Brief Definitive Report

EVIDENCE THAT ENDOGENOUS ECOTROPIC VIRUS IS NOT EXPRESSED IN AKR THYMIC LYMPHOID CELLS OF CHIMERIC HOSTS*

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The high incidence of naturally occurring lymphoma in the AKR mouse strain is dependent upon spontaneous expression of endogenous retrovirus and an intact thymus. The importance of the virus was demonstrated in the pioneering studies of Gross (1, 2), who transmitted lymphoma by inoculating filtrates of AKR tissue into newborn mice of certain low-lymphoma strains. The critical role of the thymus in AKR lymphoma was initially recognized in experiments indicating that spontaneous lymphoma is ablated by thymectomy (3).

Although the roles of both endogenous retroviruses (4–6) and the thymus (3, 7) in the AKR lymphoma have been extensively documented, analysis of cell-virus interactions within the thymus remains incomplete. AKR mice express infectious *N*-ecotropic retrovirus from about the 18th d of gestation throughout life, with similar titers in the thymus and other organs (8). In preleukemic 5- to 9-mo-old AKR mice, however, a dramatic amplification of viral antigen expression (9) and expression of polytropic mink cell focus-inducing virus (10), are seen in the thymus, but not in other organs, indicating thymus-specific expression of retrovirus information.

Selective control of virus production in the thymus has been found in hybrids of (AKR × NZB)F_1 mice, which express high levels of spontaneous retrovirus in most organs, but not in the thymus (11). Thymocytes of donor (AKR × NZB)F_1 mice that differentiate in lethally irradiated AKR and C57BR mice do express virus (12). These findings imply either that the (AKR × NZB)F_1 thymic microenvironment is restrictive of spontaneous retrovirus expression and/or that the AKR and C57BR microenvironments are permissive of virus expression in the radiation chimeras.

We have examined the role of the thymic microenvironment in the spontaneous ecotropic virus expression of AKR mice. Radiation-induced stem-cell chimeras were established by repopulating lethally irradiated B10.K mice with AKR fetal liver cells, which do not express ecotropic virus spontaneously (8). AKR thymic lymphoid cells that differentiate in the B10.K strain do not express detectable ecotropic virus. These studies indicate that the thymic microenvironment influences expression of ecotropic virus, which is prerequisite for lymphoma in AKR mice.

Materials and Methods

Mice. B10-H-2^k (B10.K) mice were raised in our own breeding colony (The Wistar Institute, Philadelphia, Pa) derived from mating pairs provided by Dr Donald Shreffler, Washington

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University School of Medicine, St Louis, Mo Adult AKR/J and AKR/J pregnant females were purchased from The Jackson Laboratory, Bar Harbor, Maine.

Radiation-induced Chimeras. B10 K and AKR/J hosts were irradiated at 3–4 mo of age with 950 and 900 R, respectively 24 h later the hosts were reconstituted with 2–10 × 10^6 AKR/J or B10.K fetal liver cells derived from 15-d-old embryos. The chimeras were housed in isolation chambers that provided filtered air and were given drinking water that contained 5 mg/liter neomycin and 50,000 U/liter polymixin B.

Virus Assay The XC assay was performed as previously described (13), using SC-1 cells (14), with modifications for enumerating cells shedding infectious virus as infectious centers (15). Mice were sacrificed at 6 and 24 wk after irradiation and reconstitution, and spleen and thymus cells were teased with blunt forceps, bone marrow cells were derived from femurs by injecting medium through the bone marrow space.

Assessment of Chimerism. Thy-1.1 antiserum was purchased from Searle Diagnostic Products, High Wycombe, England, and Thy-1.2 antiserum was kindly provided by Dr. J Sprent (University of Pennsylvania School of Medicine, Philadelphia, Pa.). A cytotoxicity assay was performed at the same time as the XC assay. Cells were harvested, and 10^4 thymocyte target cells were suspended in 50 μl of RPMI-1640 (Grand Island Biological Co , Grand Island, N. Y.) (supplemented with 10% fetal calf serum, glutamine and penicillin and streptomycin). The target cells were incubated with several dilutions of antiserum (100 μl) for 30 min, at room temperature, centrifuged, washed with fresh medium, and incubated for 45 min at 37°C with 50 μl of diluted rabbit complement. Two drops of erythrocin red were then added to each well, and an aliquot was examined for viable cells.

Results

Chimerism of Hosts. All chimeras used in these studies were reconstituted with 15-d fetal liver cells of either AKR/J or B10.K origin. Chimerism in AKR → B10.K and B10.K → AKR mice was assessed by examination of Thy-1.1 and Thy-1.2 expression in thymic cells (amenable to simple teasing, presumed to be thymic lymphoid cells) (Table I). Thymic lymphoid cells of AKR → B10.K chimeras express almost exclusively Thy-1.1 antigen, which indicates that they have been entirely repopulated by AKR donor stem cells. Similarly, the two B10.K → AKR chimeras have been repopulated by B10.K donor stem cells.

