RADIATION LEUKEMIA IN C57BL/6 MICE

I. Lack of Serological Evidence for the Role of Endogenous Ecotropic Viruses in Pathogenesis*

By JAMES N. IHLE, ROBERT McEWAN AND KATHLEEN BENGALI

(From the Basic Research Program, National Cancer Institute Frederick Cancer Research Center, Frederick, Maryland 21701)

C-type viruses are vertically transmitted, endogenous viruses of mice and the ecotropic viruses have been etiologically associated with spontaneous leukemia in AKR mice (1–4) and radiation-induced leukemias in a variety of strains of mice (5–7).

The genetics of the expression of murine leukemia viruses (MuLV)[1] in AKR mice has been extensively analyzed (8, 9), and the influence of this expression on the development of spontaneous lymphomas has been examined (10). The latter studies are particularly significant since they demonstrated that, in AKR mice genetically crossed with strains expressing low levels of virus, there is a direct correlation between the level of infectious MuLV and the incidence of lymphoma. Interestingly, however, such lymphomas are only induced by extremely high levels of infectious virus that persist throughout the life span of the mice.

Whole-body irradiation of a variety of inbred strains of mice has been shown to induce thymic lymphomas with the pathological characteristics of tumors that spontaneously arise in AKR mice (7, 11). In contrast to lymphomas in AKR mice, these lymphomas are not generally associated with high levels of infectious MuLV, and cell-free extracts from the primary lymphomas are only weakly leukemogenic with a long latency period (5, 6, 12). Continued passage of lymphoma extracts in vivo greatly enhances leukemogenic activity and decreases the latency period. From this type of propagation the radiation leukemia virus (RADLV) has been obtained and is extremely leukemogenic (7). RADLV grown in vitro is serologically cross-reactive with Gross or AKR MuLV and differs only by its Fv-1 tropism, since it is a B-tropic virus (7, 13, 14), although N-tropic viruses can also be isolated from Fv-1^b C57BL/6 mice. Although the expression of viral antigens in RADLV-induced lymphomas can be readily detected with indirect immunofluorescence, virus expression in most radiation-induced lymphomas is not detectable by this technique (15, 16). The presence of RADLV in these thymomas, however, is suggested by a variety of indirect experimental approaches (7), including the ability to obtain a B-tropic virus after continued blind passage of tumor extracts in vitro. This led to the hypothesis that primary tumors in irradiated C57BL/Ka mice are induced by the activation of a latent endogenous, ecotropic MuLV, which replicates in the tumor but at relatively low levels (7, 16).

In previous studies we analyzed the humoral immune response against endogenous

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[1] *Abbreviations used in this paper:* MuLV, murine leukemia virus; RADLV, radiation leukemia virus(es); RIP, radioimmune precipitation.

ecotropic viruses in a variety of inbred strains of mice (17–21). In general, this immune response is characterized by antibodies directed against the virion envelope antigens gp71, gp43, and p15(E) (18). The predominant natural antibody titer of sera from most inbred strains is directed against gp71 (20) and is primarily type specific for the endogenous ecotropic viruses (19, 20). As a result of the humoral immune response against gp71 most autogenous immune sera are cytotoxic against a variety of tumors replicating MuLV and can neutralize MuLV,[2] although the latter activity can only be demonstrated in vitro with rapidly harvested, unfrozen virus preparations. The antibody titer against p15(E) is generally less than the titer against gp71 and is predominantly group specific. This reaction is the basis of the cross-reactivity of autogenous immune sera with the Friend-Moloney-Rauscher group of viruses (19). The consequences of antibody against p15(E) are not known, but in general this antibody response is not associated with either neutralization or cytotoxicity.[3] The antibody titer against gp43 has been shown to be relatively low (18) and the consequences of this recognition are presently unknown.

Although the immunological properties described above suggest the potential significance of a humoral immune response in regulating virus-mediated pathogenesis, the presence of antibody also provides a convenient assay for the expression of endogenous MuLV. In particular, recent studies have demonstrated a good correlation between the presence of antibodies and the expression of infectious ecotropic MuLV in vivo (22–24). We have found that the antibody-positive phenotype segregates with the viral genome in several crosses of ecotropic virus-positive strains with strains that genetically lack this virus. The sequential sampling of individual mice for antibody can also provide information about the strain-dependent phenotypes for expression of ecotropic viruses (24). Because of the above considerations, we examined the humoral immune response in irradiated C57BL/6 mice to assess the role of irradiation in activating endogenous ecotropic viruses and to determine the influence of this immune response on the development of thymic lymphomas. The results presented here demonstrate that irradiation consistently accelerates the appearance of an immune response to the endogenous ecotropic virus but that this immune response, under various conditions, has no influence on nor correlation with the development of leukemia.

