JOURNAL OF VIROLOGY, Apr. 1976, p. 124-131
Vol. 18, No. 1

Printed in U.S.A.

Characterization of the Type and Group Specificities of the Immune Response in Mice to Murine Leukemia Viruses

J. N. IHLE,* J. J. DOMOTOR, JR., AND K. M. BENGALI

Basic Research Program, National Cancer Institute-Frederick Cancer Research Center, Frederick, Maryland 21701

Received for publication 15 October 1975

Normal sera from a variety of strains of inbred mice have precipitating antibodies to murine type C viruses that are detected by radioimmune precipitation assays. The results demonstrate that this humoral immune response is primarily directed against the AKR strain of murine leukemia virus (MuLV) proteins gp71, gp43, and p15(E). These sera also react with Friend- or Rauscher-MuLV in radioimmune precipitation assays. This reaction is not due to a separate immune response, but rather is primarily a consequence of the cross-reactivity of antibodies to the AKR strain of MuLV p15(E) with the p15(E) of these viruses. These data, using autogenous immune sera, emphasize the serological differences of the virion glycoproteins and the serological similarity of the p15(E) virion component of the viruses. Furthermore, based on the serological reactivities to the glycoproteins, the results suggest that the AKR strain of MuLV is endogenous to and expressed in mice, but that the Friend-Moloney-Rauscher virus group is not.

Murine leukemia viruses (MuLV) are endogenous in mice (2, 3, 5, 10, 21, 28) and are variably expressed in vivo, which is dependent upon factors such as strain and age (20, 21, 23, 27, 29). One consequence of this expression, however, is the development of an autogenous immune response detectable with a sensitive and quantitative radioimmune precipitation (RIP) assay using a [^{3}H]leucine-labeled intact AKR strain of MuLV (AKR-MuLV) (14, 15, 25; J. N. Ihle and M. G. Hanna, Jr., Contemp. Top. Immunobiol., in press). Analysis of immune precipitates of autogenous immune sera and labeled disrupted virions has suggested that the immune response is primarily directed against three virion envelope components: gp71, gp43, and p15(E) (15, 16). More recently, direct RIP assays using purified ^{125}I-labeled AKR-MuLV gp71 have confirmed the reactivity of these sera with this major envelope glycoprotein (13). Furthermore, competition experiments with the purified glycoprotein have suggested that this immune reaction is one of the predominant reactions detected in RIP assays with intact virions. These experiments, however, only assessed the reaction of these sera with the AKR-MuLV.

Several biologically and serologically distinct oncornaviruses have been described in mice. Ecotropic viruses are able to replicate in mouse cells. One group of ecotropic viruses has been etiologically linked to spontaneous (10) and radiation-induced (18) leukemias in mice and constitutes the Gross-type viruses. Within this group, N- and B-tropic viruses have been defined, based on host range characteristics (21). These viruses are extensively related, as determined by molecular hybridization (3). Also included in the ecotropic viruses is the Friend-Moloney-Rauscher (FMR) MuLV group. The viruses in this group are apparently not found naturally in mice and are serologically distinct from the Gross- or AKR-type viruses (6, 7, 26). Xenotropic viruses are characterized by their inability to replicate in mouse cells and by their ability to replicate in cells from a variety of other species (19). These viruses are endogenous to mice and are expressed in some strains throughout life (4, 19). They have not been linked to any spontaneous neoplasia in mice.

In a previous communication, we examined the reactivity of autogenous immune sera from 1.5-year (B6C3)F_1 [(C57BL/6 × C3H/Anf)F_1] mice with Gross, FMR, and xenotropic viruses (J. C. Lee and J. N. Ihle, J. Natl. Cancer Inst., in press). These studies demonstrated the ability of such sera to react comparably with all groups of murine viruses in RIP assays. However, these sera did not react with type C viruses of other species. In the present studies, we further examined the basis of the reactivity with Gross- and FMR-type viruses to delineate the virus types to which the autogenous immune response is directed and to better estab-

lish the molecular entities involved in cross-reactivities.

MATERIALS AND METHODS

Animals. Male (B6C3)F_1 [(C57BL/6 × C3H/Anf)F_1], C57BL/6, C3H/Anf, and BALB/c mice were bled at 18, 4, 3, and 6 months of age, respectively, and the sera were pooled. All mice were specific pathogen free.

Test sera. Blood was collected from mice by cardiac puncture. Sera were separated by centrifugation at 400 × *g* for 15 min and were stored at −70 C.

Viruses. AKR-MuLV was isolated from an established line of AKR mouse embryo cells [FIC2(16a)], which had spontaneously initiated virus synthesis (obtained initially from W. P. Rowe, National Institutes of Health). Moloney-MuLV was isolated from a chronically infected continuous-passage Swiss mouse embryo cell line. Rauscher-MuLV was purified from the JLS-V5 cell line. Gross-MuLV was kindly provided by the Office of Program Resources and Logistics, National Cancer Institute, Bethesda, Md. Friend-MuLV was obtained from the Eveline cell line, which is derived from the STU mouse strain, as previously described (E. Seifert, M. Claviez, G. Hunsmann, V. Moennig, and W. Schafer, Z. Naturforsch., in press).

