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IRRADIATION AND VIRAL LYMPHOMAGENESIS IN THYMIC GRAFTS

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IRRADIATION AND VIRAL LYMPHOMAGENESIS IN THYMIC GRAFTS¹

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SUMMARY

AKR neonatal thymus was subjected to 850 and 5000 r ^{60}Co irradiation in vivo prior to its removal for grafting to thymectomized syngeneic hosts. These irradiated grafts placed in hosts inoculated as sucklings with Gross murine leukemia virus (GMuLV) developed lymphoma. The incidence was slightly lower and the latent period longer than that found in irradiated control grafts in similar hosts. When grafts were exposed to GMuLV in vitro just prior to grafting or in vivo 24 hours before grafting, but placed in hosts not given neonatal virus inoculation, lymphoma developed with reduced incidence in the 850 r irradiated grafts and did not develop in the grafts receiving 5000 r. 5000 r was not found to alter the lymphomagenic properties of filtrates containing GMuLV.

These studies show that heavily irradiated thymic grafts can develop lymphoma in virus infected hosts, but that irradiation of grafts exposed to virus in vitro and in vivo inhibits the ability of GMuLV to produce lymphoma probably by reducing the numbers of cells available for virus replication, thus, indirectly lowering virus concentration in the thymic grafts.

IRRADIATION AND VIRAL LYMPHOMAGENESIS IN THYMIC GRAFTS

Lymphoma in the AKR mouse occurs in high incidence and is initiated in the thymus. Thymectomy prevents lymphoma in this mouse strain (1) and thymic grafts restore the potential for this animal to develop the disease (2). Pre-incubation of intact AKR thymus with Gross murine leukemia virus (GMuLV) results in a high incidence of lymphoma with a latent period shorter than spontaneous disease when such a thymus is grafted to thymectomized syngeneic hosts (3). This occurs with thymic grafts placed immediately after incubation with virus or after 7 days incubation in vivo in a diffusion chamber. The latter consist only of epithelial reticular cells. This system, i.e., using virus infected thymic grafts in AKR mice offers one of predictable in vivo virus directed malignant transformation and lends itself to a study of the relationship of lymphomagenesis to factors which alter host cell-virus interaction. In the present study, ionizing radiation at two dose levels was used. Its effect on lymphomagenesis by GMuLV in thymic grafts was evaluated.

MATERIALS AND METHODS

Mice - The animals were from the AKR strain which is maintained in this laboratory. They were housed in plastic cages and given water and pellets ad libitum.

Thymectomy - All of the experimental and control animals were thymectomized at 4 weeks of age. A combination of sodium pentobarbital and Fluothane (R) anesthesia was used and the thymus removed by gentle suction. The completeness of thymectomy was checked by the gross appearance of the anterior mediastinum at autopsy. Animals with disseminated lymphoma developing in the graft showed a characteristic appearance of enlarged thoracic nodes in the absence of a thymus.

Grafts - All of the thymuses used for grafting were from animals less than 48 hours of age. The thymuses were removed from the donor which had been sacrificed by decapitation. They were placed in Hanks' balanced salt solution with 100 units/ml of penicillin and streptomycin. Thymus was grafted under the kidney capsule of 5 week old thymectomized recipients within 1-2 hours after its removal. No attempt was made to match sexes when grafting.

Virus - The virus used in this study was Gross murine leukemia virus (GMuLV) kindly supplied by Dr. L. Gross in the form of a C3H/Bi mouse with viral induced lymphoma. Cell-free filtrates of this lymphoma were inoculated into AKR mice at 3-5 days of age and the virus maintained by passage of filtrates of developing lymphoma in this strain. These filtrates produced a 100% incidence of thymic lymphoma at 60-90 days. Filtrates were prepared by the method of Gross (4), except that Falcon Plastics 0.20 micron filters were used. GMuLV infected thymuses were prepared by 1) incubating thymus pricked 2 times with a 25 gauge needle in filtrate at room temperature for 20 minutes prior to grafting, and 2) by inoculating newborn mice with filtrate sacrificing them 24 hours later and removing the thymus for grafting.