Materials and Methods

Animals. Male C57BL/6 mice were used in these studies. All mice were specific pathogen free and were obtained from the central animal facility of the Frederick Cancer Research Center, Frederick, Md.

Viruses. Friend MuLV was obtained from the Eveline cell line, which was derived from the STU mouse strain (25). AKR MuLV was isolated from an established line of AKR mouse embryo cells that spontaneously initiated virus synthesis. C57L xenotropic virus was obtained from D17a dog sarcoma cells infected with a xenotropic virus from an X-ray-induced lymphoma of a C57L mouse. RADLV was isolated from C57BL/Ka fibroblast cultures infected with in vivo derived RADLV. These cultures were at passage 11 after infection when used and were kindly provided by Dr. A. DeCleve, Stanford University, Stanford, Calif. All viruses were purified by velocity and equilibrium density gradient centrifugation, as previously described (17, 18). Radioactive virus ([^{3}H]leucine) was prepared as previously described (17, 18).

Radioimmune Precipitation Assays. Double-antibody radioimmune precipitation (RIP) assays for antibodies against gp71 and the preparation of AKR MuLV and Rauscher MuLV gp71 have

[2] Ihle, J. N., and B. Lazar. Manuscript in preparation.

[3] Ihle, J. N., K. Bengali, and J. Domotor, Jr. Manuscript in preparation.

been previously described (20). The RIP assay against intact radioactively labeled AKR virus was performed as previously described (17) and competition RIP assays with intact virus were comparable to those previously reported (19).

Radiation. Mice were irradiated with the doses and at the ages indicated in the Results section. In general, 4-wk-old C57BL/6 mice were irradiated four times at 175 R at weekly intervals. The source was a Phillips MG 301 X-ray therapy unit (Phillips Electronic Instruments, Mount Vernon, N. Y.) with a 0.2 mm^3 filter operated at 10 mA and 300 kV.

Bone Marrow Reconstitution. The day after the last irradiation, the mice were given 1×10^7 bone marrow cells from 4-wk-old C57BL/6 mice by intravenous injection. C57BL/6 mice, 8-wk old, which had received no irradiation were also given bone marrow cells and served as controls.

Results

The development of antibody to MuLV in C57BL/6 mice after irradiation is shown in Fig. 1. In these experiments, 4-wk-old C57BL/6 mice were irradiated four times at weekly intervals with 175 R. Serum samples were collected from individual, ear-marked mice at monthly intervals starting at 1 mo of age and were assayed for the presence of antibodies to MuLV in a RIP assay. The results are plotted as the percentage of mice that developed detectable antibodies against MuLV. The immune response to MuLV in control, nonirradiated C57BL/6 mice developed slowly and by 6 mo of age approximately 50% of the mice had developed detectable antibodies. These results are comparable to those previously reported for C57BL/6 mice and are different from the serological patterns observed with other inbred strains of mice (24). The appearance of antibodies to MuLV significantly increased in irradiated C57BL/6 mice and by 2–3 mo after irradiation approximately 90% of the mice had developed detectable antibodies to MuLV. These results agree with previous results, which suggested that radiation activates latent endogenous MuLV (5–7). In our experiments this activation was manifested by the development of an immune response to the virus.

To rule out the possibility that irradiation might have suppressed the extent of the humoral immune response in irradiated mice, we examined the distribution of titers of sera from control and irradiated mice, which had detectable antibody for three consecutive months. This time point was chosen because it reflects fully developed immune capacities and in irradiated mice corresponds to the time of tumor development. The titers of immune sera from irradiated and control mice were comparable and were generally 1:1,280–2,560 (Fig. 2). Therefore, irradiation enhanced the rate of appearance of antibody against MuLV, but did not affect the peak titers.