Preparation of unlabeled AKR virus. Nonradioactive viruses were purified as described (15) from 12-h harvests of culture medium from cells grown in roller bottles. Protein concentrations were determined by the method of Lowry et al. (22).

Preparation of radioactively labeled virus. [^{3}H]leucine-labeled virus was prepared as previously described (15), except 10^{-6} M hydrocortisone was incorporated in the culture medium to enhance virus yield. All viruses were purified by a final isopycnic banding in a 15 to 50% linear sucrose gradient. In some cases, an additional step of sedimentation centrifugation was necessary to obtain homogeneous virus preparations.

The viruses were prepared under identical conditions and had similar radiospecific activities (1×10^7 to 3×10^7 counts/min per mg of protein). Purified virus pools were stored at −170 C and were used immediately upon thawing.

RIP assays against intact virus. The RIP assay against intact labeled AKR virus has been described in detail elsewhere (14). In brief, 0.1 ml of the test serum was serially diluted twofold in TNE buffer (0.05 M Tris-hydrochloride, pH 7.5, 0.1 M NaCl, 1 mM EDTA); 0.1 ml (6,000 counts/min) of labeled virus was added, and the mixture was incubated for 1 h at 37 C to allow the formation of immune complexes. Subsequently, a volume of 0.1 ml of anti-gamma globulin (Cappel anti-mouse gamma globulin) diluted 1:2 in TNE was added; the mixture was incubated again at 37 C for 1 h and finally incubated at 4 C for 2 h. The precipitates were collected by centrifugation at 1,200 × *g* for 15 min, and the supernatant was removed for the determination of radioactivity. The precipitates were washed three times with TNE, resuspended in 0.4 ml of TNE, and prepared for counting. All samples were counted in 10 ml of Aquasol (New England Nuclear Corp., Boston, Mass.) in a scintillation counter. The percentage of precipitation was expressed as the percentage of counts in the precipitate relative to the combined counts in the precipitate and in the first supernatant. A number of parameters of the RIP assay were found to affect the results, as described in a previous report (14).

Competition RIP assays were run under identical conditions, except that unlabeled virus was diluted twofold and serum was added at the indicated dilutions. The results are expressed as the percentage of precipitation occurring in the presence of unlabeled virus relative to the precipitation in the absence of competing virus.

SDS-polyacrylamide gel electrophoresis of immune precipitates. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed, as described by Weber and Osborn (34). Bromophenol blue was used as a reference standard to determine relative mobilities and was generally allowed to migrate 8 cm. Standard protein samples were used to calibrate the system as described (15). The gels were sectioned into 1-mm slices and dissolved overnight in 0.1 ml of 30% hydrogen peroxide at 75 C. The radioactivity of each gel slice was determined with a liquid scintillation counter.

To prepare immune precipitates for polyacrylamide gel electrophoresis, 25 to 50 μl of serum was allowed to react with 2×10^5 counts/min of Triton-disrupted virus and subsequently precipitated with anti-gamma globulin as described (15). The precipitates were washed four times with TNE and then sedimented through a cushion of 25% sucrose containing TNE and 0.5% deoxycholate. The precipitates were resuspended in TNE and pelleted at 1,200 × *g* for 20 min. The pellets were carefully drained dry, resuspended in 0.05 ml of 1% SDS and 1% β-mercaptoethanol in 0.01 M sodium phosphate buffer at pH 7.4, and incubated at 60 C for 1 h and then at 37 C overnight.

Viral antigens. Rauscher-MuLV gp71 and AKR-MuLV gp71 were purified as previously described (13).

Direct RIP assay. Antigens used in direct RIP assays were labeled with ^{125}I by the method of Greenwood et al. (9). The specific activity of the labeled components was approximately 5×10^3 counts/min per ng.

In direct assays, test sera were diluted twofold in 0.2 ml of assay buffer (0.05 M sodium phosphate, pH 7.0, 0.1 M NaCl, 0.001 M EDTA, 0.01% Triton X-100), and labeled antigen (2 to 4 ng) was added. The mixtures were incubated 3 h at 37 C and overnight at 4 C prior to the addition of the appropriate anti-gamma globulin antiserum (0.1 ml) (Cappel Laboratories, Downington, Pa.). The mixture was further incubated 1 h at 37 C and 3 h at 4 C and prepared as above.

Absorption of sera with viruses. To serum samples (0.1 ml), 1 mg of the appropriate virus in 0.1 ml of TNE was added. The mixtures were incubated 45 min at 37 C and 45 min at 4 C, and the virus was removed by centrifugation (30,000 rpm, 1 h). The procedure was repeated once, and the sera were stored at −70 C.