Irradiation - A 10,000 curie ^{60}Co source was used. Normal or virus infected newborn mice were placed in disposable plastic tubes and irradiated with a dose of 850 r (470 r/min, 38 cm from center of source), or 5000 r (4,450 r/min, 12.5 cm from center of source). Immediately after irradiation the animals were killed by decapitation and the thymuses removed.

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Sacrifice - The grafted animals were sacrificed when they developed generalized lymphoma or at 365 days after grafting. They were considered to have thymic lymphoma when they presented the gross appearance of a massive enlargement of the graft which frequently covered the kidney. These animals also had enlargement of all of the lymph nodes, liver and spleen. Microscopically, the tumor was composed of uniform sized lymphoblasts which were found to completely replace the graft and directly invade the kidney containing the graft. They effaced the architecture of the lymph node and spleen. Tumor was found invading the liver and opposite kidney in many instances. The microscopic morphology was identical to that found in spontaneous thymic lymphoma of AKR mice. Non-thymic lymphoma was the diagnosis when the animal showed a gross and microscopic picture similar to that above, but the thymic graft was small and not replaced by lymphoma cells. Reticulum cell sarcoma was characterized by a massive enlargement of the spleen with white nodules of tumor; such nodules were frequently present in the liver. The thymic grafts were small; some contained tumor cells. Microscopically this tumor was composed of large immature cells with pleomorphic nuclei.

Histology - Tissues were fixed in Bouin's fluid and cut at 5 microns. They were stained with hematoxylin and eosin.

RESULTS

Regenerative Capacity of Irradiated Thymic Grafts

The effect of an irradiation dose of 850 and 5000 r on the regenerative capacity of thymic grafts implanted under the kidney capsule was determined. Newborn mice were irradiated, their thymuses removed and grafted to 6-week old intact mice. The animals were sacrificed at 7 and 14 days after grafting. The size and morphologic appearance of their grafts was compared to that found in a previous study of regenerating newborn AKR thymic grafts (5). At 7 days, 4 grafts exposed to 850 r were found to be considerably smaller in size than normal grafts, and contained clusters of epithelial reticular cells with scattered small lymphocytes; lymphoblasts were seen in 2 of the 4 grafts. Seven grafts receiving 5000 r were evaluated at 7 days. These grafts were smaller than the 850 r grafts. Three grafts were composed of epithelial reticular cells only, and 4 grafts showed a scattered infiltrate of small, dark lymphocytes in the epithelial graft. Non-irradiated grafts all show normal thymic morphology by 7 days (5).

At 14 days all of the 4, 850 r irradiated grafts showed the histologic appearance of normal thymus, and those receiving 5000 r (6 grafts) showed some of the histologic features of thymus and also increased numbers of lymphoblasts with mitotic figures. They were smaller in size than the 850 r grafts.

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Effect of Irradiation on Development of Lymphoma in Thymic
Grafts Exposed to GMuLV

The animals in this study were divided into four groups. The experimental design for all of the groups is presented as table 1, and the results summarized in table 2. Group 1 animals were given GMuLV at 3-5 days of age, thymectomized at 4 weeks of age, and grafted with irradiated newborn thymus. The controls for this group were grafted with non-irradiated thymus. Ten of 12 mice that received 850 r irradiated grafts developed a typical thymic lymphoma between 66 and 188 days after grafting. The lymphomas massively enlarged the graft and disseminated to the lymphoid organs, liver, and opposite kidney. Of the remaining 2 mice, 1 developed a non-thymic lymphoma at 340 days after grafting and the other was found to have a reticulum cell sarcoma when sacrificed at 365 days. Of the 11 mice receiving 5000 r irradiated grafts, 7 developed typical thymic lymphoma involving the graft from 15 to 330 days after grafting. The remaining 4 mice had no lymphoma, but demonstrated small thymic grafts when sacrificed at 365 days. Seventeen mice received non-irradiated grafts and all developed thymic graft lymphoma from 16 to 79 days after grafting.