Previous studies have shown that the immune response to MuLV has both type-specific and group-specific immunological recognition of MuLV (19). In order to establish the serological characteristics in irradiated C57BL/6 mice, we examined the antibody specificity by competition assays. The results of typical virus competition assays are shown in Fig. 3. In these assays, sera from irradiated C57BL/6 mice were reacted at limiting dilutions with labeled AKR MuLV, and the ability of in vitro passaged RADLV (a B-tropic C57BL/Ka virus), an AKR N-tropic virus, a xenotropic virus from C57L mice, and Friend MuLV to compete was examined. Both the in vitro passaged RADLV and the AKR N-tropic virus competed completely and equivalently, suggesting that the major antigenic determinants involved in the immunologic recognition of these viruses

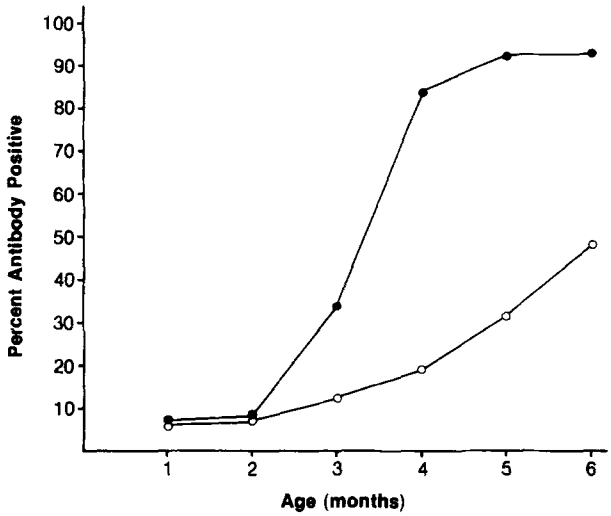

FIG. 1. Development of antibody against MuLV in control and irradiated C57BL/6 mice. Groups of 50 C57BL/6 mice at 1 mo of age were either irradiated with 175 R four times at weekly intervals, (●—●); or served as unirradiated controls, (○—○). Beginning at 1 mo of age and subsequently at monthly intervals serum samples were collected from individual mice by tail bleeding and were tested in a RIP assay at a 1:40 serum dilution for antibodies against MuLV. The results are plotted as the percentage of mice having developed detectable antibodies against MuLV vs. age.

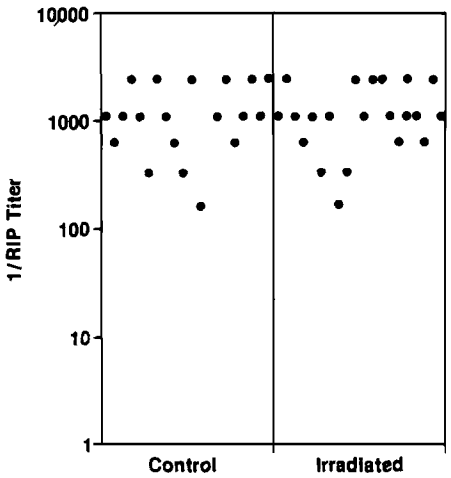

FIG. 2. Antibody titer of control and irradiated C57BL/6 mice against MuLV. Sera from individual control C57BL/6 mice and mice irradiated at 1 mo with 175 R four times at weekly intervals, which had been positive for antibodies against MuLV for 3 mo, were titered in a RIP assay, as previously described (17, 18). The titer of a serum is that dilution giving 50% of maximal precipitation of labeled virus.

by C57BL/6 mouse sera were identical. In contrast, neither the xenotropic C57L virus nor Friend MuLV could compete in this assay, demonstrating that the humoral immune response detectable in RIP was specifically directed against the endogenous ecotropic virus. These results agree with previous results using

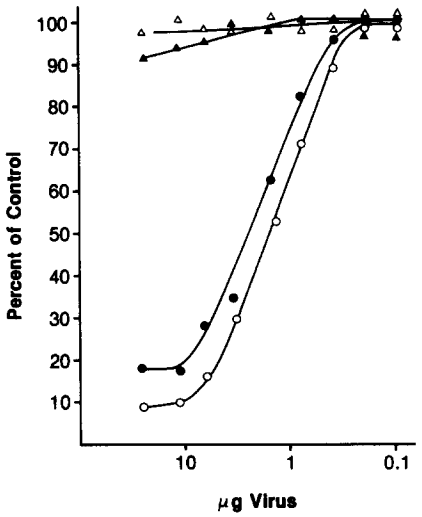

FIG. 3. Competition RIP assays of a serum from an irradiated C57BL/6 mouse. Serum from an irradiated C57BL/6 mouse was reacted with [^{3}H]leucine-labeled AKR MuLV at a dilution of 1:200 in the presence of decreasing concentrations of AKR MuLV, (○—○); in vitro pasaged RADLV, (●—●); C57L xenotropic virus, (△—△); and Friend MuLV, (▲—▲), as previously described (19). Control precipitation in the absence of competing antigen was approximately 70%. Comparable curves were obtained with various additional individual sera.

sera from irradiated C57BL/6 mice (26) and are also comparable to the results obtained with sera from nonirradiated control C57BL/6 mice (19).