RESULTS

Pooled sera from 1.5-year (B6C3)F_1, 4-month C57BL/6, 3-month C3H/Anf, and 6-month BALB/c mice were used in these experiments. The titers of these sera against various MuLV in RIP assays using intact virions are shown in Table 1. All sera had precipitating titers against AKR-MuLV and the FMR group of viruses. In general, however, the titer against the FMR group of viruses was slightly lower for a particular serum than the titer against AKR-MuLV. The titers of sera from the various strains were variable, and these variations have been shown to be a function of both strain and age (14, 25; Ihle and Hanna, Contemp. Top. Immunobiol., in press).

The comparable precipitation of the FMR viruses and the AKR virus could be due to unique antibody populations specific to each virus type, or it could be due to cross-reactivity of antibody populations specific to one virus type. To examine these possibilities, we initially used a series of RIP competition assays. In these assays, limiting dilutions of sera were reacted with [^{3}H]leucine-labeled virions, and unlabeled intact viruses were used as the competing antigen. Although this measures the predominant immunological reaction, it can clearly distinguish cross-reactivity from unique immune recognition. Typical competition curves for (B6C3)F_1 sera are shown in Fig. 1. When limiting dilutions of sera are reacted with labeled AKR-MuLV in the presence of increasing amounts of unlabeled AKR-MuLV, the precipitation of the labeled virus is progressively inhibited. In this reaction approximately 0.2 μg of virus was required for 20% competition. In contrast, Friend-MuLV did not compete in this reaction, even at the highest concentrations. Rauscher-MuLV competed only marginally, with 20% competition occurring at approximately 100 μg of virus.

TABLE 1. *RIP titers of autogenous immune sera*

Serum	RIP titer against[a]:		
	AKR-MuLV	Friend-MuLV	Rauscher-MuLV
(B6C3)F_1	1,280	640	640
C3H/Anf	320	80	80
BALB/c	80	80	80
C57BL/6	640	320	320

[a] Titers were determined using the respective [^{3}H]leucine-labeled viruses (1×10^7 to 3×10^7 counts/min per mg of protein) in RIP assays, as described in the text. Titers are the reciprocal of the serum dilution giving 50% precipitation of the labeled virus.

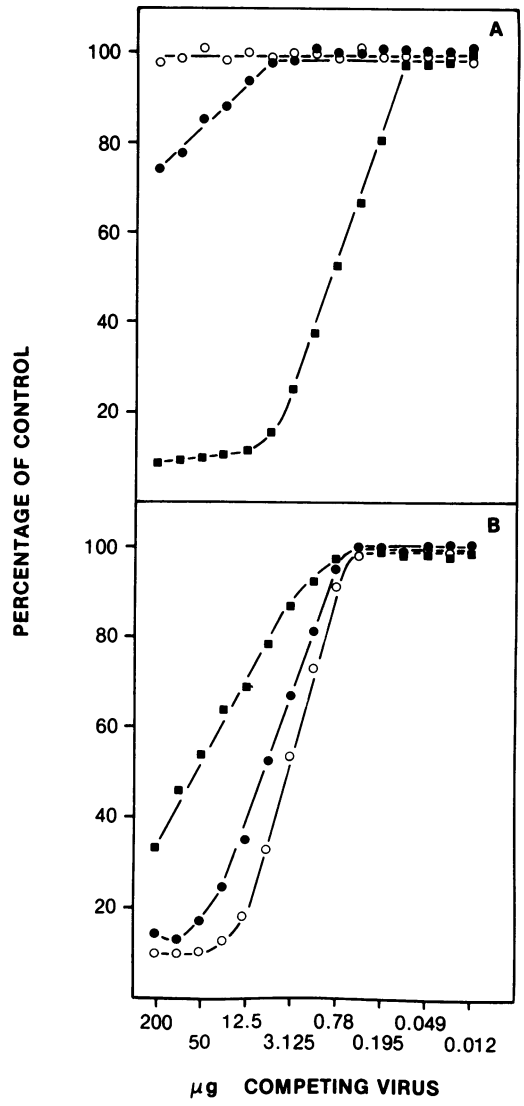

FIG. 1. *Competition RIP assays of (B6C3)F_1 sera. Sera from 1.5-year (B6C3)F_1 mice were reacted with [^{3}H]leucine-labeled AKR-MuLV (A) or Friend-MuLV (B) at serum dilutions of 1:300 and 1:100, respectively, in the presence of decreasing amounts of unlabeled AKR-MuLV (■), Rauscher-MuLV (●), or Friend-MuLV (○). In control reactions in the absence of competing virus, approximately 70% of the labeled virus was precipitated.*

The results of comparable experiments analyzing the reaction of (B6C3)F_1 sera with labeled Friend-MuLV are also shown in Fig. 1. As expected, unlabeled Friend-MuLV can completely inhibit this reaction, in a manner comparable to the above, with 20% competition occurring at approximately 1 μg of virus. In addition, Rauscher-MuLV can inhibit the reaction

in a similar manner, with 20% competition occurring with approximately 2 μg of virus. However, AKR-MuLV also significantly inhibited the precipitation of Friend-MuLV, although the slope was somewhat displaced to higher viral concentrations and 20% inhibition required approximately 6 to 7 μg of the virus. The inability of Friend- or Rauscher-MuLV to compete for the precipitation of AKR-MuLV and the ability of AKR-MuLV to compete for the precipitation of Friend-MuLV suggest that the antigenic determinants recognized on the FMR viruses by (B6C3)F_1 sera are shared with AKR-MuLV; but, in addition, antigenic determinants exist on AKR-MuLV that are not present on the FMR viruses.

