ng/mg of tissue protein, was also detected

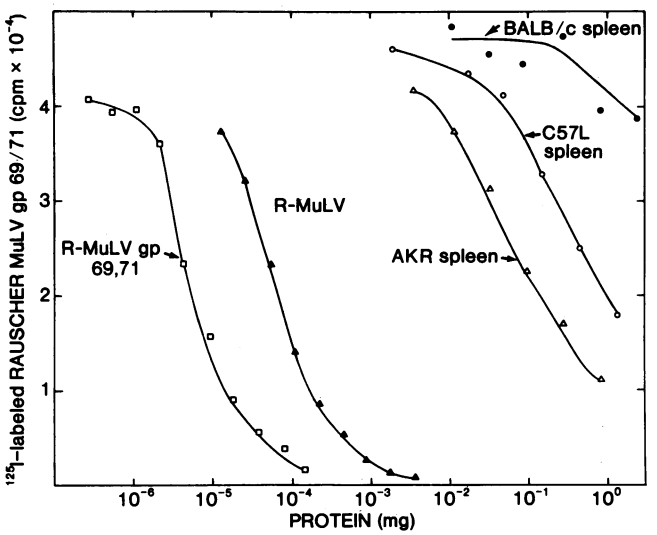

FIG. 2. Radioimmunoassay of mammalian C-type virus gp69/71 protein in mouse spleen extracts. The assay was carried out as described in Fig. 1, except that the reaction mixture contained 2 ng of ^{125}I-labeled Rauscher MuLV gp69/71 (7.8×10^4 cpm/ng), rabbit antiserum to FeLV (1:1500 final dilution), and competing protein as indicated.

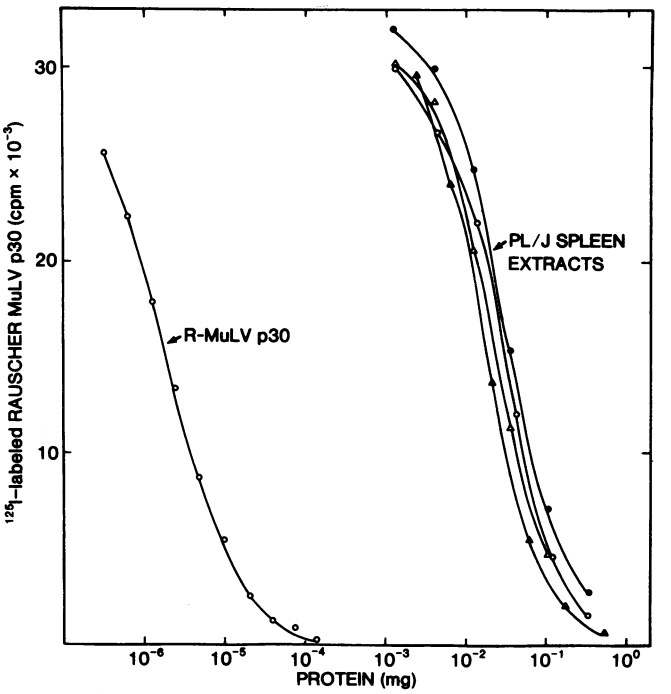

FIG. 3. Comparison of p30 concentration in individual spleens. Radioimmunoassay was performed as described in Fig. 1, with ^{125}I-labeled p30 of 8.7×10^4 cpm/ng.

in BALB/c mice, whereas with this strain little or no gp69/71, less than 1 ng/mg of tissue protein, was found.

In repeated assays of the spleen extract, the precision of this assay was very high, with a variation no greater than 10%. Assay of individual spleens of the same strain of mice in most cases gave a narrow range of values. For example, with four spleens of PL/J mice, the values for p30 were from 60 to 100 ng/mg of total protein and for gp69/71, from 40 to 50 ng/mg of total protein (Figs. 3 and 4). Six individual BALB.B spleens were tested and all showed less than 1 ng of gp69/71 per mg of protein. With seven C57L mice, however, the range for p30 was much greater, with values from 0.5 to 15 ng/mg of protein.

