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The humoral immune response of NIH Swiss and SWR/J mice to vaccination with formalinized AKR or Gross murine leukemia virus

(C-type virus vaccine/antibody)

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ABSTRACT **The humoral immune responses of NIH Swiss and SWR/J mice immunized with formalin-inactivated AKR or Gross murine leukemia virus, respectively, were examined. Both immune sera had high titers of antibodies detectable in radioimmune precipitation assays using [^{3}H]leucine-labeled AKR virus and in radioimmunoassays using purified virion components. The predominant antibody titers were directed against gp71 and p15(E). The immune response against gp71 was predominantly type-specific, whereas the reactivity with p15(E) was predominantly group-specific. A weak immune response against p15 was also detected. Both sera were cytotoxic against cells replicating the AKR-Gross virus type but not against cells replicating Friend murine leukemia virus. This cytotoxicity could be specifically blocked with purified gp71 of AKR murine leukemia virus. Sera from immune NIH Swiss mice neutralized AKR virus, but did not neutralize Rauscher, Scripps, or wild mouse leukemia virus.**

Ecotropic murine leukemia viruses (MuLV) are endogenous to various inbred strains of mice and have been etiologically linked to spontaneous and radiation-induced thymic lymphomas in these mice (1, 2). Although the regulation of expression of these viruses is complex and involves various genetic factors, one component of this regulation is the ability to mount a systemic humoral immune response to the virus (3–5). This autogenous immune response has been examined in various strains of mice and, in general, is characterized by precipitating antibodies to the envelope components gp71, gp43, and p15(E) of the virus (5–8). The natural immunological reactivities appear type-specific against the major glycoprotein, gp71, reacting only with the gp71 of AKR MuLV and not with gp71 of Rauscher or Friend MuLV. In contrast, natural antibodies have group specificity against p15(E) and via this crossreactivity precipitate Rauscher or Friend MuLV (7, 9). The efficacy of this immune response in regulating pathogenesis is not totally understood, although the ability of this response to neutralize infectious virus (Ihle *et al.*, unpublished) and to be cytotoxic against virus-replicating cells suggests a beneficial function (10). Clearly, however, experiments designed to alter this immune response are warranted.

Several investigators have examined the effect of virus immunization in mice (11–13); however, the characteristics of the immune response were not determined. More recently, several studies have been directed toward examining the response of various strains of mice to purified gp71 of Friend MuLV(14–17). The results of these experiments have demonstrated that such immunizations can protect against Friend MuLV-induced pathogenesis in STU mice (1), but that similar immunizations in other strains provided no protection from endogenous MuLV-mediated pathogenesis (17). These results were apparently the consequence of a predominantly type-specific humoral immune response to gp71 of Friend MuLV, since antibodies induced by gp71 immunization did not crossreact with the glycoprotein of the endogenous AKR-type MuLV (15, 16). Furthermore, the immune response was relatively weak and, in general, the titers of precipitating antibodies were significantly lower than the naturally occurring antibodies to the endogenous MuLV.

The present experiments were undertaken to examine, quantitatively and qualitatively, the humoral immune response of mice to immunization with formalinized virus vaccines to better correlate any protective effects with specific immunological reactions. The results demonstrate that of the techniques tested for immunization to date, this protocol induced the strongest humoral immune response, and that, in general, the qualitative parameters of this immune response are similar to those of the autogenous immune response.

Abbreviations: MuLV, murine leukemia virus(es); $NaDodSO_4$, sodium dodecyl sulfate.

MATERIALS AND METHODS

Test Sera. Sera were generally separated by centrifugation at 400 × *g* for 15 min and were stored at −70°. All sera were heat-inactivated at 56° for 30 min.

Immunization. Weanling MAI-NIH Swiss female mice were inoculated with freshly banded formalin-treated AKR virus vaccine with equal parts of complete Freund's adjuvant. Sera were collected on day 87 and were tested for complement fixation, neutralization, and viral precipitation activity. SWR/J male mice were inoculated with freshly banded formalin-treated Gross leukemia virus vaccine with equal parts of complete Freund's adjuvant intraperitoneally and subcutaneously. Booster doses were administered on days 14, 28, 45, and 56. Sera were taken on days 42, 55, and 89.