The predominant immunological reaction obtained with most autogenous immune sera is against the ecotropic viral gp71 and is type specific (19, 20). Since the glycoprotein of RADLV appears completely homologous to AKR viral glycoprotein (accompanying paper), we examined sera from irradiated C57BL/6 mice for the presence of antibodies capable of reacting with AKR MuLV gp71. As demonstrated in Table I, immune sera from irradiated C57BL/6 mice had demonstrable antibodies capable of precipitating AKR MuLV gp71. In contrast, none of these sera had detectable reactivity against Rauscher MuLV gp71 (data not shown). Moreover, there was a good correlation between positivity in the RIP assay and the presence of antibody capable of reacting with AKR MuLV gp71. These results are similar to those previously reported (14, 26) and further suggest that the immune response detectable in irradiated C57BL/6 mice is specifically directed against the endogenous ecotropic virus.

The correlation between the development of a humoral immune response and lymphoma is shown in Tables II and III. In Table II the representative serological histories of individual mice that died from lymphoma are tabulated. In general, three distinct types of immunological patterns were observed during the course of irradiation and tumor development: (*a*) One group of mice showed no detectable immune response throughout the course of the experiment and later died; (*b*) a second group transiently developed a humoral immune response, but later died without any detectable antibodies; (*c*) most mice, however, developed a persistent humoral immune response and later died with detectable

TABLE I

Comparison of Titers of Sera from Irradiated C57BL/6 Mice against Labeled AKR MuLV and AKR MuLV gp71

Serum no.	Titer*	
	AKR MuLV	AKR MuLV gp71
1	0	<1:10
2	0	<1:10
3	1:640	1:20
4	1:640	1:20
5	1:640	1:40
6	1:1,280	1:40
7	1:1,280	1:80
8	1:1,280	1:80

* Sera were titered as described in Materials and Methods. Serum titers against AKR MuLV are defined as the serum dilution precipitating 50% of the labeled virus. Serum titers against AKR MuLV gp71 are defined as the serum dilution precipitating 25% of the maximum antigen precipitated with a xenogenic antiserum against AKR MuLV gp71.

TABLE II

*Anti-Viral Antibody Distribution During the Life Span of Individual Irradiated C57BL/6 Mice that Died from Lymphomas**

Mouse no.	Antibody titer‡						
	Age (mo)						
	1	2	3	4	5	6	Titer at death
1	0	0	0	0	0	0	0
2	<1:40	0	0	0	0		0
3	0	0	0	0	>1:40	0	0
4	0	0	0	<1:40	>1:40	<1:40	0
5	0	0	<1:40	<1:40	0		0
6	<1:40	>1:40	0	0	0	0	0
7	0	0	0	0	<1:40		1:160
8	0	0	0	0	<1:40	>1:40	1:160
9	<1:40	0	<1:40	<1:40	>1:40	<1:40	1:320
10	0	0	<1:40	>1:40	>1:40		1:1,280
11	0	0	0	<1:40	>1:40	>1:40	1:2,560
12	0	0	0	<1:40	<1:40		1:2,560
13	<1:40	0	<1:40	0	>1:40	>1:40	1:1,280
14	0	<1:40	<1:40	0	0	>1:40	1:1,280
15	0	0	0	0	0	>1:40	1:640

* C57BL/6 mice were tail bled at monthly intervals and serum collected for RIP assays. To induce leukemia, mice were irradiated with 175 R four times at weekly intervals starting at 1 mo of age. Death from thymomas occurred during the 5th and 6th mo of age and sera were collected from moribund mice for RIP assays.

‡ Relative antibody titers against MuLV were determined by RIP assays at a 1:40 serum dilution. Sera precipitating greater than 50% of the labeled virus were considered to have titers >1:40, whereas sera precipitating less than 50% of the virus but more than 10% above background were considered to have titers of <1:40.

