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Natural Immunity in Mice to Structural Polypeptides of Endogenous Type C RNA Viruses

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The immunological responses of inbred mice to structural components of one class of endogenous virus were investigated by means of radioimmunoassays utilizing highly purified viral proteins. Naturally occurring antiviral antibodies were demonstrated only in those strains possessing information for induction of a mouse cell-tropic endogenous virus. Moreover, these antibodies invariably appeared subsequent to the detection of spontaneous replication of this virus in the same animal. The immune responses elicited were much stronger against endogenous viral gp70 than p30, consistent with previous findings of tolerance in the mouse to the major structural antigen of its endogenous virus. However, the demonstration of an immune response to p30 under conditions of both natural and experimental immunization establishes that tolerance to this viral antigen can be overcome.

Information coding for type C RNA viruses exists within the DNA of mouse cells (1, 15). In some inbred mouse strains, endogenous viruses, infectious for mouse cells, are spontaneously activated and replicate in the animal. In such situations, there is an associated incidence of lymphoreticular neoplasia that is correlated with the age of onset and level of the endogenous virus expression (16, 21). These findings have led to questions as to whether the host immune system, either naturally or following experimental manipulation, might be involved in the control or prevention of tumors associated with these endogenous viruses.

Early studies, indicating the expression of structural antigens of endogenous viruses in embryonic mouse tissues, suggested the possibility that the mouse might be tolerant to its endogenous viruses or at least to some viral components (8, 9). More recently, the detection of immune complexes containing type C viral antigens in kidneys of the AKR strain (19), and of antibodies that precipitate intact mouse type C viruses (11, 18) in sera of several strains of mice, has suggested that the mouse possesses the ability to immunologically recognize certain viral antigens. Serological reactivity against the virus has primarily been measured by neutralization (9), or immunoprecipitation of radioactively labeled virus (11, 18). The development of radioimmunological techniques for purified type C viral structural polypeptides has made it possible to more precisely quantitate immunological reactivity against specific viral components. In the present report, the immune responses of different mouse strains to the major structural polypeptide, p30, and the envelope glycoprotein, gp70, of a representative mouse cell-tropic endogenous virus have been investigated both under natural conditions and after immunization.

MATERIALS AND METHODS

Mice. Inbred BALB/c Cr mice were obtained from the production colonies at Microbiological Associates, Walkersville, Md. Inbred C57BL/6, C57BL/10, DBA, and NIH Swiss mice were obtained from the National Institutes of Health, Bethesda, Md.

Cells. Cells were grown in Dulbecco modified Eagle medium supplemented with 10% calf serum (Colorado Serum Co., Denver, Colo.) in petri dishes (100 by 20 mm) (Falcon Plastics, Los Angeles, Calif.). A wild mouse embryo line, SC-1, which is highly sensitive to replication of both N- and B-tropic viruses (6), was provided by J. Hartley, National Institute of Allergy and Infectious Diseases.

Viruses. The Rauscher strain of murine leukemia virus (R-MuLV) and a woolly monkey type C virus were obtained as a twice-banded, 1,000-fold concentrates of tissue culture fluids from the Frederick Cancer Research Center, Frederick, Md. Sucrose gradient-purified AKR-murine leukemia virus (AKR-MuLV) and the Rickard strain of feline leukemia virus (FeLV) were obtained from Electro-Nucleonics, Inc., Bethesda, Md. Prototype class I and class II endogenous type C viruses of BALB/c mouse cells, including the N-tropic virus, BALB:virus-1 (23), the xenotropic virus, BALB:virus-2 (2) and a prototype

class III xenotropic virus of the NIH Swiss strain (24) have been described. Avian myeloblastosis virus (AMV), obtained as a 1,000-fold concentrate from serum, was provided by J. Beard, Life Sciences, Fla.

Inactivated virus preparations used as immunogens. Virus inactivation was performed as previously described (12). All immunizations described in this study were given subcutaneously in a three-injection schedule on days 0, 14, and 28, in doses of 200, 100, and 100 μg of viral proteins, respectively. The relative amounts of p30 and gp70 antigens as compared with total viral protein in each virus preparation were similar (R. L. Peters, R. M. Donahoe, B. Sass, M. Kende, and G. J. Kelloff, J. Natl. Cancer Inst., in press). Primary immunizations included emulsification of the antigen 1:1 with Freund complete adjuvant. In the second and third injections, the viral immunogen was diluted 1:1 with phosphate-buffered saline, pH 7.0. The humoral responses of immunized mice were evaluated by radioimmunological analysis of sera obtained 14 to 21 days after the third immunizing injection.