Viruses. Viruses used in the radioimmune precipitation assays included AKR virus purified from an established line of AKR mouse embryo cells, which had spontaneously initiated virus synthesis. These cells were initially obtained from Dr. W. P. Rowe, National Institutes of Health. Friend MuLV, purified from the Eveline cell line (18), was provided by W. Schäfer, Tübingen, Germany. Moloney MuLV was purified from an established NIH Swiss fibroblast cell line chronically infected with Moloney MuLV. Rauscher MuLV was purified from the JLS-V5 cell line.

Preparation of Radioactively Labeled Virus. [^{3}H]Leucine-labeled virus was prepared as described (3), except 1 μM hydrocortisone was incorporated in the culture medium to enhance virus yield. All viruses were purified by a final isopycnic banding in a 15–50% linear sucrose gradient.

All other viruses were prepared under identical conditions and had similar radiospecific activities of 1 to 3 × 10^7 cpm/mg of protein. Purified virus pools with acceptable specific activity were stored at −70°, and then used immediately upon thawing.

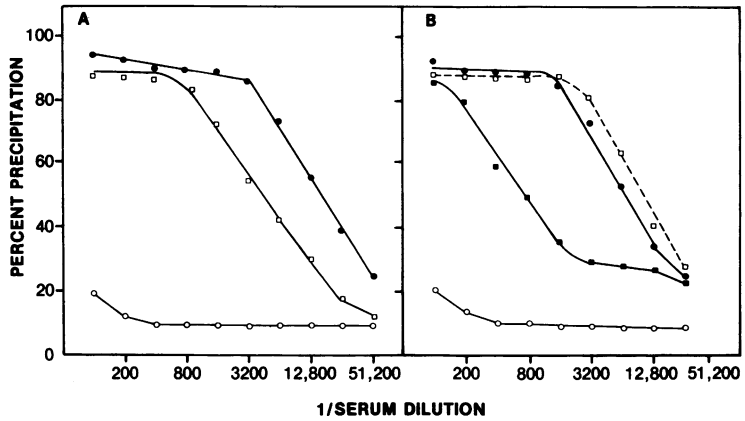

FIG. 1. Radioimmune precipitation assays of control and immune sera. (A) Control (○) and immune (●) NIH Swiss sera were titered against [^{3}H]leucine-labeled AKR MuLV. The titration of autogenous immune sera from 1-year (B6C3)F_1 mice (□) is shown for comparison. (B) Control (○) and immune sera from SWR/J mice at 42 (●), 55 (□), and 89 (■) days after immunization were titered against [^{3}H]leucine-labeled AKR MuLV.

Radioimmune Precipitation Assay Against Intact Virus. The radioimmune precipitation assay against intact radioactively labeled AKR virus has been described (3).

To prepare immune precipitates for polyacrylamide gel electrophoresis, 0.025–0.050 ml of serum was allowed to react with 2×10^5 cpm of Triton-disrupted virus and subsequently precipitated with antiserum against mouse immunoglobulin as described (5). The precipitates were resuspended in 0.05 ml of 1% sodium dodecyl sulfate ($NaDodSO_4$), 1% 2-mercaptoethanol in 0.01 M sodium phosphate buffer at pH 7.4; incubated at 60° for 1 hr; and finally, incubated at 37° overnight.

$NaDodSO_4$-Polyacrylamide Gel Electrophoresis of Immune Precipitates. The electrophoresis was performed as described by Weber and Osborn (19). The gels were sectioned into 1-mm slices, and dissolved overnight in 30% hydrogen peroxide at 75°. The radioactivity of each gel-slice fraction was determined with a liquid scintillation counter.

Virus Proteins. The glycoprotein (gp71) and the major internal protein (p30) from AKR and Rauscher MuLV were purified as described (6). Other AKR MuLV structural proteins, p10, p12, and p15, were purified with virions disrupted by lithium diiodosalicylate by Sephadex G-200 and DEAE-Sephadex chromatography (Ihle *et al.*, in preparation). Antigens were iodinated as described (6). The virion components were all homogeneous, as determined by $NaDodSO_4$-polyacrylamide gel electrophoresis of iodinated samples.