These results and a summary of additional competition experiments are shown in Table 2. With (B6C3)F_1 sera, the reaction with labeled AKR-MuLV could also be completely inhibited with Gross-MuLV but could not be inhibited with Moloney-MuLV. Furthermore, the reaction with Friend-MuLV could be completely inhibited by all the viruses examined. However, neither of these reactions could be inhibited with murine mammary tumor virus or rat leukemia virus (data not shown).

In addition to (B6C3)F_1 sera, we have also examined autogenous immune sera from other strains. These results are summarized in Table 2. All the sera examined gave comparable patterns of competition. In particular, immune precipitation of AKR-MuLV could be inhibited by AKR-MuLV but not by FMR viruses. Furthermore, immune precipitation of Friend-MuLV by these autogenous immune sera was inhibited by the FMR viruses, as well as by AKR-MuLV. Therefore, the pattern of reactivity of autogenous immune sera from various strains of mice is the same and suggests that the immune response in these strains of mice is directed against the AKR-MuLV.

To further examine the specificities of these autogenous immune sera, (B6C3)F_1 sera were absorbed with AKR-, Friend-, or Rauscher-MuLV, as described above. These sera were subsequently titered in RIP assays with various viruses. The results are summarized in Table 3. Sera absorbed with AKR-MuLV lost all reactivity detectable in RIP against AKR-MuLV and Friend-MuLV. Sera absorbed with Friend-MuLV failed to react with Friend- or Rauscher-MuLV in RIP assays, but they had titers comparable to controls against AKR-MuLV. These data are consistent with the results of the competition experiments and again suggest that

TABLE 3. *Comparisons of antibody titers of autogenous immune sera and absorbed sera against Friend- and AKR-MuLV virions and glycoproteins*

Serum	RIP titer against[a]:		RIA titer against[b]:	
	Friend-MuLV	AKR-MuLV	Rauscher gp71	AKR gp71
C3H/Anf	80	80	10	80
C57BL/6	320	640	10	160
BALB/c	80	80	10	80
(B6C3)F_1	640	1,280	10	320
(B6C3)F_1 absorbed with:				
AKR-MuLV	0	0	0	0
Friend-MuLV	0	1,280	0	320
Rauscher-MuLV	0	1,280	0	320

[a] RIP assays against [^{3}H]leucine-labeled viruses were performed as described in the text.

[b] Direct RIA against purified, ^{125}I-labeled glycoproteins, as described in the text. Titers are expressed as the reciprocal of the serum dilution required for 50% precipitation of the labeled antigen.

TABLE 2. *Competition RIP assays using intact virions and various autogenous immune sera*

Serum	Labeled MuLV	μg required for 20% competition[a]				
		Competing MuLV				
		AKR	Moloney	Friend	Rauscher	Gross
(B6C3)F_1	AKR	0.39	>100	>200	100	5.12
	Friend	6.25	3.12	1.56	1.56	3.12
C3H/Anf	AKR	0.20	>200	100	ND[b]	ND
	Friend	0.20	3.12	0.20	ND	ND
C57BL/6	AKR	1.56	ND	>200	100	ND
	Friend	3.12	ND	1.56	1.56	ND
BALB/c	AKR	0.78	>200	200	ND	ND
	Friend	1.56	3.12	1.56	ND	ND

[a] (B6C3)F_1, C3H/Anf, C57BL/6, and BALB/c sera were reacted against labeled AKR-MuLV at dilutions of 1:400, 1:40, 1:100, and 1:40 and against labeled Friend-MuLV at dilutions of 1:200, 1:40, 1:80, and 1:40, respectively, in RIP competition assays, as described in the text. The results are summarized as the amount (micrograms) of competing virus required to effect 20% competition.

[b] ND, Not determined.

these autogenous sera have antibodies specific for the Gross type of ecotropic viruses, some of which are capable of cross-reacting with the FMR viruses.