As measured with this assay, the differences in concentration of the p30 protein between young (6 weeks) and middle-aged (6–12 months) AKR and BALB/c mice was no greater than 2-fold. Nevertheless, all of the mice used in these studies were between 6 and 10 weeks old, as others have reported an effect of age on the concentration of viral protein antigens (12, 24). The concentration of the viral proteins was also measured in the thymus, lymph nodes, and liver of AKR and BALB/c mice; the highest concentrations were found in the spleen, with little or no protein detected in the liver.

Strain Distribution. Both the viral p30 and gp69/71 components were found in most of a large number of different strains tested, including many low leukemia strains (Table 1). Extracts from high leukemia, high MuLV strains (AKR, C58/J, PL/J) contained high concentrations of both proteins, and the ratio of the two was close to that of virion particles. The low incidence strains usually showed low but measurable concentrations of these proteins, but there were many exceptions to the ratio of concentrations that would be expected from the ratio in virions.

BALB/c mice (*H-2*^{d}) and their congenic *H-2*^{b} (BALB.B) and *H-2*^{k} (BALB.K) partners showed low concentrations of p30 and, more interestingly, virtually no gp69/71. The results with RF/J mice were similar. The low concentration of gp69/71 in these BALB/c spleen extracts was not due to the presence of an inhibitor, as these extracts added with Rauscher MuLV did not block competition by the viral protein. The absence of gp69/71 in BALB/c mice was dominant in F_1 progeny of crosses with AKR and DBA/2, both of which express a high level of the viral protein, strongly suggesting a genetically controlled suppression of gp69/71 in BALB/c. Thymus-deficient BALB/c-*nu/nu* (nude) mice, in contrast, showed an easily detectable concentration of gp69/71 typical of other low leukemia strains, which suggests that the absence of the protein in normal BALB/c mice may result from immunologic suppression.

The C57L, C57BL/10Sn (B10), and C57BL/6 (B6) strains showed a consistently low but readily detectable concentration of gp69/71, but in these and related strains there was wide variation in the concentration of p30. This variation was observed as well in different individuals from a single strain, C57L. In these strains the apparent concentration of p30 was lower, in some markedly lower, than what would be expected from the concentration of gp69/71. Similar findings were obtained with strains A and P/J. In contrast, B10.BR (*H-2*^{k}) congenic mice showed a much higher level of p30 than did the B10 (*H-2*^{b}) mice, and this high concentration of p30 was dominant in an F_1 cross between the two strains. The finding of an anomalously high level of p30 in spleens of B10.BR mice, alone among several *H-2*-congenic strains on a B10 background examined, suggests that the *H-2* region might be involved in this phenomenon. Studies by W. P. Rowe (personal communication) have demonstrated infectious, B-tropic MuLV in tissue extracts of all B10.BR mice from our own colony (but in only 1/17 B10.BR mice obtained from the Jackson Laboratory); this could possibly account for the elevated p30 in these mice, but does not explain the relatively low level of gp69/71. The apparent notable increase in concentration of gp69/71 in B6-G$_{IX}^+$ and low

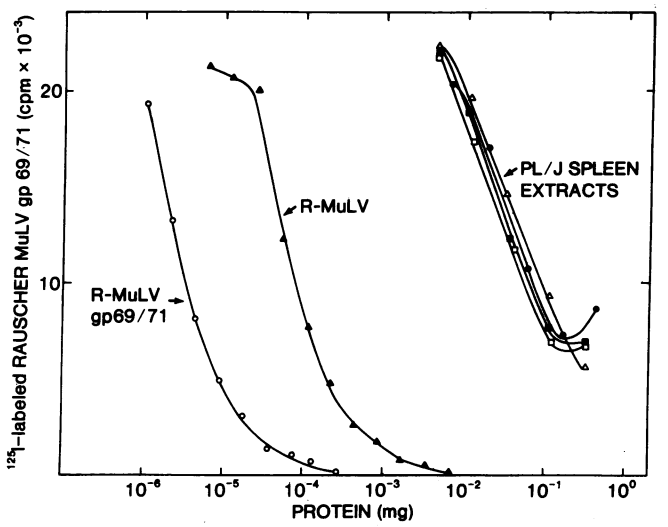

FIG. 4. Comparison of gp69/71 concentration in individual spleens. Radioimmunoassay was performed as described in Fig. 2, with ^{125}I-labeled gp69/71 of 3.2×10^4 cpm/ng.

concentration of p30 in the congenic B6-*H-2*^{k} are additional findings which remain to be confirmed and studied further.