Assay for type C virus release by spleen cells. Spleen cell suspensions were prepared by mincing spleens and drawing cells sequentially through 18-, 21-, and 26-gauge hypodermic needles (Becton, Dickinson and Co., Rutherford, N.J.). A total of 5×10^7 nucleated spleen cells were co-cultivated with 10^5 SC-1 fibroblasts, pretreated for 18 h with 2 μg of polybrene per ml (22). After 3 weeks of incubation at 37°C, culture fluids were concentrated 100-fold by centrifugation and assayed for RNA-dependent DNA polymerases activity. Reaction mixtures containing 0.05 M Tris-hydrochloride, pH 7.8, 0.06 M potassium chloride, 0.002 M dithiothreitol, 0.2 mM manganese acetate, 0.02 absorbance units at 260 nm of poly(rA)·oligo(dT_{12-18}), 2×10^{-5} M [^{3}H]TTP (5,000 cpm/pmol), and 0.5% Triton X-100, in a volume of 0.05 ml, were incubated at 37°C for 60 min. DNA synthesis was measured as previously described (22).

Antisera. Antisera from goats immunized with detergent-disrupted viruses, as well as anti-goat immunoglobulin G and anti-mouse immunoglobulin G prepared in swine, were generously provided by R. Wilsnack through the Office of Resources and Logistics, National Cancer Institute.

Analysis of mouse sera for antibodies to type C viral polypeptides. The isolation of the major structural polypeptides (p30) of R-MuLV, AKR-MuLV, FeLV, AMV, and the woolly monkey virus, as well as the 70,000-molecular weight glycoproteins (gp70) of R-MuLV and AKR-MuLV, have been described in detail (7, 24, 26). Similar methods were used for the isolation and characterization of FeLV gp70. Viral polypeptides were radioactively labeled with ^{125}I (Amersham-Searle, Arlington Heights, Ill.) at specific activities of 5 to 20 μCi/μg by the chloramine T method of Greenwood et al. (5). Mouse serum and ^{125}I-labeled antigen were incubated for 3 h at 37°C and a further 18 h at 4°C in 0.2-ml reaction mixtures containing 0.01 M Tris-hydrochloride, pH 7.8, 0.001 M EDTA, 0.4% Triton X-100, 1% bovine serum albumin, 0.1% normal goat serum, and 0.01 M NaCl (p30) or 0.2 M NaCl (gp70). After addition of 0.05 ml of undiluted pig anti-mouse immunoglobulin G to each tube to precipitate antigen-antibody complexes, reaction mixtures were incubated 3 h at 4°C and centrifuged at 2,500 rpm for 15 min. Supernatants were aspirated, and the radioactivity in the precipitate was measured in a Searle 1285 gamma counter.

Radioimmunoassay for type C viral polypeptides. Competition radioimmunoassay for p30 and gp70 structural polypeptides of prototype endogenous type C viruses of mouse cells were performed as described previously (7, 24). Viral protein concentrations were determined by the method of Lowry et al. (17).

RESULTS

Development of natural precipitating antibodies to endogenous type C viral gp70 and p30 in BALB/c mice. BALB/c mouse cells contain information of at least three distinguishable endogenous viruses. The first is an inducible virus (class I), which is perferentially infectious for NIH Swiss mouse cells (1, 23). The second (class II) virus is xenotropic in host range and is also inducible both spontaneously and by chemicals (2; S. A. Aaronson and J. R. Stephenson, Biochim. Biophys. Acta Rev. Cancer, in press). Antigens of a third (class III) virus are expressed at high levels in the absence of virus release (26). Although infectious virus of this class has not been isolated from BALB/c mice, a xenotropic virus that contains antigens immunologically indistinguishable from those expressed by virus-negative BALB/c cells has been isolated from certain other mouse strains. Viruses obtained from a large number of other inbred strains have been shown to fit within this classification system (25).

In the present studies, attempts were made to detect naturally occurring antibodies directed against these endogenous viruses using ^{125}I-labeled gp70 or p30 of the class I virus as probes. These antigens are known to cross-react with the analogous proteins of each of the other endogenous virus classes (7, 25). As shown in Table 1, precipitating activity against class I viral gp70 was first demonstrated in sera of BALB/c animals at 6 months of age. By 15 to 22 months, sera from all BALB/c mice tested exhibited higher levels of reactivity with antibody titers ranging from 1:80 to 1:500. In contrast, none of the same sera demonstrated detectable reactivity (<1:10) against class I viral p30. The specificity of the reactivity against mouse endogenous viral gp70 was further indicated by the absence of detectable precipitating activity against ^{125}I-labeled FeLV gp70 or p30 (Table 1).