Direct Radioimmunoassay. Test sera were diluted 2-fold in 0.2 ml of PNE (0.05 M sodium phosphate at pH 7.0, 0.1 M NaCl, 1 mM EDTA) containing 0.01% Triton X-100. Labeled virion proteins were then added (1–2 ng), and the mixture was incubated 3 hr at 37° and overnight at 4°. Immune complexes were precipitated by the addition of 0.1 ml of rabbit antisera against mouse immunoglobulins. This mixture was allowed to incubate 1 hr at 37° and 2 hr at 4°. The precipitates were subsequently collected and washed as above. When necessary, purified BALB/c immunoglobulins were used as carrier immunoglobulins.

Serum Cytotoxicity. Various tissue culture cell lines, AKR (HP), AKR (LP) RLδ1, and Eveline, were used as target cells in the serum cytotoxicity assay. Except for RLδ1, which was maintained as a suspension culture, other cell cultures were trypsinized and resuspended in RPMI 1640 supplemented with 10% fetal calf serum, which was used throughout this assay. Target cells, adjusted to 10^7 cells per ml, were treated with 100–150 μCi of ^{51}Cr (New England Nuclear, Boston, Mass.) at 37° for 45 min. They were washed thoroughly and resuspended to 10^6 cells per ml. Serum samples were diluted as required; 0.1 ml of this dilution was added to 0.1 ml of target cells, and the reaction was allowed to proceed for 30 min at 37°. Fresh guinea pig serum (0.05 ml) as a source of complement was then added and followed by a second 30-min incubation at 37°. One milliliter of unlabeled medium was then added to each culture and the cells were sedimented at $400 \times g$ for 10 min. Supernatant was transferred, and radioactivity was measured in a Searle automatic gamma counter. Target cells incubated with guinea pig complement alone acted as a baseline control; four freeze-thaw cycles of target cells represented maximum release. Percent lysis was calculated based on those proper controls. In blocking experiments, samples of blocking agent were added to the reaction mixture at the beginning of the first incubation.

RESULTS

The titers of viral antibodies of sera from control and formalinized virus-immunized NIH Swiss and SWR/J mice were determined with a radioimmune precipitation assay. Typical titrations are shown in Fig. 1, and the results are summarized in Fig. 2. Control sera from NIH Swiss or SWR/J mice had low amounts of or no detectable antibodies by this assay. However, immunization of NIH Swiss mice with formalinized AKR MuLV or immunization of SWR/J mice with formalinized Gross MuLV induced high titers of antibodies. At the peak of the response, titers of approximately 1:12,800 were obtained, which are considerably higher than the titers of a relatively high autogenous immune sera from normal 1-year (B6C3)F_1 mice. In SWR/J mice, the induced immune response was relatively low at 42 days, was maximal at 55 days, and subsequently decreased sharply such that by 89 days the titer was 1:800.

Each immune serum was also examined in terms of the predominant antibody type. In these experiments specific antisera to mouse 19S and 7S immunoglobulins were used in the radioimmune precipitation assays to precipitate immune complexes. As demonstrated in Table 1, the predominant immunoglobulin reacting in the assay with AKR MuLV was a 7S antibody, although lower but detectable reactivity was associated with the 19S class of immunoglobulins.

The ability of these sera to crossreact with Rauscher or Moloney MuLV was also examined. Both immune sera had high titers of antibodies capable of reacting with these viruses, which was similar to the results seen with AKR MuLV. This cross-

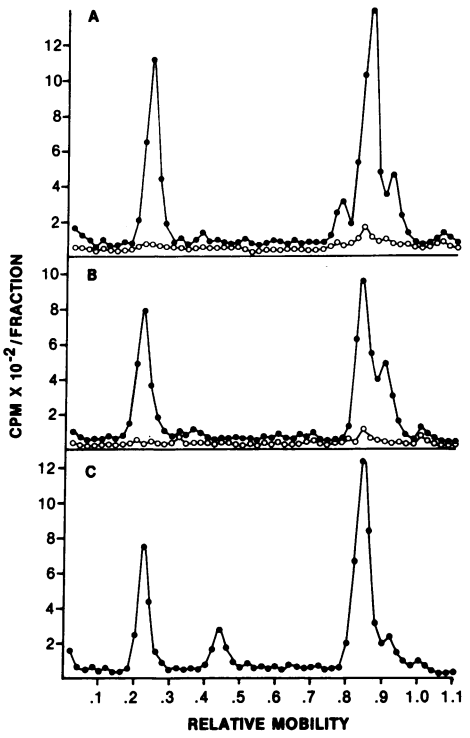

FIG. 2. NaDodSO$_4$-polyacrylamide gel electrophoresis profiles of immune precipitates of control and immune sera with [^{3}H]leucine-labeled AKR MuLV. (A) Control (○) and immune (●) NIH Swiss sera. (B) Control (○) and immune (55 days) (●) SWR/J sera. (C) The titration of autogenous immune sera from 1-year (B6C3)F$_1$ mice is shown for comparison.