Using immune precipitation of disrupted AKR-MuLV virions, followed by SDS-polyacrylamide gel electrophoresis, we have previously demonstrated antibodies in these sera that are specific for gp71, gp43, and p15(E) (15). Similar immunological reactivities with Rauscher-MuLV (Lee and Ihle, J. Natl. Cancer Inst., in press) were demonstrated using this assay. However, since these reactions were done at a single, high-serum concentration, the degree of cross-reactivity against various antigens could not be assessed. Therefore, we examined normal and absorbed sera for reactivity against purified AKR-MuLV gp71 and Rauscher-MuLV gp71. Typical titrations are shown in Fig. 2, and the results are summarized in Table 3. As previously demonstrated, sera from $(B6C3)F_1$ and C3H/Anf mice had titers against AKR-MuLV gp71 (13). Similarly, BALB/c and c57BL/6 sera had precipitating titers against purified AKR-MuLV gp71. In general, the titer of a particular serum was lower against the glycoprotein than against the intact virus. This difference is due to the greater sensitivity of the virus RIP (Ihle et al., unpublished data). However, the relative differences in the titers of these sera against intact virus were similar to the differences seen in the assays using the glycoprotein. These sera, however, had only a weak reactivity against purified Rauscher-MuLV gp71, indicative of the serological differences between the FMR and AKR-MuLV glycoproteins (13) and demonstrating that the reactivity of these autogenous immune sera with the AKR-MuLV glycoprotein is primarily type specific.

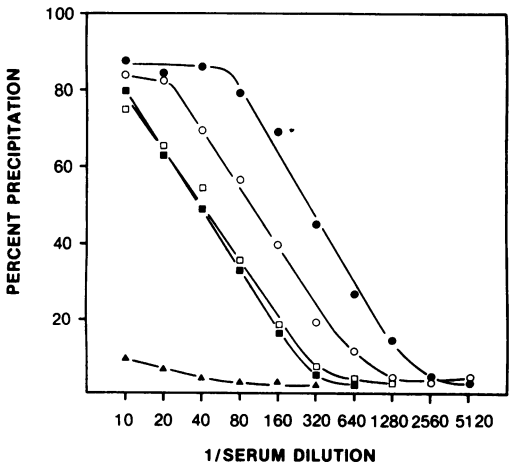

FIG. 2. *RIA of ^{125}I-labeled AKR-MuLV gp71 by normal mouse sera. Direct RIAs were performed, as described in the text, using sera from $(B6C3)F_1$ (●), C57BL/6 (○), C3H/Anf (□), and BALB/c (■) mice. The reaction of $(B6C3)F_1$ sera with ^{125}I-labeled Rauscher-MuLV gp71 (▲) is shown for comparison.*

The reactivities of absorbed sera from $(B6C3)F_1$ mice with the AKR-MuLV gp71 were also summarized in Table 3. Sera absorbed with AKR-MuLV lost all precipitating titers against labeled AKR-MuLV gp71, whereas sera absorbed with Rauscher- or Friend-MuLV retained their original titers. Interestingly, the weak reactivity of these sera with Rauscher-MuLV gp71 was completely lost after absorption with any of the viruses. This observation suggests that this weak reactivity detectable with Rauscher-MuLV gp71 may represent a small amount of group-specific recognition of these autogenous immune sera, although the predominant response is clearly type specific.

The above data demonstrate that the gp71 molecules are at most only slightly cross-reactive; however, these data do not exclude gp43 or p15(E). To examine these possibilities, we reacted absorbed $(B6C3)F_1$ sera with disrupted [^{3}H]leucine-labeled AKR-MuLV and analyzed the immune precipitates by SDS-polyacrylamide gel electrophoresis. Sera that were mock-absorbed had profiles comparable to those previously reported for normal sera (15). The majority of the radioactivity is in a 1.5×10^4 molecular weight component. This has been previously demonstrated to be p15(E) (16). The two smaller peaks have been shown to be glycoproteins and constitute AKR gp71 and gp43 (15). The differences in the radioactivity reflect the relative contribution of these components in the virion (15). Sera that had been absorbed with AKR-MuLV and reacted with disrupted [^{3}H]leucine-labeled AKR-MuLV lost all their reactivity by this assay (Fig. 3). In contrast, sera absorbed with Rauscher-MuLV gave profiles that had comparable amounts of gp71 and gp43 but significantly reduced amounts of p15(E). Identical profiles were obtained with sera absorbed with Friend-MuLV (data not shown). These data not only substantiate the lack of significant cross-reactivity of the FMR and AKR glycoproteins but moreover suggest that significant cross-reactivity does exist between the p15(E) proteins. Consequently, the predominant immunological cross-reactivity detected by the RIP assay of autogenous immune sera with the FMR viruses is found against p15(E).