DBA/2 mice showed high levels of gp69/71, 10-fold greater than that of p30, whereas congenic DBA/2-*Fv-1*^{b} mice showed the opposite pattern. In the cross DBA/2 × BALB/c the low level of gp69/71 of BALB/c was dominant.

Mice of the 129-G_{IX}^- strain showed no detectable levels of either p30 or gp69/71 proteins, although the congenic 129 (G_{IX}^+) showed appreciable levels of both. The absence of these proteins in 129-G_{IX}^- mice is a recessive trait, since F_1 hybrids with AKR and DBA/2 showed moderate levels of both.

DISCUSSION

The chief conclusion that can be drawn from these studies is that the cellular expression of the viral p30 and gp69/71 proteins is not coordinately linked. In the different strains the ratio of the two proteins varied from a large excess of p30, as found in the BALB/c, B10.BR and RF/J strains, to a large excess of gp69/71, as found in most B10, B6, DBA/2, and P/J strains. This finding has been made independently as well, in collaboration with J. Bilello and with H. Freedman of this institution, in studies of cell lines that are found to express high concentrations of gp69/71 and no detectable p30. The mechanism of this independent expression of viral proteins, possibly involving transcription, translation, or degradation, remains to be determined. One possibility relating to the segmental structure of the oncornavirus genome is that the p30 and gp69/71 genes reside in different nucleic acid segments.

Several genes appear to influence the relative expression of these proteins: (*a*) Genes of the "V" category [e.g., *Akv-1* and *Akv-2* (9, 10)] are probably the basis of the high levels of the substances found in the spleens of mice of high leukemia strains. (*b*) The inverse expression of the two proteins in DBA/2 and DBA/2-*Fv-1*^{b} mice suggests that the *Fv-1* gene, known to play an important regulatory role in virus expression, strongly influences the expression of specific viral proteins; however, since the DBA/2-*Fv-1*^{b} strain is at present only partially congenic with DBA/2, this conclusion remains tentative. (*c*) The high level of p30 found in tissues of B10.BR (*H-2*^{k}) and (B10.BR × B10)F_1 (*H-2*^{k}/*H-2*^{b}) by comparison with tissues of other strains of the B10 congenic series (differing from B10.BR only in the *H-2* region of the genome) would at first glance suggest that a gene governing the p30 molecule might be in close proximity to *H-2*. On the other hand, Rowe's observation that mice of our subline of B10.BR also express a relatively high level of infectious MuLV in their tissues may signify either that these mice are expressing a virus inherited by means of a "V" gene, perhaps linked to *H-2*, or that the virus is present in the mice by virtue of horizontal infection among members of the colony. The *H-2* locus is known to play a significant role in susceptibility to *leukemogenesis* by MuLV (25), but there is no compelling

TABLE 1. *Expression of MuLV p30 and gp69/71 in spleens of inbred mice*

Strain	p30 (ng viral protein per mg spleen protein*)	gp69/71 (ng viral protein per mg spleen protein*)
High leukemia		
AKR	105 ± 13 (5)	60 ± 20 (2)
C58/J	210	100
PL/J	75 ± 9 (4)	48 ± 3 (4)
Low leukemia		
BALB/c	4 ± 2 (3)	<1 ± 0 (2)
†BALB/c-*nu/nu*	5	10
†BALB/c-+/*nu*	5	<1
BALB.B	6 ± 1 (2)	0.6 ± 0.1 (6)
BALB.K	5	<1
C57L	6 ± 1.8 (7)	6 ± 2 (2)
B10 (C57BL/Sn)	1	11
B10.BR	80	10
B10.A	1	12
B10.D2new	0.5	10
B10.A(2R)	3	20
B6 (C57BL/6)	20	25
‡B6-G_{IX}^+	23 ± 0 (2)	100 ± 0 (2)
B6-H-2^k	1	20
DBA/2	20 ± 3 (4)	175 ± 25 (4)
DBA/2-Fv-1^b	85 ± 6 (5)	55 ± 9 (5)
‡129	6 ± 1 (3)	10 ± 1 (3)
‡129-G_{IX}^-	<0.1 ± 0 (3)	<1.0 ± 0 (3)
P/J	1	10
A	7	15
RF/J	6	<1
F_1 hybrids		
C57L × AKR	133 ± 33 (3)	50
BALB/c × AKR	32	4
BALB.B × AKR	40	3
BALB/c × DBA/2	11 ± 1 (5)	3 ± 0.4 (5)
B10 × B10.BR	181 ± 5 (3)	40 ± 0 (3)
‡DBA/2 × 129-G_{IX}^-	10	25
‡129-G_{IX}^- × AKR	80	25