The kinetics of the appearance of anti-gp70 reactivity in BALB/c mice were compared with

TABLE 1. *Development of natural precipitating antibody to type C viral p30 and gp70 in BALB/c mice*

Age (mo)	Serum no.	Antisera titers for binding ^{125}I-labeled antigens of[a]: AKR-MuLV p30	AKR-MuLV gp70	FeLV p30	FeLV gp70
1	2312	<10	<10	<10	<10
	2313	<10	<10	<10	<10
	2314	<10	<10	<10	<10
	2315	<10	<10	<10	<10
	2316	<10	<10	<10	<10
6	2317	<10	20	<10	<10
	2318	<10	<10	<10	<10
	2319	<10	<10	<10	<10
	2320	<10	<10	<10	<10
	2321	<10	40	<10	<10
9	2322	<10	<10	<10	<10
	2323	<10	<10	<10	<10
	2324	<10	20	<10	<10
	2325	<10	20	<10	<10
	2326	<10	20	<10	<10
15	2327	<10	160	<10	<10
	2328	<10	320	<10	<10
	2329	<10	160	<10	<10
	2330	<10	80	<10	<10
	2331	<10	80	<10	<10
22	2332	<10	80	<10	<10
	2333	<10	320	<10	<10
	2334	<10	500	<10	<10
	2335	<10	320	<10	<10
	2336	<10	160	<10	<10

[a] Sera were assayed at serial twofold dilutions for ability to bind 10,000 cpm of ^{125}I-labeled viral antigen. Results are expressed as the reciprocal of the highest dilution at which 10% (1,000 cpm over a background of 50 cpm) of the appropriate ^{125}I-labeled antigen was precipitated and represent mean values from three separate determinations.

the time course of spontaneous type C virus expression by spleen cells of the same mice. Spleen cells from individual animals at different ages were co-cultivated with an equal number of SC-1 cells, a continuous mouse embryo line highly permissive for growth of ecotropic mouse type C viruses (6). The level of virion-associated reverse transcriptase activity in tissue culture fluids was assayed at 3 weeks as described in Materials and Methods. The amount of enzyme activity was shown in reconstruction experiments to be proportional to the virus input. Figure 1 illustrates that release of detectable levels of virus by spleen cells preceded the development of measurable antibody to viral gp70.

Naturally occurring immune responses against endogenous viral gp70 and p30 in additional mouse strains. The levels of naturally occurring anti-p30 and anti-gp70 were next compared in 15-month-old mice of several other strains. These included three strains which, like BALB/c, have previously been shown to contain information for class I virus as well as xenotropic (class II and III) viruses (25). In addition, the NIH Swiss strain, only known to contain information for class III xenotropic virus (25), was tested. At the time of serum collection, the level of class I virus in spleen cells of the same animals was also measured. As shown in Table 2, the serum titers against viral gp70 and p30 in C57BL/10 and DBA mice closely resembled those observed with BALB/c mice of the same age. Although reactivity against class I viral gp70 ranged from 1:20 to 1:320, there was no detectable anti-p30 reactivity in the same sera.

C57BL/6 mice generally exhibited much higher levels of natural antibody capable of precipitating viral gp70, with titers as high as 1:1,600. In addition, C57BL/6 mice demonstrated lower but readily detectable anti-p30 activity. In the one C57BL/6 animal that did not show serological reactivity against either viral polypeptide, attempts to detect virus in cultured spleen cells were unsuccessful (Table 2). It should be noted that despite much higher levels of precipitating antibody to viral gp70 in

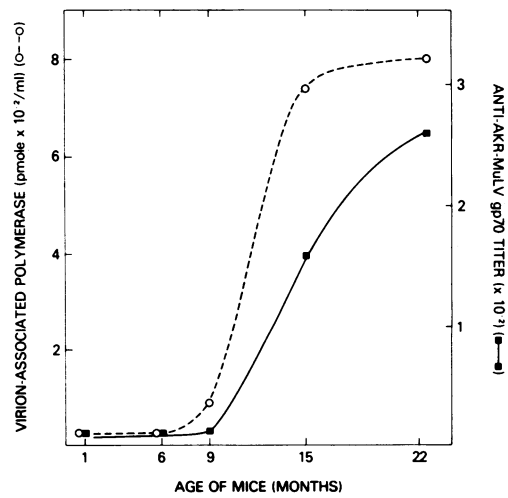

FIG. 1. *Comparison of the kinetics of appearance of antibody to AKR-MuLV gp70 in sera of BALB/c mice and the release of infectious type C virus by spleen cells of the same mice. Data were determined as described in Materials and Methods and are expressed as mean values from five individual mice per point.*

TABLE 2. *Analysis of sera of different mouse strains for natural antibody to type C viral p30 and gp70*

Strain	Serum no.	Antisera titers for binding ^{125}I-labeled antigens of[a]: AKR-MuLV p30	AKR-MuLV gp70	FeLV p30	FeLV gp70	Infectious virus associated with spleen cells[b]: expressed as virion-associated polymerase activity (pmol/ml)
C57BL/10	2103	<10	160	<10	<10	320
	2104	<10	80	<10	<10	80
	2105	<10	40	<10	<10	120
	2106	<10	320	<10	<10	40
	2107	<10	80	<10	<10	310
DBA	2180	<10	80	<10	<10	210
	2109	<10	40	<10	<10	190
	2110	<10	20	<10	<10	450
	2111	<10	160	<10	<10	340
	2112	<10	160	<10	<10	80
C57BL/6	2113	20	800	<10	<10	250
	2114	40	1000	<10	<10	40
	2115	<10	<10	<10	<10	<0.2
	2116	80	1200	<10	<10	350
	2117	40	1600	<10	<10	80
NIH Swiss	2118	<10	<10	<10	<10	<0.2
	2119	<10	<10	<10	<10	<0.2
	2110	<10	<10	<10	<10	<0.2
	2111	<10	<10	<10	<10	<0.2
	2112	<10	<10	<10	<10	<0.2

[a] Antibody titers, expressed as the reciprocal of the serum dilution required to bind 10% of the ^{125}I-labeled antigen, were determined as described in the footnote to Table 1.