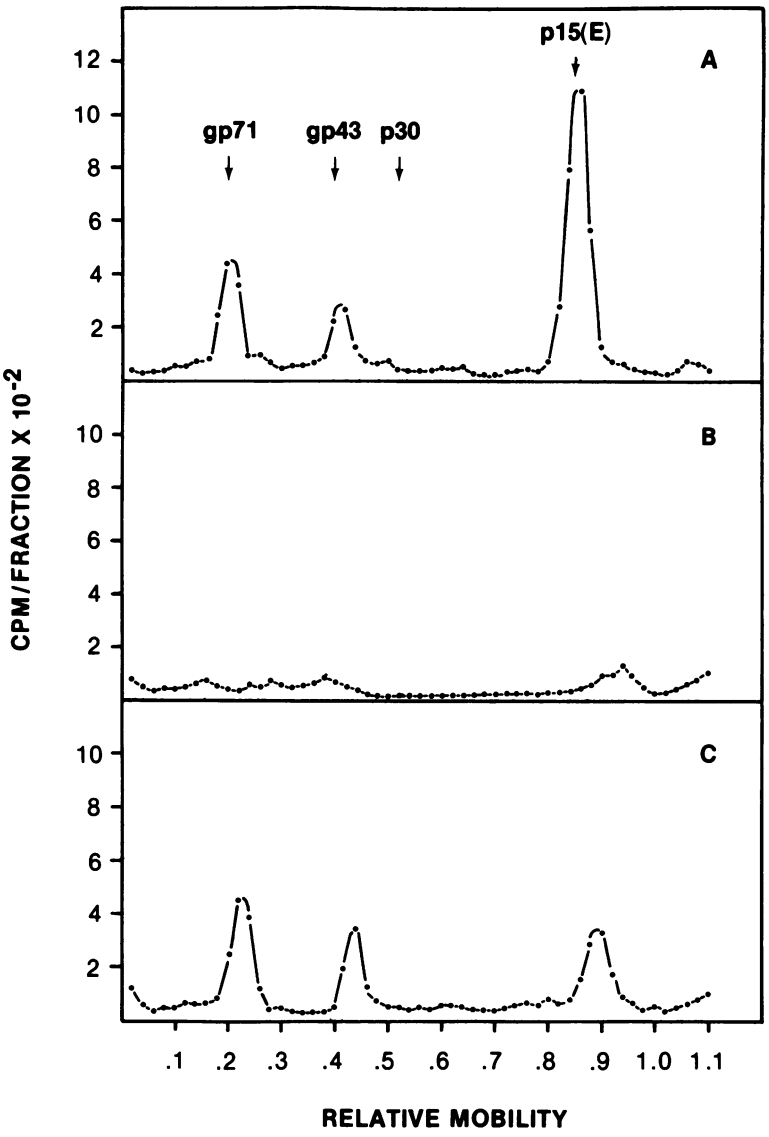

FIG. 3. *SDS-polyacrylamide gel electrophoresis of immune precipitates. Sera from (B6C3)F$_1$ mice were mock-absorbed (A) or absorbed with AKR-MuLV (B) or Rauscher MuLV (C) and reacted with disrupted [^{3}H]leucine-labeled AKR-MuLV, as described in the text. The resulting immune complexes were precipitated with an antiglobulin serum, disrupted with SDS, and subjected to polyacrylamide gel electrophoresis.*

DISCUSSION

The existence of an autogenous immune response in a variety of strains of mice has several significant consequences, the most important of which is the possibility of immunological surveillance against the virus or against virus replicating cells. This is further emphasized by our present results and those obtained previously (13), which demonstrate that one of the predominant immunological responses is against the AKR-MuLV gp71, although the reactivity to p15(E) is only slightly lower in titer, as discussed below. The immune response to gp71, however, is highly type specific and is in contrast to studies employing heterologous antisera to FMR virus glycoproteins that have emphasized the group-specific reactivity of antisera to this envelope component (24, 31–38). The significance of the type-specific antibodies in these autogenous immune sera is not clear. Studies analyzing the specificities of antibodies

in heterologous antisera to the FMR glycoproteins have suggested that both group- and type-specific reactions with the glycoprotein can neutralize virus infectivity (12, 31). Therefore, it is somewhat unusual that significant neutralization of ecotropic viruses has not been detected with autogenous immune sera (1, 11), although high neutralizing activity is detected against the xenotropic virus which is antibody independent (1; J. A. Levy, J. N. Ihle, O. Oleszko, and R. D. Barnes, Proc. Natl. Acad. Sci. U.S.A., in press). This may be due in part to the differences in titers; for example, in direct precipitation assays these heterologous antisera have titers of 1:15,000 to 25,000, whereas in the neutralization assays these same sera have neutralization titers of only approximately 1:200 to 400. In contrast, autogenous immune sera have titers of at most 1:320 to 640 in direct precipitation assays. Therefore, the quantitative differences in titer may be more important than the qualitative differences in considering the lack of neutralization by autogenous immune sera.

The consequences of a type-specific response may be significant in considering serum-mediated cytotoxicity. The expression of gp71 has been reported to occur on both budding viruses and on the cell surface of virus replicating cells (8; J. N. Ihle, J. C. Lee, J. Longstreth, and M. G. Hanna, Jr., submitted for publication; H. Schwarz, M. Claviez, G. Hunsmann, V. Moennig, and W. Schafer, Virology, in press). However, preliminary data suggest that the antigenic determinants expressed on the cell surface sites are limited to some group and interspecies type of determinants (Ihle et al., submitted for publication). Consequently, the type-specific antibodies of autogenous immune sera fail to react with the cell surface sites. Therefore, the antibody reactive sites for such cytotoxicity in vitro may be a function of the relative concentration of such sites on a particular target cell.