TABLE 1. Experimental design

Group	Day 3-5	Day 28	Day 35 - Kidney capsule thymic graft
1	MuLV I.P.	Thymectomy	0-24 hr. Thymus given 850 r, 5000 r, or 0 r.
2	MuLV I.P.	Thymectomy	0-24 hr. Thymus given 850 r, 5000 r, or 0 r. Incubated <u>in vitro</u> with MuLV.
3	----	Thymectomy	0-24 hr. Thymus given 850 r, 5000 r, or 0 r. Incubated <u>in vitro</u> with MuLV.
4	----	Thymectomy	48 hr. Thymus from mouse given MuLV I.P. 24 hr previously. Given 850 r, 5000 r, or 0 r.

TABLE 2. Effect of irradiation on development of lymphoma
in thymic grafts exposed to GMuLV in AKR mice

Group	Irrad. Dose r	No. of mice with thymic lymphoma/ No. of mice grafted	Latent period (days after grafting)*
1	850	10/12	111 ± 37
	5000	7/11	161 ± 102
	0	17/17	56 ± 22
2	850	14/14	118 ± 86
	5000	8/11	139 ± 79
	0	12/12	66 ± 4
3	850	5/15	193 ± 70
	5000	0/13	---
	0	14/14	73 ± 15
4	850	3/12	94, 119, 340
	5000	0/15	---
	0	10/13	117 ± 85

*Mean ± S.D.

In group 4, the thymic grafts were taken from neonatal animals given virus 24 hours previously. Irradiation was carried out just prior to removal of the thymus for grafting to thymectomized hosts. Three of 12 animals receiving 850 r grafts developed typical thymic lymphoma involving the graft. The remaining animals had a normal thymus when they were sacrificed at 365 days. None of the 15 mice receiving 5000 r irradiated grafts developed lymphoma. All showed small thymic grafts when sacrificed in good health at 365 days. Ten of 13 control mice showed typical thymic graft lymphoma at 63-312 days. The remaining 3 mice were healthy with grafts present when sacrificed at 365 days.

Effect of Irradiation on the Infectivity of GMuLV

Cell-free filtrate was irradiated with 5000 r ^{60}Co and inoculated into ten, 3 day old AKR hosts. Three control animals received the same filtrate which had not been exposed to ionizing radiation. All of the animals in both groups developed lymphoma. It occurred between 55-97 days after inoculation in the irradiated group, and 59-80 days in the control group.

DISCUSSION

The demonstration in these studies that newborn thymus grafted under the kidney capsule shortly after exposure to 850 and 5000 r of ionizing radiation could survive, indicates that certain thymic cells are resistant to these doses. It has been shown that thymic lymphocytes are virtually destroyed by exposure to a radiation dose of 800 r (6). Also, studies have demonstrated that the lymphocyte population of thymic grafts is replaced by host lymphocytes within the first few weeks after grafting and that epithelial reticular cell viability is necessary for this to take place (7,8). It can be assumed, therefore, that this is the case in the present experiments, i.e., that some of the epithelial reticular cells survive the irradiation insult and direct the population of grafts by host lymphocytes. Such a conclusion was made in previous studies where thymic grafts exposed to 2000 r were evaluated (7). However, it was demonstrated subsequently, that epithelial reticular cells exposed as a whole thymus in vitro to 2000 r ^{60}Co , do sustain some damage. It was found that the ability of such irradiated thymuses, when grafted, to restore the capacity for homograft immunity of neonatally thymectomized mice, was impaired 3 weeks after grafting. Thymic implants given 500 r had the ability to restore the immunologic defects of the neonatally thymectomized recipients. Both types of graft in this study had regained normal thymic morphology at 3 weeks (9).