reactivity is also observed with autogenous immune sera and has been shown to be due to the immune response to p15(E) (7), a virion envelope component shared by MuLV (20, 21). As demonstrated below, NIH Swiss and SWR/J immune sera have similar crossreactive antibodies to p15(E) that account for the above results, and consequently the reactivity with Rauscher or Moloney MuLV provides a relative measure of the titer against p15(E).

The antigenic specificity of this immune response was examined by reacting immune and control sera with disrupted [^{3}H]leucine-labeled AKR MuLV virions and analyzing the immune precipitates by NaDodSO$_4$-polyacrylamide gel electrophoresis as described (5). As demonstrated in Fig. 2, both

Table 1. Radioimmune precipitation titer

Serum	AKR	Rauscher	Moloney
B6C3F$_1$	1:3,200	1:1,280	1:1,280
NIH Swiss (normal)	<1:10	<1:10	<1:10
NIH Swiss (immune)	1:12,800	1:12,800	1:12,800
7 S	1:6,400	—*	—
19 S	1:400	—	—
SWR/J (normal)	<1:10	<1:10	<1:10
SWR/J (immune)			
day 42	1:6,400	—	—
7 S	1:6,400	—	—
19 S	<1:80	—	—
SWR/J (immune)			
day 55	1:12,800	1:6,400	1:12,800
7 S	1:12,800	—	—
19 S	1:80	—	—
SWR/J (immune)			
day 89	1:800	—	—
7 S	1:800	—	—
19 S	<1:80	—	—

Radioimmune precipitation assays were performed as described in *Materials and Methods* with [^{3}H]leucine-labeled AKR, Rauscher, and Maloney MuLV. The specfic radioactivity of each virus was approximately 3 × 10^7 cpm/mg of protein. Titers are expressed as the serum dilution giving 50% of maximum precipitation of the labeled virus.

* Not determined.

immune sera reacted with comparable virion components as detected by this assay. The predominant reactivities appeared to be with components migrating in the positions of gp71 and p15(E), which are similar to the reactivities seen with most autogenous immune sera as described (5) and as shown here for comparison in Fig. 2C. In contrast, however, neither serum precipitated significant amounts of gp43. In addition, both immune sera precipitated material migrating slightly faster than p15(E), as well as a minor peak migrating somewhat more slowly than p15(E). Control sera from NIH Swiss and SWR/J mice only weakly reacted in this assay.

The reactivity of these sera with purified MuLV components was also examined. Typical titrations are shown in Fig. 3 and the results are summarized in Table 2. Both groups of immune sera had high titers of antibody capable of precipitating AKR MuLV gp71 in direct radioimmunoassay. In contrast, these immune sera had only low titers of antibodies capable of reacting with Rauscher MuLV gp71, demonstrating that the

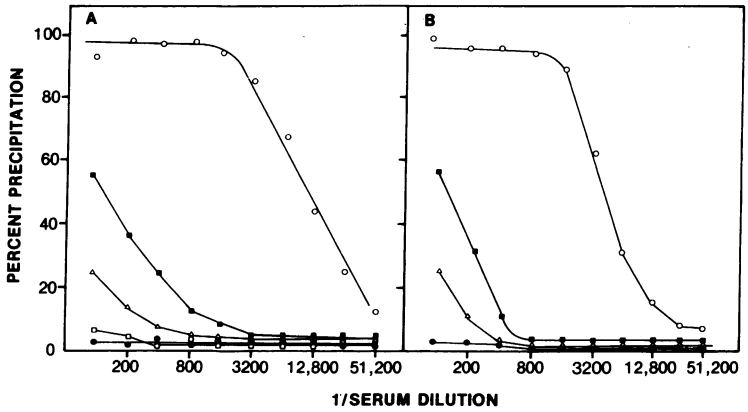

FIG. 3. Radioimmunoassays of immune NIH Swiss and SWR/J sera. NIH Swiss (A) and SWR/J (55 days) (B) sera were titered against AKR MuLV gp71 (○), p15 (■), p30 (●), and p12 (□) and Rauscher MuLV gp71 (△). Titrations with Rauscher MuLV p30 and AKR MuLV p10 were identical to (●).