[b] A total of 5×10^7 nucleated spleen cells were co-cultivated with the SC-1 assay cell line for 5 weeks, culture fluids were changed, and 24 h later, assayed for poly(rA)·oligo(dT)-directed poly(dT) synthesis. Results are expressed as picomoles of [^{3}H]TMP incorporated per milliliter and represent the mean values of two separate determinations.

sera of C57BL/6 mice than in the other strains, the amounts of infectious virus associated with spleen cells were similar. As further indicated in Table 2, sera from mice of the NIH Swiss strain failed to demonstrate antibody capable of precipitating either p30 or gp70. Moreover, attempts to isolate this virus from NIH Swiss spleen cells by co-cultivation with the SC-1 line, as in previous studies (22), were unsuccessful. These findings suggested that the natural immune response to type C viral p30 or gp70 in strains other than NIH Swiss was directed against class I virus.

Virus specificity of anti-gp70 reactivity in sera of normal BALB/c mice. The gp70's of three prototype endogenous mouse type C viruses can be differentiated in competition immunoassays using antisera prepared against each virus to precipitate the homologous viral ^{125}I-labeled gp70 (7). In such assays, only the gp70 of virus of the same class is able to fully compete in the homologous gp70 immunoassay (7). In an attempt to identify the virus to which the natural anti-gp70 reactivity of BALB/c sera was directed, a competition immunoassay was developed utilizing sera of 15-month-old BALB/c mice to precipitate class I viral gp70. The relative abilities of three prototype endogenous viruses to compete in this assay were compared. Viruses were first standardized on the basis of their reactivities in an immunoassay for the group-specific antigenic determinants of mouse type C virus gp70 (data not shown).

As shown in Fig. 2, class I virus competed 95 to 100% in this assay, whereas class II and III viruses were able to compete no more than 50% of the ^{125}I-labeled gp70, even at 10-fold higher concentrations. These findings indicate that sera of BALB/c mice recognize antigenic determinants specific to class I endogenous virus and argue that the natural immune response detectable as precipitating antibody to viral gp70 is directed against spontaneously activated class I endogenous virus.

Comparison of immune responses elicited in BALB/c mice after active immunization with type C virus isolates of different species. The above results indicated that natural im-

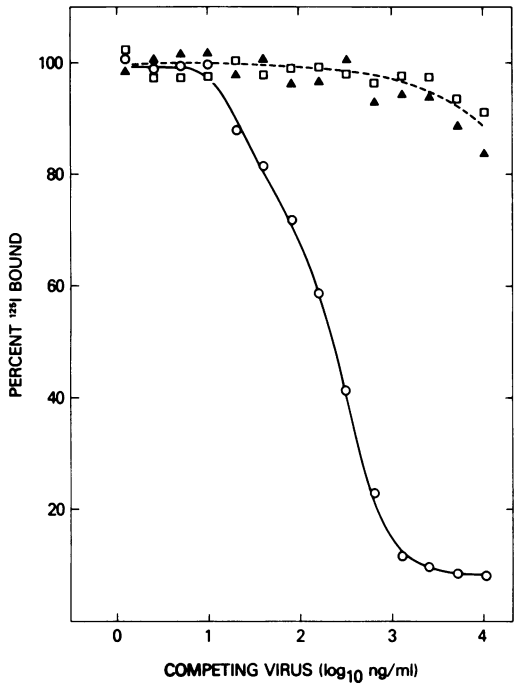

FIG. 2. *Comparison of immunological reactivities of prototype endogenous mouse type C viruses in competition immunoassay in which pooled sera from nonimmunized 15-month-old BALB/c mice were used to precipitate ^{125}I-labeled AKR-MuLV gp70. Viruses were disrupted by incubation at 37°C for 30 min in 1% Triton X-100, and viral antigens were assayed at serial twofold dilutions for their ability to compete with ^{125}I-labeled AKR-MuLV gp70 for binding limiting antibody. Competing viruses included the following: class I (BALB:virus-1) (○); class II (□); and class III (NIH Swiss virus) (▲).*

mune sera, obtained from mice of several strains, invariably precipitated viral gp70 at higher titer than p30. In contrast, antisera prepared by direct immunization of heterologous hosts with type C viruses have generally been found to possess higher titers against p30 (unpublished observations). The relatively poor immunological reactivity of mice against mouse type C viral p30 could reflect relative tolerance to this viral polypeptide (9) or, alternatively, the inadequate exposure of this antigen under conditions of natural immunization. To examine these possibilities, a comparison was made of the response of 3-month-old BALB/c mice to immunization with Formalin-inactivated and disrupted AKR-MuLV, a prototype class I virus, or type C viruses increasingly unrelated to AKR-MuLV, including R-MuLV, the woolly monkey virus, and AMV.