In addition to its potential efficacy, an immune response provides a convenient marker for virus expression and has the specificity necessary to identify the inducing virus. Our results suggest that in the strains examined the immune response is induced by an AKR or Gross type of MuLV and specifically is not induced by an FMR virus. In contrast, when mice are immunized with either Rauscher virus or Friend-MuLV gp71, they develop type-specific antibodies against the FMR glycoproteins (Ihle et al., unpublished data). These observations suggest that the FMR glycoproteins are not endogenous in mice but do not resolve the question of their origin.

The results demonstrate that only the antibodies directed against p15(E) cross-react with both AKR-MuLV and the FMR viruses. Consequently, the assays with the FMR viruses provide a quantitative assay for these antibodies. Furthermore, since the absorption experiments with FMR viruses failed to significantly reduce the titer against the AKR virus and, as reported previously (13), purified AKR gp71 can specifically compete for the precipitation of intact AKR virus at limiting serum dilutions, the titer against the AKR virus provides a quantitative measure of the antibodies against AKR-MuLV gp71. Consequently, a comparison of the titers of a serum against the FMR viruses and the AKR virus provides a relative measure of these two antigenic reactivities. Such a comparison of the sera used here suggests that the titers of most autogenous immune sera have only, at most, a slightly lower titer against p15(E) than against AKR-MuLV gp71.

Our study, showing the ability of antibodies to p15(E) in autogenous immune sera to cross-react with both AKR-MuLV and the FMR viruses, supports previous work that shows a similar cross-reactivity with various viruses. Schäfer et al. (30) have purified p15(E) from Friend-MuLV and have serologically demonstrated significant cross-reactivity of antisera to this component with a number of viruses. This component has also been shown to be localized on the virion envelope (16). Subsequent studies have identified p15(E) in a variety of viruses and have emphasized its common occurrence (17). The significance of this virion component in neutralization is not known, and studies employing immunoelectron microscopy have demonstrated that it is not expressed in the cell surface but only on budding virions (Ihle et al., submitted for publication; Schwarz, Claviez, Hunsmann, Moennig, and Schafer, Virology, in press). Consequently, the biological significance of this immune reactivity is unknown. Nevertheless, the results presented here provide a more detailed understanding of the autogenous immune response that is necessary to further understand its role in vivo.

ACKNOWLEDGMENTS

This research was supported by Public Health Service Contract NO1-CO-25423 from the National Cancer Institute to Litton Bionetics, Inc.

LITERATURE CITED

1. **Aaronson, S. A., and J. R. Stephenson.** 1974. Widespread natural occurrence of high titered neutralizing antibodies to a specific class of endogenous murine type-C virus. Proc. Natl. Acad. Sci. U.S.A. 71:1957-1961.
2. **Aaronson, S. A., G. J. Todaro, and E. M. Scolnick.** 1971. Induction of murine C-type viruses from clonal