Table 2. Radioimmunoassays against purified virus proteins

Protein	NIH Swiss Normal	NIH Swiss Immune	SWR/J Normal	SWR/J Immune
AKR MuLV				
gp71	0	1:10,000	0	1:3,200
p30	0	0	0	0
p15	0	1:100	0	1:100
p12	0	0	0	0
p10	0	0	0	0
Rauscher MuLV				
gp71	0	<1:100	0	<1:100
p30	0	0	0	0

Radioimmunoassays were performed as described in *Materials and Methods* using iodinated purified virion components. The specific radioactivities of the antigens were between 1 and 3 × 10^4 cpm/ng, and generally 1–2 ng were used per assay. Titers are expressed as the serum dilution giving 50% of maximum precipitation of each antigen.

immune response to gp71 was predominantly type-specific. These results are not unexpected since recent studies have demonstrated significant differences in the serological relationship of these glycoproteins (6). Immune sera did not react with p30 from Rauscher or AKR MuLV nor with p10 or p12 of the AKR virus. These results are in agreement with the NaDodSO$_4$-polyacrylamide gel electrophoresis profiles of the immune precipitates. However, although not clearly detected by the NaDodSO$_4$-polyacrylamide gel electrophoresis profiles, both sera had a low but significant titer against p15. This reaction has also been previously detected by gel diffusion reactions of AKR p15 with similar immune sera (22). This antigen is serologically unique from p15(E) and migrates on gels slightly faster than p15(E). The other AKR MuLV antigens, gp43 and p15(E), were not available for comparable analysis. Nevertheless, the results taken together demonstrate that the predominant titers of these immune sera are against gp71 and p15(E), although the relative titer of the latter can only be inferred from the reactivity with Rauscher or Moloney MuLV.

Table 3. Serum cytotoxicity of control and immune NIH Swiss and SWR/J sera

Serum	Dilution	% Cytotoxicity AKR*	RLσ1	Eveline
NIH Swiss (control)	(1:10)	2.71†	2.32	1.07
	(1:100)	1.04	—‡	—
NIH Swiss (immune)	(1:10)	78.04	38.84	1.64
	(1:100)	18.64	3.35	—
	(1:1000)	1.11	—	—
SWR/J (control)	(1:10)	0.28	1.11	2.07
	(1:100)	0.21	—	—
SWR/J (immune)				
Day 42	(1:10)	1.47	1.07	2.74
	(1:100)	−0.22	—	—
Day 55	(1:10)	41.57	32.07	6.73
	(1:100)	1.57	—	—
Day 89	(1:10)	0.78	4.11	7.03
	(1:100)	0.65	—	—

* Cell lines tested included an established AKR fibroblast cell line replicating AKR MuLV, RLσ1 cells derived from a radiation-induced lymphoma in BALB/c mice and replicating an endogenous BALB virus, Eveline cells that were derived from STU mice, and one replicating Friend MuLV.

† Serum cytotoxicity was determined by a ^{51}Cr release assay as described in *Materials and Methods*.

‡ Not determined.

Table 4. Blocking of cytotoxicity of NIH Swiss immune serum with purified glycoproteins

AKR MuLV gp71 (μg)	% Cytotoxicity*	Rauscher MuLV (μg)	% Cytotoxicity*
0	28.46 (0.79)	0	27.32 (0.49)
1	8.72 (0.08)	1	26.31 (0.68)
5	1.46 (0.02)	—†	—
10	−5.43 (0.03)	10	25.22 (0.73)

Serum cytotoxicity was determined by a ^{51}Cr release assay as described. Blocking activity was determined by incubating NIH Swiss immune sera (1:100) with the appropriate antigen at 37° for 30 min prior to the cytotoxicity assay.

* Percent cytotoxicity ± standard error.

† Not determined.