Mice identified with /J were obtained from the Jackson Laboratory.

* Values represent either single determinations with a pool of three to four spleens or the mean (±SEM) values of determinations with individual spleen extracts; the number in parentheses is the number of extracts examined.

† Mice obtained by the courtesy of Dr. Seung-il Shin, Albert Einstein College of Medicine.

‡ Mice obtained by the courtesy of Dr. Elisabeth Stockert, Sloan-Kettering Institute.

evidence that the locus is an important factor governing susceptibility to virus infection. (*d*) The genetically recessive lack of expression of both viral antigens in 129-G_{IX}^- mice is particularly important in comparison with the presence of low but significant levels of the antigens in mice of the congenic parental strain, 129 (G_{IX}^+). The genomes of these two strains differ mainly at the *Gv-1* locus (26), the dominant gene governing the leukemia-associated G_{IX} cellular antigen. Our findings suggest that *Gv-1* might also be closely linked to structural genes governing p30 and gp69/71 molecules. Thus, it will be of interest to determine if other MuLV-associated functions might also be found to be closely linked, as would be expected if G_{IX} were, in fact, a (perhaps defective) "V" gene. (*e*) One or more unidentified, dominant genes (not closely linked to *H-2*) of the BALB/c (and perhaps RF/J) strain appears to be capable of suppressing almost completely the expression of the gp69/71 molecule. Since this suppression is relaxed in the presence of the *nude* trait, its mechanism may be immunological in nature.

Another host mechanism that also would possibly control the concentration of antigen measured by antibody binding in the radioimmunoassay is the presence of endogenous antibodies. High titers of antibodies might bind sufficient antigen to mask the presence of competing protein. For this reason we have measured antibody concentration in both the spleen extracts and serum of these animals. Antibodies to the gp69/71 protein are detected. However, by actual measurement of free antibody titer and by reconstruction experiments adding spleen extracts with known amounts of viral protein, it was ascertained that at dilutions where competing protein was detected, the concentration of these endogenous mouse antibodies was not sufficient to significantly affect the quantitative measurement of labeled antigen. With some strains showing the highest antibody titers, such as the PL/J (Fig. 2), the most pronounced effects were a slight reduction in the slope of the inhibition curve and an actual blocking of inhibition by excess antibody at high concentrations of spleen proteins, between 0.1 and 1.0 mg. This is interpreted as showing an excess of tissue antibody at these high protein concentrations and an equilibrium between the added ^{125}I-labeled antigen and tissue antigen. It should be emphasized, however, that these experiments measure free antigen, and any viral protein completely saturated by antibody would theoretically not be detected.

An additional consideration in these experiments is that only those viral p30 or gp69/71 proteins that contain common interspecies determinants, as is known to occur with Rauscher, Friend, Moloney, Kirsten, Gross, and AKR viruses (23), will be measured quantitatively in this assay. If there are murine viruses of a different group that contain markedly different interspecies determinants, the p30 and gp69/71 of these viruses might not be detected. If this were the case, the apparent reduced concentration of viral proteins could reflect the expression of such different endogenous viruses. At present, this is a theoretical consideration, as no such different group of endogenous murine viruses has been demonstrated.

It remains to confirm and expand the implied genetic control mechanisms revealed by our studies. Further, it will be of interest to extend this work to include detection of other major MuLV components as soon as adequate assays for these substances become available. It is obvious that genes regulating MuLV expression are of great potential importance in the elucidation of mechanisms of leukemogenesis and of the control of this disease.

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