TABLE III
*Incidence of Radiation-Induced Lymphomas Among C57BL/6 Mice of Different Antibody Phenotypes**

Antibody phenotype	Total, no./total	Lymphoma, no./total	No lymphoma, no./total	Lymphoma incidence
				%
No antibody	4/49	3/32	1/17	75
Transient antibody	9/49	6/32	3/17	66.7
Persistent antibody	36/49	23/32	13/17	63.9
Combined	49/49	32/49	17/49	65.3

* C57BL/6 mice were irradiated with 175 R four times at weekly intervals beginning at 1 mo of age. All mice were individually tested for antibodies against MuLV at monthly intervals starting at 1 mo of age. Tumor-bearing mice were those that died of thymomas 5 mo after the last irradiation, whereas tumor-free mice were those surviving 5 mo after the last irradiation. Antibody phenotypes were: no antibody, lack of detectable antibody during the entire experimental period; transient antibody, detectable antibody only occasionally during the course of the experiment; and persistent antibody, antibody detectable consistently from the time of its first appearance (see Table I). Antibody positivity was determined by RIP assays at a 1:40 serum dilution. Sera precipitating virus above background levels were considered positive.

levels of antibody to MuLV. The distribution of these types of immune responses in mice dying of lymphomas and mice living at least 5 mo postirradiation is summarized in Table III. Only 4/49 mice never developed any detectable antibody throughout the period of observation; nevertheless, within this group 3/4 mice developed lymphomas and died. A larger group of mice (9/49) developed detectable antibody transiently during the period of observation, and the frequency of tumors in such mice was approximately 67%. The majority (36/49) of mice developed a persistent immune response to the virus and, of these 36, approximately 64% developed tumors. Of the mice with thymomas, approximately 28% were antibody negative and 72% were antibody positive at death. These results suggest that there is little or no correlation between the development and maintenance of an immune response to the virus and the occurrence of lymphoma.

Kaplan (27) has demonstrated an age-associated incidence of lymphomas in irradiated C57BL/6 mice. We, therefore, examined the correlation of age and the development of a humoral immune response. As illustrated in Fig. 4, there were no apparent differences in either the rate of development of an immune response in mice irradiated at 4, 8, 12, or 16 wk of age or in the range of titers of anti-viral antibodies among the various age groups (data not shown). In contrast, age profoundly influenced the incidence of irradiation-induced lymphomas (Table IV). At 6 mo postirradiation, the incidence of thymomas was 75, 53, 25, and 20% for the 4-, 8-, 12-, and 16-wk-old groups, respectively. Although these results are comparable to those initially reported by Kaplan (27), they suggest that the age dependence for induction of thymomas is not associated with differences in the immune response to the virus.

Previous studies have demonstrated that bone marrow reconstitution of irradiated mice can suppress the development of leukemias (28). We, therefore, examined bone marrow-reconstituted mice for differences in the appearance and titer of antibodies to MuLV. As illustrated in Table V, bone marrow reconstitu-

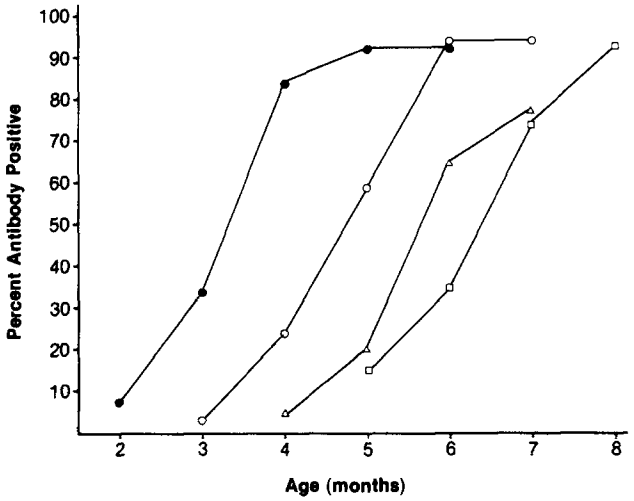

FIG. 4. Age dependency of the radiation-induced appearance of antibody against MuLV. Groups of antibody-negative 1-, (●—●, 20 mice); 2-, (○—○, 15 mice); 3-, (△—△, 20 mice); or 4-mo-old (□—□, 20 mice) C57BL/6 mice were irradiated with 175 R four times at weekly intervals. Sera were collected at monthly intervals and tested at a 1:40 serum dilution in the RIP assay for antibodies against MuLV. The results are presented as the percentage of mice developing an immune response against MuLV vs. age.

TABLE IV
*Thymoma Incidence in C57BL/6 Mice Irradiated at Various Ages**

Age at irradiation	Lymphoma incidence					
	Months postirradiation					
	1	2	3	4	5	6
mo						
1	0/20	7/20	13/20	15/20	15/20	15/20
2	0/15	1/15	3/15	7/15	8/15	8/15
3	0/20	0/20	1/20	2/20	4/20	5/20
4	0/20	0/20	2/20	3/20	4/20	4/20

* C57BL/6 mice at 1–4 mo of age were irradiated four times at weekly intervals with 175 R.