The efficacy of this immune response was examined by determining the ability of these sera to be cytotoxic against various virus-replicating cells. As demonstrated in Table 3, sera from either strain, at a time when high radioimmune precipitation titers were present, were significantly cytotoxic against AKR and RLδ1 cells. These sera, however, were not cytotoxic against the Friend MuLV-producing cell line Eveline. These results are in agreement with the type specificity of the immune response to the glycoprotein. The antigenic specificity of this cytotoxicity is demonstrated in Table 4. The cytotoxicity of immune sera from NIH Swiss mice against AKR cells could be completely blocked by prior inoculation with AKR MuLV gp71. As above, the type specificity of this immune response is demonstrated by the inability of gp71 from Rauscher MuLV to give comparable blocking. These results, therefore, demonstrate that these sera are cytotoxic and type-specific and that this cytotoxicity and its specificity are due to the characteristics of the immune response to AKR MuLV gp71.

The ability of these sera to neutralize various MuLV is illustrated in Table 5. Immune sera from NIH Swiss mice neutralized AKR MuLV but did not neutralize Rauscher or other MuLVs. Since significant neutralization has only been associated with an immune response to gp71 (23, 24) and not with p15(E) (9), these results are compatible with the radioimmunoassay data, which demonstrate a type-specific response to the glycoprotein.

DISCUSSION

The experiments reported here were undertaken to characterize the immune response of mice to immunization with formalinized virus vaccines. The most evident consequence of such immunizations was the induction of a relatively strong immune response as determined by a number of parameters, including radioimmune precipitation assays, radioimmunoassays against the AKR MuLV gp71, cytotoxicity, and neutralization. The response is quantitatively stronger than the majority of autogenous immune sera from various inbred strains of mice (3–5) and is approximately 100- to 500-fold higher than the immune response induced in BALB/c and C57BL/6 mice immunized against Friend MuLV gp71 (15–17). Furthermore, in our experience, this response is considerably stronger than that obtained by immunization with tumor cells or nonformalinized virus vaccines.

The response was in part characterized by high titers of

Table 5. Specific reduction of AKR XC plaques by immune antisera from NIH Swiss weanling mice vaccinated with freshly banded formalin-treated AKR vaccine

Serum	Reciprocal of serum dilutions	XC plaques produced by specified viruses (%)*			
		AKR†	SLV‡	WM§	RLV¶
15037-38	10	97	29	60	39
	20	60	16	47	7
	40	28	0	42	0
	80	0	0	39	0
54263	10	100	NT‖	42	1
	20	99		35	0
	40	82		2	0
	80	33		0	0
54435	10	100	8	1	10
	20	80	1	0	0
	40	64	0	0	0
	80	34	0	0	0
55445	10	100	32	0	38
	20	85	5	0	35
	40	19	0	0	36
	80	17	0	0	0

* Significant neutralization is 70% reduction of plaque formation at any dilution. Values are percent of foci neutralized.
† Endogenous virus from AKR leukemia.
‡ Scripps leukemia virus (a variant of Moloney leukemia virus).
§ Wild mouse virus (LC strain from Dr. Murray Gardner).
¶ Rauscher leukemia virus.
‖ Not tested.

predominantly type-specific antibodies against the AKR MuLV gp71. The type specificity of the immune response of mice to MuLV glycoproteins has been a consistent characteristic observed in autogenous immune sera (6, 7) and in immune sera from mice immunized with tumor cells (unpublished). This response was not unexpected, however, since recent results (6) have demonstrated that distinct serological differences exist between purified gp71 from AKR MuLV and Friend or Rauscher MuLV, even in terms of heterologous antisera against the purified glycoproteins. The consequences of this type specificity, however, are extremely important in any consideration of immunoprophylaxis, since gp71 recognition is important in virus neutralization and, as demonstrated here, in cytotoxicity. Thus, as has been evident in previous reports (15–17), immunization for protection will require the appropriate viral preparation or glycoprotein of the virus under study.

A strong humoral immune response was also detected against p15(E), an antigen also recognized in an autogenous immune response. This immune response is predominantly group-specific and is the basis of the crossreactivity of these sera with Rauscher or Moloney MuLV (7). These results are compatible with the observation that p15(E) is a common virion envelope component of murine C-type viruses, which has predominantly group- and interspecies-serological characteristics. The consequences of an immune response to p15(E) are not known, although xenogeneic antisera to p15(E) only weakly neutralizes the virus (25).

The only additional immune response detectable was a weaker reaction with AKR MuLV p15. This antigen is unique from p15(E) and migrates slightly faster than p15(E) on Na-DodSO_4-polyacrylamide gel electrophoresis. In addition, p15 is a type-specific antigen. The reaction of similar immune sera with purified AKR MuLV p15 has also been previously detected by gel diffusion (22) and in this study was demonstrated to be type-specific. The consequences of an immune response to p15 are unknown, although with the availability of the purified antigen and specific antisera, this question can be approached.