As shown in Table 3, the immune response to AKR-MuLV p30 was the weakest observed, with precipitating antibody titers only as high as 1:80. The strength of the immune response of BALB/c mice against viral p30 appeared to increase as the relatedness of the virus to the endogenous mouse type C virus diminished. For example, R-MuLV, a laboratory strain of mouse type C virus, whose p30 shares group-specific antigenic determinants with those of AKR-MuLV (24), elicited precipitating antibody to its p30 at titers of 1:320 and 1:640 in two different animals. There was relatively weak cross-reactivity against AKR-MuLV p30 (titer 1:80). The precipitating antibody titers against the homologous p30, after immunization with the woolly monkey virus or AMV, were 1:1,100 and 1:1,900, and 1:2,100 and 1:2,500, respectively. The specificity of each response was indicated by the lack of detectable cross-reactivity with p30's of the other viruses. These findings demonstrate that the BALB/c mouse can immunologically recognize the major structural polypeptide, p30, of one of its endogenous viruses, but indicate that the immunological response to this polypeptide is much weaker than that elicited by analogous polypeptides of type C viruses of other species.

Comparison of the immunological responses to p30 and gp70 after immunization of individual BALB/c and C57BL/6 mice with inactivated AKR- and R-MuLV. The responses of 3-month-old BALB/c and C57BL/6 mice to immuniation with Formalin-inactivated, disrupted AKR-MuLV or R-MuLV were investigated to further compare the immunity elicited by disrupted viruses with that acquired naturally in older animals of each strain. As shown in Table 4, BALB/c mice exposed to disrupted AKR-MuLV demonstrated low but significant titers (1:10 to 1:40) of precipitating antibody against AKR- and R-MuLV p30's. The same sera demonstrated higher titers, ranging from 1:40 to 1:320, against the two mouse type C viral gp70's. Immunization with R-MuLV led to much higher levels of precipitating antibodies directed against both R-MuLV p30 and gp70. In fact, there was as much cross-reacting precipitating antibody against AKR-MuLV polypeptides after immunization with R-MuLV as was elicited by immunization with AKR-MuLV. With R-MuLV, the immune response to gp70 was much stronger than against p30.

The responses of individual C57BL/6 mice to immunization with either AKR- or R-MuLV (Table 5) were generally five- to eightfold greater against both p30 and gp70 than observed with BALB/c mice. As was found with the BALB/c strain, the relative response to gp70 was much better than against p30. These results are consistent with the patterns of natu-

TABLE 3. *Analysis of the ability of BALB/c mice to immunologically respond to type C viral p30 antigens*

Sera tested	Serum no.	Antiserum titer for binding p30 antigen of[a]:			
		AKR-MuLV	R-MuLV	Woolly monkey virus	AMV
Immunized with					
AKR-MuLV[b]	1010	80	20	<10	<10
	1011	40	40	<10	<10
R-MuLV	1012	80	320	<10	<10
	1013	80	640	<10	<10
Woolly monkey virus	1014	<10	<10	1,600	<10
	1015	<10	<10	1,900	<10
AMV	1016	<10	<10	<10	2,500
	1017	<10	<10	<10	2,100
Nonimmunized controls	1018	<10	<10	<10	<10
	1019	<10	<10	<10	<10

[a] Antisera titers, expressed as the reciprocal of the serum dilution required to bind 10% of the ^{125}I-labeled antigen, were determined as described in Table 1.

[b] Formalin-inactivated, Tween-ether-disrupted virus was prepared as previously described (R. L. Peters, R. M. Donahoe, B. Sass, M. Kende, and G. J. Kelloff, J. Natl. Cancer Inst., in press). Subcutaneous immunizations were given in a three-injection schedule to 3-month-old mice on days 0, 14, and 28 in doses of 200, 100, and 100 μg of viral protein, respectively. All primary immunizations involved emulsification of the viral immunogen with Freund complete adjuvant. The viral immunogen was diluted 1:1 in saline for the second and third injections. The humoral responses of the immunized mice were evaluated by radioimmunoassays of sera obtained 14 to 21 days following the final immunization.