lines of virus-free BALB/3T3 cells. Science 174:157.
3. Callahan, R., R. E. Benveniste, M. M. Lieber, and G. J. Todaro. 1974. Nucleic acid homology of murine type-C viral genes. J. Virol. 14:1394–1403.
4. Callahan, R., M. M. Lieber, and G. J. Todaro. 1975. Nucleic acid homology of murine xenotropic type C viruses. J. Virol. 15:1378–1384.
5. Chattopadhyay, S. K., W. P. Rowe, N. M. Teich, and D. R. Lowry. 1975. Definitive evidence that the murine C-type virus inducing locus AKv-1 is viral genetic material. Proc. Natl. Acad. Sci. U.S.A. 72:906–910.
6. Eckner, R. J., and R. A. Steeves. 1972. A classification of the murine leukemia viruses: neutralization of pseudotypes of Friend spleen focus-forming virus by type specific murine antisera. J. Exp. Med. 136:832–850.
7. Gomard, E., J. C. Leclerc, and J. P. Levy. 1973. Murine leukemia and sarcoma viruses: further studies on the antigens of the viral envelope. J. Natl. Cancer Inst. 50:955–961.
8. Grant, J. P., D. D. Bigner, P. J. Fischinger, and D. P. Bolognesi. 1974. Expression of murine leukemia virus structural antigens on the surface of chemically induced murine sarcomas. Proc. Natl. Acad. Sci. U.S.A. 71:5037.
9. Greenwood, F. C., W. M. Hunter, and J. W. Glover. 1963. The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. Biochem. J. 89:114–123.
10. Gross, L. 1951. Pathogenic properties and vertical transmission of the mouse leukemia agent. Proc. Soc. Exp. Med. 78:342–348.
11. Hanna, M. G., Jr., J. N. Ihle, B. L. Batzing, R. W. Tennant, and C. Schenley. 1975. Autogenous immunity to endogenous RNA tumor virus: assessment of reactivity to virus envelope and virus-induced cell-surface antigens. Cancer Res. 35:167–171.
12. Hunsmann, G., V. Moennig, L. Pister, E. Seifert, and W. Schäfer. 1974. Properties of mouse leukemia viruses. VIII. The major viral glycoprotein of Friend leukemia virus. Seroimmunological, interfering and hemagglutinating capacities. Virology 62:307–318.
13. Ihle, J. N., T. Denny, and D. P. Bolognesi. 1976. Purification and serological characterization of the major envelope glycoprotein from AKR murine leukemia virus and its reactivity with autogenous immune sera from mice. J. Virol., 17:727–736.
14. Ihle, J. N., and M. G. Hanna, Jr. 1973. Autogenous immunity to endogenous RNA tumor virus: radioimmune precipitation assay of mouse serum antibody levels. J. Exp. Med. 138:194–208.
15. Ihle, J. N., M. G. Hanna, Jr., L. E. Roberson, and F. T. Kenney. 1974. Autogenous immunity to endogenous RNA tumor virus: identification of antibody reactivity to select viral antigens. J. Exp. Med. 136:1568–1581.
16. Ihle, J. N., M. G. Hanna, Jr., W. Schäfer, G. Hunsmann, D. P. Bolognesi, and G. Huper. 1975. Polypeptides of mammalian oncornaviruses. III. Localization of p15 and reactivity with natural antibody. Virology 63:60–67.
17. Ikeda, H., W. Hardy, Jr., E. Tress, and E. Fleissner. 1975. Chromatographic separation and antigenic analysis of proteins of the oncornaviruses. I. Identification of a new murine viral protein, p15(E). J. Virol. 16:53–61.
18. Kaplan, H. F. 1967. On the natural history of the murine leukemias: presidential address. Cancer Res. 27:1325.
19. Levy, J. A. 1973. Xenotropic viruses: murine leukemia viruses associated with NIH Swiss, NZB and other mice strains. Science 182:1151–1153.
20. Lilly, F. 1972. Mouse leukemia: a model of a multiple-gene disease. J. Natl. Cancer Inst. 49:927–934.
21. Lilly, F., and T. Pincus. 1973. Genetic control of murine viral leukemogenesis advances. Cancer Res. 17:231–273.
22. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
23. Meier, H., B. A. Taylor, M. Cherry, and R. T. Huebner. 1973. Host-gene control of type-C RNA tumor virus expression and tumorigenesis in inbred mice. Proc. Natl. Acad. Sci. U.S.A. 70:1450–1455.
24. Moennig, V., H. Frank, G. Hunsmann, I. Schneider, and W. Schafer. 1974. Properties of mouse leukemia viruses. VII. The major viral glycoprotein of Friend leukemia virus. Isolation and physiochemical properties. Virology 61:100.
25. Nowinski, R. C., and S. L. Kaehler. 1974. Antibody to leukemia virus. Widespread occurrence in inbred mice. Science 185:869–971.
26. Old, L. J., E. A. Boyse, and E. Stockert. 1964. Typing of mouse leukemias by serological methods. Nature (London) 201:777–778.
27. Peters, R. L., J. W. Hartley, G. J. Spahn, L. S. Rabstein, C. E. Whitmire, H. C. Turner, and R. J. Huebner. 1972. Prevalence of the group-specific (gs) antigen and infectious virus expressions of murine C-type RNA viruses during the life span of BALB/cCR mice. Int. J. Cancer 10:283–289.
28. Rowe, W. P., J. W. Hartley, M. R. Lander, W. E. Pugh, and N. Tiech. 1971. Noninfectious AKR mouse embryo cell lines in which each cell has the capacity to be activated to produce infectious murine leukemia virus. Virology 46:866–876.
29. Rowe, W. P., and T. Pincus. 1972. Quantitative studies of naturally occurring murine leukemia virus infection of AKR mice. J. Exp. Med. 135:429–436.
30. Schäfer, W., G. Hunsmann, V. Moennig, F. De-Nororha, D. P. Bolognesi, R. W. Green, and G. Huper. 1975. Polypeptides of mammalian oncornaviruses. II. Characterization of a murine leukemia virus polypeptide (p15) bearing interspecies reactivity. Virology 63:48–59.
31. Steeves, R. A., M. Strand, and J. T. August. 1975. Structural proteins of mammalian oncogenic RNA viruses: murine leukemia virus neutralization by antisera prepared against purified envelope glycoprotein. J. Virol. 14:187–189.
32. Strand, M., and J. T. August. 1973. Structural proteins of oncogenic RNA viruses: *interspec II*, a new interspecies antigen. J. Biol. Chem. 248:5627–5633.
33. Strand, M., and J. T. August. 1975. Structural proteins of mammalian oncogenic RNA viruses: multiple antigenic determinants of the major internal protein and envelope glycoprotein. J. Virol. 13:171–180.
34. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406–4412.