The combined results demonstrate that immunization with formalinized virus vaccines can induce a strong humoral immune response. The characteristics of this response *in vitro* (i.e., neutralization and cytotoxicity) clearly suggest that such immunizations can be efficacious in controlling viral burdens *in vivo* and possibly in controlling pathogenesis. Examination of this question directly *in vivo* should provide definitive results to assess the usefulness of virus vaccines in controlling *endogenous* virus-mediated pathogenesis.

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1. Gross, L. (1951) *Proc. Soc. Exp. Med.* **78**, 342–348.
2. Kaplan, H. F. (1967) *Cancer Res.* **27**, 1325–1340.
3. Ihle, J. N., Yurconic, M., Jr. & Hanna, M. G., Jr. (1973) *J. Exp. Med.* **138**, 194–208.
4. Nowinski, R. C. & Kaehler, S. L. (1974) *Science* **185**, 869–871.
5. Ihle, J. N., Hanna, M. G., Jr., Roberson, L. E. & Kenney, F. T. (1974) *J. Exp. Med.* **136**, 1568–1581.
6. Ihle, J. N., Denny, T. P. & Bolognesi, D. P. (1976) *J. Virol.* **17**, 727–736.
7. Ihle, J. N., Domotor, J. J., Jr. & Bengali, K. M. (1976) *J. Virol.* **18**, 124–131.
8. Lee, J. C. & Ihle, J. N. (1975) *J. Natl. Cancer Inst.* **55**, 831–838.
9. Ihle, J. N., Hanna, M. G., Jr., Schäfer, W., Hunsmann, B., Bolognesi, D. P. & Hüper, G. (1975) *Virology* **63**, 60–67.
10. Martin, S. E. & Martin, W. J. (1975) *Nature* **256**, 498–499.
11. Fink, M. A. & Rauscher, F. J. (1964) *J. Natl. Cancer Inst.* **32**, 1075–1082.
12. Mayyasi, S. A., Foster, H. S., Bultone, L. M., Wright, S. B. & Shibley, G. P. (1968) *Proc. Soc. Exp. Biol. Med.* **128**, 1088–1092.
13. Kirsten, W. H., Stefanski, E. & Panem, S. (1974) *J. Natl. Cancer Inst.* **52**, 983–985.
14. Hunsmann, G., Moennig, V. & Schäfer, W. (1975) *Virology* **66**, 327–329.
15. Ihle, J. N., Collins, J. J., Lee, J. C., Fischinger, P. J., Moennig, V., Shäfer, W., Hanna, M. G., Jr. & Bolognesi, D. P. (1976) *Virology*, in press.
16. Ihle, J. N., Lee, J. C., Collins, J. J., Fischinger, P. J., Pazmino, N., Moennig, V., Schäfer, W., Hanna, M. G., Jr. & Bolognesi, D. P. (1976) *Virology*, in press.
17. Ihle, J. N., Collins, J. J., Lee, J. C., Fischinger, P. J., Pazmino, N., Moennig, V., Schäfer, W., Hanna, M. G., Jr. & Bolognesi, D. P. (1976) *Virology*, in press.
18. Seifert, E., Claviez, M., Frank, H., Hunsmann, G., Schwarz, H. & Schäfer, W. (1975) *Z. Naturforsch. Teil C* **30**, 698–700.
19. Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412.
20. Schäfer, W., Hunsmann, G., Moennig, V., de Noronha, F., Bolognesi, D. P., Green, R. W. & Hüper, G. (1975) *Virology* **63**, 48–49.
21. Ikeda, H., Hardy, W., Jr., Tress, E. & Fleissner, E. (1975) *J. Virol.* **16**, 53–61.
22. Gilden, R. V. (1975) *Adv. Cancer Res.* **22**, 157–201.
23. Hunsmann, G., Moennig, V., Pister, L., Seifert, E. & Schäfer, W. (1974) *Virology* **62**, 307–318.
24. Steeves, R. A., Strand, M. & August, J. T. (1975) *J. Virol.* **14**, 187–189.
25. Fischinger, P. J., Schäfer, W. & Bolognesi, D. (1976) *Virology* **71**, 169–184.