TABLE 4. *Analysis of sera of immunized BALB/c mice for precipitation of AKR- and R-MuLV p30 and gp70*

Mice	Serum no.	Antiserum titer for binding[a]:			
		AKR-MuLV		R-MuLV	
		p30	gp70	p30	gp70
Immunized with[b]					
AKR-MuLV	2389	10	80	20	80
	2390	20	160	20	40
	2391	10	160	40	320
	2392	20	40	10	80
	2393	40	80	10	160
	2394	40	160	20	80
R-MuLV	2404	20	40	40	320
	2405	20	160	160	640
	2407	40	80	80	4,000
	2408	40	80	160	8,000
	2409	20	40	10	1,280
	2411	10	320	80	640
Nonimmunized	2379	<10	<10	<10	<10
	2381	<10	<10	<10	<10
	2382	<10	<10	<10	<10

[a] Antisera titers, expressed as the reciprocal of the serum dilution required to bind 10% of the ^{125}I-labeled antigen, were determined as described in Table 1.

[b] Immunogen preparation and immunization was as described in Table 3.

ral immunity to p30 and gp70 observed with C57BL/6 and BALB/c mice (Tables 1 and 2) and argue against the possibility that the much higher levels of anti-gp70 reactivity under conditions of natural immunization reflect a lack of exposure to p30.

Expression of p30 and gp70 in BALB/c mouse cells during embryogenesis. The above-described preferential immunological response to gp70 as compared with p30 suggested that mice possess a greater degree of immunological tolerance to endogenous viral p30 than gp70. One possible reason for this might be a much higher level of expression of p30 relative to gp70 during embryogenesis. To test this possibility, cell extracts of embryos at different ages, as well as extracts of spleen, liver, and thymus of 3-month-old mice, were analyzed for p30 and gp70 expression by competition immunoassay. The results, summarized in Table 6, indicate that, whereas the levels of p30 and gp70 expression were about twofold higher in embryo than in adult tissues, the relative levels of the two polypeptides were similar. Confirming previous reports (7), the type-specific antigenic determinants of gp70's partially purified from both embryonic and adult BALB/c tissues were indistinguishable from those of class III endogenous virus (data not shown).

DISCUSSION

Evidence that mice and chickens are immunologically tolerant to the major internal structural components of their endogenous type C

TABLE 5: *Analysis of sera of immunized C57BL/6 mice for precipitation of AKR- and R-MuLV p30 and gp70*

Mice	Serum no.	Antiserum titer for binding[a]: AKR-MuLV p30	AKR-MuLV gp70	R-MuLV p30	R-MuLV gp70
Immunized with[b]					
AKR-MuLV	2809	20	160	40	640
	2810	40	320	80	640
	2811	40	640	80	160
	2812	80	320	40	320
	2813	20	80	40	320
	2814	40	640	80	640
R-MuLV	2803	40	320	80	8,000
	2804	80	640	160	16,000
	2805	80	80	80	4,000
	2806	160	1,280	320	32,000
	2807	40	640	80	8,000
	2808	80	1,280	160	2,500
Nonimmunized	2790	<10	<10	<10	<10
	2791	<10	<10	<10	<10
	2792	<10	<10	<10	<10

[a] Antisera titers, expressed as the reciprocal of the serum dilution required to bind 10% of the ^{125}I-labeled antigen, were determined as described in Table 1.

[b] Immunogen preparation and immunization was as described in Table 3.

TABLE 6. *Expression of type C RNA viral p30 and gp70 in BALB/c mouse tissues during embryogenesis*[a]

Tissue extracts of:	Viral polypeptide concn (ng/mg cell protein) p30	gp70
Mouse embryos		
14 day	60	40
16 day	40	30
19 day	50	30
Adult tissues		
Spleen	20	15
Liver	15	10
Thymus	20	20

[a] Tissue extracts, prepared as previously described (7, 25) were tested in group-specific immunoassays for MuLV p30 and gp70 utilizing antiserum to AKR-MuLV to precipitate ^{125}I-labeled R-MuLV viral p30 and gp70, respectively. Results are expressed as nanograms of viral antigen per milligram of total cell protein and represent mean values of three separate determinations. Protein concentrations were determined by the method of Lowry et al. (17).

viruses (9) derives, first, from information that viral antigens are expressed during embryogenesis (4, 8). In addition, early studies indicated that immunization with the homologous virus elicited neutralizing antibodies against the envelope antigen of the virus in the absence of any detectable immune response to the internal structural components (9, 13). In contrast, immunization with a heterologous type C virus resulted in high-titered antibody to the major internal antigen of that virus (9). Thus, it was reasoned that the natural host was specifically tolerant to the major internal component of its endogenous virus, presumably due to exposure to it during embryonic development (8, 9).

More recent evidence has indicated that mice can naturally mount an immune response to certain endogenous viral antigens. Oldstone et al. (19) showed that antibodies to viral antigens were present in kidneys of AKR mice in the form of antigen-antibody complexes. Subsequently, by measuring immunoprecipitation of radioactively-labeled intact MuLV, antibodies to three major structural polypeptides, possessing molecular weights of 68,000, 43,000, and 17,000, respectively, were detected in sera of mice of many inbred strains (11). In addition, Ihle et al. (10) have recently reported antibody to ^{125}I-labeled AKR-MuLV gp70 in several inbred strains of mice. Mouse sera also contain a high-titered neutralizing activity directed against xenotropic endogenous viruses (1), although there is evidence indicating that this reactivity may not be a known immunoglobulin (14). In general, there has been little, if any, evidence to indicate that natural immunity to endogenous viruses is directed against the major viral structural component, p30.