TABLE V
*Thymoma Incidence in Control and Bone Marrow-Reconstituted C57BL/6 Mice**

Group	B.M.	Irradiated	Lymphoma incidence							
			Months postirradiation							
			1	2	3	4	5	6	7	8
1	–	–	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
2	+	–	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
3	–	+	0/15	0/15	0/15	5/15	11/15	12/15	12/15	12/15
4	+	+	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15

* 4-wk-old C57BL/6 mice were used at the start of the experiments in all groups. Irradiation (175 R) was given four times at weekly intervals. Bone marrow reconstitution was at 2 mo of age after the last irradiation with 1×10^7 bone marrow cells from 4-wk-old C57BL/6 mice.

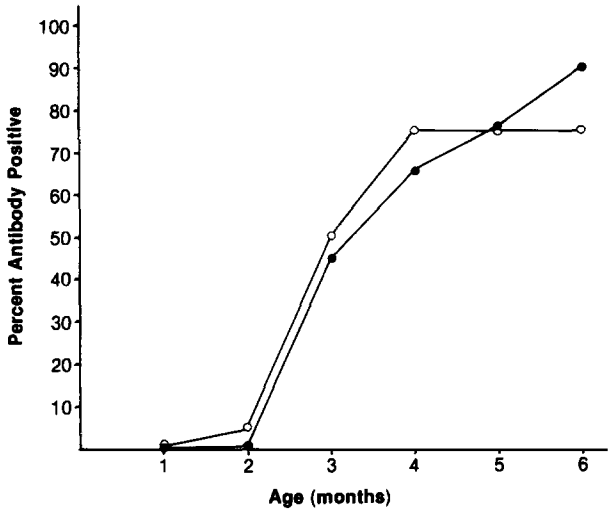

FIG. 5. Influence of bone marrow reconstitution on the appearance of antibodies against MuLV after irradiation. C57BL/6 mice were irradiated at 1 mo of age with 175 R four times at weekly intervals. One group of mice (15) was given 1×10^7 syngeneic bone marrow cells immediately after the last irradiation, (○—○); and a second group of mice was not reconstituted, (●—●). Serum was collected at monthly intervals by tail bleeding and assayed at a 1:40 serum dilution for antibodies against MuLV in the RIP assay. The results are presented as the percentage of mice developing an immune response against MuLV vs. age. Control nonirradiated, and nonirradiated bone marrow-treated mice developed antibodies identical to the controls illustrated in Fig. 1.

tion completely suppressed the occurrence of thymic lymphomas. This suppression occurred without any detectable difference in the rate of appearance of a humoral immune response against the virus (Fig. 5). Moreover, when sera from mice, which had developed a humoral immune response against the virus, were titered, bone marrow-reconstituted mice generally had titers of 1:320-640 (data not shown), which are only slightly less than the titers of control mice. These results suggest that the suppression of leukemia by bone marrow reconstitution is not related to its potential influence on the humoral immune response against the virus.

The effect of the cumulative dose of irradiation on lymphomas and the humoral immune response is shown in Fig. 6. Mice given either two, three, or four treatments of 175 R developed comparable immune responses both in the rate and extent of appearance of antibody. In contrast, with mice given 175 R once, only approximately 35% of the population developed detectable antibodies but at a rate comparable to the other groups. Sera from mice of all groups were also titered, and no significant differences were observed (data not shown). The incidence of lymphoma, as shown in Table VI, decreased with a decreasing cumulative dose. Thus, as above, there was no correlation between the appearance of an immune response to MuLV and development of lymphomas.