In the present study, when the irradiated thymic grafts were given to mice previously infected with GMuLV, they developed lymphoma as did non-irradiated control thymic grafts. The incidence of lymphoma was somewhat less, however, in the 5000 r irradiated grafts. The latent period was prolonged in both irradiated groups when compared to that of controls. Exposing the grafts to virus in vitro, prior to implantation under the renal capsule, did not increase the incidence of or shorten the latent period to the appearance of lymphoma in these infected hosts. These data reveal that the irradiated grafts are fully capable of developing lymphoma when they recover in virus infected hosts and imply that the surviving epithelial reticular cells provide a nidus for reconstitution of a typical AKR thymus, i.e., one that develops lymphoma in the presence of GMuLV. The decrease in lymphoma incidence in the 5000 r irradiated grafts suggests that the reduction in numbers of epithelial reticular cells, which probably occurs after the heavy irradiation, results in a smaller thymus with fewer cells, providing less chance for lymphoma development. In fact, throughout the experiment the 5000 r non-lymphomatous grafts were found to be smaller than those receiving 850 r or than non-irradiated thymus grafted in a similar manner.

When neonatal thymus was irradiated just prior to virus exposure and implanted in non-virus infected AKR hosts, the incidence of thymic lymphoma was low in the animals receiving 850 r irradiated grafts and lymphoma did not develop in the mice grafted with 5000 r irradiated thymus. Irradiation of grafts with an opportunity for in vivo infection to become established in the thymic cells, i.e., 24 hours after infection of the donor mouse, also resulted in a low incidence of lymphoma development in these grafts. Lymphoma occurred in high incidence in non-irradiated thymic grafts exposed to virus in vivo and in vitro and grafted to non-virus infected hosts.

These results could be explained in several ways. Heavy irradiation might have had a direct effect on the virus. The demonstration of unaltered lymphomagenic properties of GMuLV exposed to 5000 r is against this possibility. The irradiation could have blocked the initial infection of cells by GMuLV. The observation of a similar striking reduction of lymphoma incidence by irradiation of grafts of established viral infection to that found in grafts exposed at the time of initial infection suggests that this is not the case. The most likely explanation of the results observed is that the irradiation induced a reduction in numbers of thymic cells susceptible to, or productively infected with virus, thus lowering the amount of virus present in the graft. This cell decrease could involve both the thymic lymphocytes, which provide a source of virus to infect lymphocytes repopulating the graft, and/or the more radio-resistant epithelial reticular cells which are known to support viral replication (3), and regulate the size, lymphoid composition, and function of the graft.

One can conclude from these studies that lymphoma can develop in thymic grafts given 850 and 5000 r of ionizing radiation when the host animal has high levels of GMuLV produced by inoculation of sucklings with this agent. However, lymphoma development in thymic grafts, infected with virus, in vivo and in vitro, can be inhibited by these same doses of irradiation when the concentration of virus in the host animal is low. Therefore, the amount of virus available to the thymus from established host infection, as well as the numbers of thymic cells which can support viral infection in the thymic graft, must be major factors regulating lymphoma-genesis in this system.

In group 2, the only change in protocol was that the grafts were exposed to GMuLV in vitro for 20 minutes before grafting to the virus infected hosts. All of 14 mice receiving the 850 r graft developed thymic graft lymphoma 29-349 days after grafting. Eight of 11 mice receiving the 5000 r irradiated thymuses developed thymic lymphoma at 72-305 days; 3 animals were found to have small thymic grafts when sacrificed at 365 days. All of the 12 control animals (those receiving virus infected but non-irradiated grafts) developed typical thymic graft lymphoma at 57-70 days after grafting.

In group 3, animals were thymectomized and received grafts infected in vitro with virus, but were not given virus as newborns. Fifteen animals received 850 r irradiated grafts; 5 of them developed thymic graft lymphoma at 141-308 days after grafting. Two animals had a reticulum cell sarcoma involving the spleen, with a normal appearing graft at 364 days. The remaining 8 mice were healthy with grafts present at 364 days. None of the 13 animals receiving 5000 r virus infected grafts developed thymic lymphoma. One animal had a reticulum cell sarcoma greatly enlarging the spleen which appeared to secondarily involve a very small thymic graft. The remaining 12 animals had small grafts composed of epithelial cells and lymphocytes when sacrificed at 365 days after grafting. All of 14 non-irradiated virus infected control grafted mice developed thymic graft lymphoma at 52-98 days after grafting.

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