Investigation of immunity to endogenous viruses in the mouse system has taken on increasing complexity with the demonstration that several partially related endogenous viruses exist within mouse cells (25). Moreover, each different structural component of these viruses possesses a range of antigenic determinants that varies in the degree of cross-reactivity with the analogous antigens of other endogenous viruses (7). For example, p30 is a very broadly reactive antigen, whereas gp70 contains highly type-specific antigenic determinants (7, 28). These findings are of importance in interpreting the present studies as well as results of previous investigations.

The appearance of precipitating antibodies to purified viral structural antigens in the strains examined here correlated with the presence in vivo of infectious virus of one endogenous virus class. In a strain, NIH Swiss, that lacks information for induction of class I virus (23, 25), there was no evidence of antibody to either p30 or gp70. In contrast, individual mice of BALB/c and C57BL/6 strains, which are known to contain this virus (25), demonstrated immune responses to viral antigens only after the appearance of detectable class I endogenous virus in

their spleens. Competition immunoassays made it possible to show that BALB/c sera contained antibodies cross-reactive specifically with antigenic determinants of class I viral gp70. These findings all imply that the naturally occurring precipitating antibody to gp70 was directed against endogenous virus of this class.

In confirmation of previous reports (9, 13), active immunization of mice with heterologous type C viruses led to much stronger immune responses against the respective major structural antigens of these viruses than was observed against mouse viral p30 in response to immunization with a prototype mouse endogenous virus. Moreover, whenever an immune response to mouse endogenous virus was elicited, the level of immunity was invariably much stronger against gp70 than against p30. These findings are consistent with the concept that the mouse is relatively tolerant to its endogenous viral p30. The present findings that antibody to endogenous viral p30 could be elicited not only experimentally but even under conditions of natural exposure establish that tolerance to the major structural antigen of the endogenous virus can be overcome.

The explanation for the much stronger immune response to endogenous viral gp70 than p30 in animals whose embryonic tissues were shown to express gp70 at levels comparable to those of p30 may not be readily apparent. It should be noted in this regard that the gp70 expressed by such tissues, as shown here and in previous studies (7), was primarily that of a class III rather than a class I endogenous virus. Thus, the host may be able to recognize, as foreign, antigenic determinants specific to class I viral gp70, which are not normally expressed during embryogenesis. A more direct examination of this question will require quantitative comparisons of the levels of immunity that can be elicited directly in response to the gp70's of each endogenous virus class.

The present studies indicate that R-MuLV, a type C virus that has diverged antigenically from known endogenous mouse viruses (7, 24), was able to elicit as strong an immune response against class I viral antigens as did class I endogenous virus itself. The use of partially related viruses as immunogens may thus provide an alternative approach in efforts aimed at breaking tolerance to those viral antigens to which the host is not naturally responsive. Whether such experimental manipulation can reduce the incidence of natural tumors in inbred mouse strains remains to be determined, as does the role of natural immunity to endogenous viral antigens in disease.