Discussion

The existence and characteristics of a humoral immune response against endogenous ecotropic viruses in a variety of inbred strains of mice have been

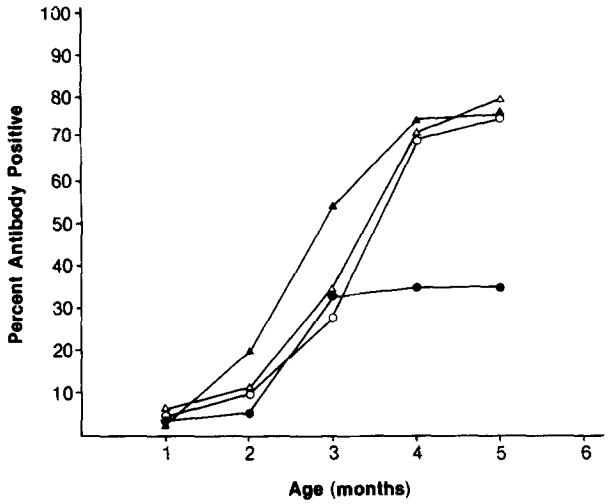

FIG. 6. Influence of dose of irradiation on the appearance of antibodies against MuLV. C57BL/6 mice at 1 mo of age were given one, (●—●); two, (○—○); three, (▲—▲); or four, (△—△) treatments of 175 R at weekly intervals. Sera were collected at monthly intervals and tested at a 1:40 serum dilution for antibodies against MuLV with the RIP assay. The results are presented as the percentage of mice developing an immune response against MuLV vs. age.

TABLE VI

*Incidence of Lymphoma in C57BL/6 Mice Given Various Doses of Irradiation**

Treatment	Thymoma incidence				
	Months postirradiation				
	1	2	3	4	5
1 × 175 R	0/25	0/25	0/25	0/25	0/25
2 × 175 R	0/25	0/25	2/25	3/25	3/25
3 × 175 R	0/25	0/25	0/25	1/25	1/25
4 × 175 R	0/17	0/17	3/17	7/17	12/17

* C57BL/6 mice, 1 mo of age, were given one to four treatments of 175 R at weekly intervals.

amply demonstrated (17–21). The present study was undertaken to determine the influence of this immune response on endogenous ecotropic virus-mediated disease. Radiation-induced leukemia in C57BL/6 mice was chosen for various reasons including: (*a*) the lack of chronic viremia such as that found in the AKR mouse, which might preclude the detection of free antibody; (*b*) the existing hypothesis that radiation activates latent endogenous viruses, which in turn induce leukemia; and (*c*) the availability of a variety of experimental manipulations that influence the development of leukemia, some of which have been interpreted as being immunological. The results of our experiments were (*a*) radiation consistently accelerates the appearance of an immune response to the endogenous ecotropic virus, and (*b*) this immune response, under various conditions, has no correlation with nor influence on the development of leukemia.

The ability of irradiation to accelerate the natural appearance of the humoral immune response to MuLV is probably a consequence of the activation of

endogenous MuLV. This conclusion is supported by recent results, which have demonstrated a correlation between the presence of antibody and infectious ecotropic MuLV (22, 23) and by genetic experiments designed to map the viral genome of a variety of inbred strains of mice and to study the phenotypes for virus expression (reference 24 and footnote 3). Therefore, our results are compatible with earlier work, (5–7, 12), which suggested that one consequence of irradiation was the activation of endogenous MuLV. Our results also suggest that this activation requires only minimal doses of irradiation, is independent of age, and is independent of experimental manipulations which suppress leukemia, such as bone marrow reconstitution.

Although radiation accelerated the appearance of antibody against MuLV, the humoral immune response had no apparent influence on the incidence of leukemia. Thus, irrespective of whether an immune response developed or persisted after irradiation, the incidences of leukemia were comparable. These results are in contrast to previous studies which suggested immune regulation of leukemia via viral antigenic determinants (29, 30). The occurrence of leukemia in irradiated mice, which never developed detectable antibodies against MuLV, is of particular interest. In these cases, either there was insufficient viral expression to stimulate an immune response, or in these mice there was no detectable antibody because of viremia. As demonstrated in the accompanying paper, viremia was not detectable in such mice. The most likely explanation for the lack of antibody in these mice is that there was an insufficient level of activation of MuLV to induce an immune response. Clearly, however, radiation still effectively induces leukemia.

Another characteristic immune response in irradiated mice was the transient development of antibody against MuLV. This pattern is also characteristic of nonirradiated C57BL/6 mice and, in general, is characteristic of the immune response in inbred strains in which antibody against MuLV appears slowly during their life span.[3] This type of response could be due to either transient viremia or waning of the immune response due to a lack of persistent virus expression. That this response is not due to viremia is indicated in the accompanying paper. Thus, we feel that the transient appearance of antibody probably reflects the transient expression of virus with subsequent waning of the immune response. This conclusion is supported by the observation that introduction of a constitutively expressed viral genome into C57BL/6 mice by the cross C57BL/6 × AKR or C57BL/6 × C3H results in the appearance of antibodies early in life, at high titers, which persist for the life span of the hybrid (J. N. Ihle, K. Bengali, and J. Domotor, Jr., unpublished data). Nevertheless, the results demonstrate that whether or not the immune response persists in irradiated mice, there is no influence on the incidence of leukemia.