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LITERATURE CITED

1. **Aaronson, S. A., J. W. Hartley, and G. J. Todaro.** 1969. Mouse leukemia virus: spontaneous release by mouse embryo cells after long-term *in vitro* cultivation. Proc. Natl. Acad. Sci. U.S.A. 64:87-93.
2. **Aaronson, S. A., and J. R. Stephenson.** 1973. Independent segregation of loci for activation of biologically distinguishable RNA C-type viruses in mouse cells. Proc. Natl. Acad. Sci. U.S.A. 70:2055-2058.
3. **Aaronson, S. A., and J. R. Stephenson.** 1974. Widespread natural occurrence of high-titered neutralizing antibodies to a specific class of endogenous mouse C-type virus. Proc. Natl. Acad. Sci. U.S.A. 71:1957-1961.
4. **Dougherty, R. M., H. S. Di Stefano, and F. K. Roth.** 1967. Virus particles and viral antigens in chicken tissues free of infectious avian leukosis virus. Proc. Natl. Acad. Sci. U.S.A. 58:808-816.
5. **Greenwood, F. C., W. M. Hunter, and J. S. Glover.** 1963. The preparation of ^{131}I-labelled human growth hormone of high specific activity. Biochem. J. 89:114-123.
6. **Hartley, J. W., and W. P. Rowe.** 1975. Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia viruses. Virology 65:128-134.
7. **Hino, S., J. R. Stephenson, and S. A. Aaronson.** 1976. Radioimmunoassays for the 70,000 molecular weight glycoproteins of endogenous type-C viruses: viral antigen expression in normal mouse tissues and sera. J. Virol. 18:933-941.
8. **Huebner, R. J., G. J. Kelloff, P. S. Sarma, W. T. Lane, H. C. Turner, R. V. Gilden, S. Oroszlan, H. Meier, D. D. Myers, and R. L. Peters.** 1970. Group-specific antigenic expression during embryogenesis of the genome of the C-type RNA virus: implications for ontogenesis and oncogenesis. Proc. Natl. Acad. Sci. U.S.A. 67:366-376.
9. **Huebner, R. J., P. S. Sarma, G. J. Kelloff, R. V. Gilden, H. Meier, D. D. Myers, and R. L. Peters.** 1971. Immunological tolerance to RNA tumor virus genome expressions: significance of tolerance and prenatal expressions in embryogenesis and tumorigenesis. Ann. N.Y. Acad. Sci. 181:246-271.
10. **Ihle, J. N., J. J. Domotor, and K. M. Bengali.** 1976. Characterization of the type and group specificities of the immune response in mice to murine leukemia viruses. J. Virol. 18:124-131.
11. **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. 139:1568-1581.
12. **Kelloff, G. J., R. L. Peters, R. M. Donahoe, I. Ghazzauli, B. Sass, R. M. Nims, and R. J. Huebner.** 1976. An approach to C-type virus immunoprevention of spontaneously occurring tumors in laboratory mice. Cancer Res. 36:622-630.
13. **Kelloff, G., and P. Vogt.** 1966. Localization of avian tumor virus group-specific antigen in cell and virus. Virology 29:377-384.
14. **Levy, J. A., J. N. Ihle, O. Oleszko, and R. D. Barnes.** 1975. Virus-specific neutralization by a soluble nonimmunoglobulin factor found naturally in normal mouse sera. Proc. Natl. Acad. Sci. U.S.A. 72:5071-5075.
15. **Lieber, M. M., and G. J. Todaro.** 1975. Mammalian type-C RNA viruses, p. 91-130. *In* F. F. Becker (ed.),

Cancer: a comprehensive treatise, vol. 2. Plenum Press, New York.
16. **Lilly, F., M. L. Duran-Reynals, and W. P. Rowe.** 1975. Correlation of early murine leukemia virus titer and H-2 type with spontaneous leukemia in mice of the BALB/c X AKR cross: a genetic analysis. J. Exp. Med. 141:882-889.
17. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:266-275.
18. **Nowinski, R. C., and S. L. Kaehler.** 1974. Antibody to leukemia virus: widespread occurrence in inbred mice. Science 185:869-871.
19. **Oldstone, M. B. A., T. Aoki, and F. Dixon.** 1972. The antibody response of mice to murine leukemia virus in spontaneous infection: absence of classical immunologic tolerance. Proc. Natl. Acad. Sci. U.S.A. 69:134-138.
20. **Peters, R. L., R. M. Donahoe, and G. J. Kelloff.** 1975. Assay in the mouse for delayed type hyper-sensitivity to murine leukemia virus. J. Natl. Cancer Inst. 55:1089-1095.
21. **Peters, R. L., G. J. Spahn, L. S. Rabstein, G. J. Kelloff, and R. J. Huebner.** 1973. Oncogenic potential of murine C-type RNA virus passaged directly from naturally occurring tumors of the BALB/c Cr mouse. J. Natl. Cancer Inst. 51:621-630.
22. **Stephenson, J. R., and S. A. Aaronson.** 1976. Induction of an endogenous B-tropic type-C RNA virus from SWR/J embryo cells in tissue culture. Virology 70:352-359.
23. **Stephenson, J. R., and S. A. Aaronson.** 1972. A genetic locus for inducibility of C-type virus in BALB/c cells: the effect of a nonlinked regulatory gene on detection of virus after chemical activation. Proc. Natl. Acad. Sci. U.S.A. 69:2798-2801.
24. **Stephenson, J. R., S. A. Aaronson, P. Arnstein, R. J. Huebner, and S. R. Tronick.** 1974. Demonstration of two immunologically distinct xenotropic RNA type-C viruses of mouse cells. Virology 61:244-248.
25. **Stephenson, J. R., R. K. Reynolds, S. R. Tronick, and S. A. Aaronson.** 1975. Distribution of three classes of endogenous type-C RNA viruses among inbred strains of mice. Virology 67:404-414.
26. **Stephenson, J. R., S. R. Tronick, and S. A. Aaronson.** 1974. Isolation from BALB/c mouse cells of a structural polypeptide of a third endogenous type-C virus. Cell 3:347-353.
27. **Stephenson, J. R., R. Wilsnack, and S. A. Aaronson.** 1973. Radioimmunoassay for avian C-type virus group-specific antigen: detection in normal and virus-transformed cells. J. Virol. 11:893-899.
28. **Strand, M., and J. T. August.** 1974. Structural proteins of mammalian oncogenic RNA viruses: multiple antigenic determinants of the major internal protein and envelope glycoprotein. J. Virol. 13:171-180.