The lack of correlation of the serological data with the development of lymphomas could have been due to the inability of our assays to detect antibodies serologically specific for the endogenous ecotropic virus associated with radiation leukemia. This does not appear to be the case, however, since virus competition assays demonstrated that, in terms of the humoral immune response, in vitro passaged RADLV was serologically equivalent to the AKR-type virus. In addition, these assays have demonstrated that the immune response is specifically directed against the ecotropic viruses and not the xenotropic viruses

of mice. Thus, assuming the ecotropic virus is the causative agent of radiation lymphomas, we might have expected a correlation.

A lack of correlation between antibody and the incidence of lymphoma could have been due in part to the absence of overt ecotropic virus expression in the majority of radiation-induced lymphomas (accompanying paper). This observation, however, does not preclude the virus as the etiological agent, in that once transformation has occurred overt expression may not be necessary and, in fact, in an immunologically competent animal the expression of viral antigens may be selected against. The data presented here, however, suggest that if virus expression is at all required, then this expression might not be sufficient to induce an immune response and, as shown in the accompanying paper, that even in the absence of a humoral immune response, thymomas may fail to express MuLV. Thus, from a seroepidemological perspective there is no demonstrable correlation between endogenous ecotropic viruses and radiation-induced lymphomas.

Our results are of particular significance when considering the potential immunological mechanisms in the induction of radiation leukemia, and in general demonstrate that the radiation regimen used in these experiments is not immunosuppressive insofar as development of the humoral immune response against endogenous MuLV is concerned. Of particular interest was the observation that although bone marrow reconstitution of irradiated mice did not significantly alter the immune response to MuLV, it completely suppressed subsequent leukemia. Therefore, neither radiation-induced leukemia nor its suppression by bone marrow reconstitution appears to be due to their influence on the humoral immune response against MuLV.

The results presented here and in the accompanying paper also suggest that any immunological approach via viral antigens to control radiation-induced leukemia will not be successful. In fact, previous attempts using passive immunization against MuLV have proven equivocal (31) and attempts at immunization have not succeeded (32). This conclusion is also supported by the lack of an influence of the humoral immune response as demonstrated here, in spite of the fact that this immune response has been shown to be capable of being cytotoxic for virus-replicating cells and of neutralizing MuLV. The efficacy of an immunological approach is also questioned by the lack of overt virus expression in the majority of radiation-induced leukemias, as shown in the accompanying paper.

In conclusion, the results have demonstrated the lack of a serological relationship between the immune response to endogenous ecotropic MuLV and the development of thymic lymphomas, and reasonably question the etiological role of endogenous MuLV in radiation-induced leukemias in C57BL/6 mice. Clearly, however, additional experiments are necessary to further examine the role of these viruses in radiation leukemia.

Summary

The humoral immune response against endogenous ecotropic murine leukemia viruses (MuLV) was examined in irradiated and control C57BL/6 mice. Control mice developed antibodies against MuLV slowly throughout life. In contrast, within 2-3 mo after irradiation 90% of irradiated C57BL/6 mice had

developed detectable antibodies against MuLV. The characteristics of this immune response, however, were identical in control and irradiated mice in terms of peak titers, specificity for endogenous ecotropic MuLV, and reactivity against the ecotropic viruses' glycoprotein (gp71). Moreover, the rate of appearance of antibodies against MuLV in irradiated mice and the peak titers were generally not affected by age at irradiation, dose of irradiation (two, three, or four treatments of 175 R), or bone marrow reconstitution.

Although the ability of irradiation to accelerate the appearance of antibody in a population of C57BL/6 mice suggested activation of endogenous ecotropic MuLV, there was no apparent correlation between the appearance of this immune response or its persistence and the development of lymphoma. Thus, the incidence of lymphoma was comparable in mice that: (*a*) developed no immune response; (*b*) developed an immune response only transiently after irradiation; or (*c*) developed an immune response which persisted until death from lymphoma. Moreover, experimental conditions that alter the ability of irradiation to induce leukemia, such as age, dose, or bone marrow reconstitution did so without significantly altering either the rate of appearance of a humoral immune response to MuLV or its peak titers. The results, therefore, fail to demonstrate any seroepidemological relationship between endogenous ecotropic MuLV and radiation-induced leukemia.

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