Virus-producing Cells in Radiation-induced Chimeras. Individual chimeras were tested for the presence of cells producing ecotropic virus in the spleen, bone marrow, and thymus at 6 or 24 wk after reconstitution (Table I). Fetal liver cells from both strains produced no ecotropic virus detectable in the XC assay (data not shown), in agreement with previous studies (8). High numbers of virus-producing cells were present in organs of normal AKR mice as well as in those from AKR → AKR chimeras, whereas B10.K and B10.K → B10.K mice showed no cells producing virus in any of the organs tested. AKR → B10.K chimeras showed no detectable virus-producing cells in the thymus, which indicates that AKR thymocytes that repopulate the B10.K thymuses do not express virus spontaneously. Spleen and bone marrow of the AKR → B10.K chimeras contained virus-producing cells in numbers similar to nonmanipulated AKR mice. These virus-producing cells are, most likely, of AKR origin because B10.K mice do not produce virus and irradiation does not induce virus production in B10.K → B10.K chimeras. In contrast to the AKR → B10.K chimeras, the number of virus-producing cells in the thymus, spleen, and bone marrow of B10.K → AKR chimeras is similar to the high numbers found in 6- and 24-wk-old normal AKR and AKR → AKR mice.

Incidence of Thymoma. 11 mo after reconstitution with AKR fetal liver cells, none (0/42) of the surviving AKR → B10.K chimeras had developed thymomas, whereas

TABLE I

Expression of Thy-1 Antigens and Infectious Virus in Thymocytes from AKR → B10.K and B10.K → AKR Chimeras

Mouse tested	Weeks after reconstitution	Cytotoxic index with *		Infectious center titers‡		
		Anti-Thy-1.1	Anti-Thy-1.2	Thymus	Spleen	Bone marrow
					$\times 10^{-5}$	
B10.K	6§	98	2	<0.01	<0.01	<0.01
AKR	6§	8	94	63	63	50
AKR → B10.K						
No. 22	6	4	97	<0.01	6.3	4.0
No. 14	6	4	95	<0.01	10	NT‖
No. 29	6	4	NT	<0.01	6.3	4.0
No. 32	6	3	97	<0.01	32	4.0
No. 5	6	4	100	<0.01	4.0	4.0
B10.K	24§	94	3	<0.01	<0.01	<0.01
AKR	24§	3	97	63	32	100
AKR → AKR	24	NT	NT	10	10	20
B10.K → B10.K	24	NT	NT	<0.01	<0.01	<0.01
B10.K → AKR						
No. 1	24	96	4	40	130	100
No. 2	24	98	2	79	100	79
AKR → B10.K						
No. 27	24	6	94	<0.01	0.063	1
No. 97	24	4	6	<0.01	0.063	2.5
No. 99	24	6	95	<0.01	0.40	0.63

* Data expressed as percentage of live cells after incubation with complement and antiserum. At least 100 cells were counted to determine these percentages.

‡ Data registered as number of infectious centers in the XC assay/number of cells used to infect assay culture.

§ Normal B10.K and AKR mice were studied at 6 and 24 wk of age.

‖ Not tested.

89% (32/36) of age-matched AKR mice had developed thymomas during this same period.

Discussion

AKR thymus lymphoid cells produce no endogenous *N*-ecotropic retrovirus when these cells differentiate from stem cells in the thymic microenvironment of *H-2*-compatible, nonvirus-shedding B10.K mice. The absence of spontaneous virus production appears specific to the thymus as virus production is seen in spleen and bone marrow cells of probable AKR origin in the AKR → B10.K chimeras. Virus production is not seen in AKR thymic lymphoid cells despite the presence of *Akv-1* and *Akv-2* loci, which lead to spontaneous production of virus in AKR mice.

An AKR thymic influence on virus expression by thymic lymphoid cells was described by Datta et al. (12) with the finding that thymocytes from (AKR × NZB)F_1 mice, which normally express no virus, express ecotropic and xenotropic viruses and virus antigens when they mature in an AKR thymus. The results of Datta et al. (12) and those presented here, taken together, are consistent with a hypothesis that virus expression in the chimera is dependent upon initial retrovirus production by AKR thymic stromal cells, i.e., the natural history of AKR lymphoma may involve virus production by thymic lymphocytes only after infection by ecotropic virus produced in radioresistant, nonlymphoid thymic cells.

Further evidence that endogenous retrovirus infection of the thymus originates in the thymic stromal cells is derived from an unrelated line of investigation of embryonic thymus in Swiss/ICR mice (16). Type-C retrovirus particles are found in embryonic

tissue (17), including thymic stromal and lymphoid cells (18). In Swiss mouse embryo thymus, thymic stromal cells with budding type-C virus particles were seen at 11.5 d of gestation, whereas lymphoblasts were not seen until 13.5 d, and budding virus was not seen in lymphoid cells until 15.5 d of gestation (16). These experiments are again consistent with the hypothesis that virus production in the thymus occurs initially in the stromal cells.

In related experiments, Datta et al. (12) found that chimeras derived by repopulation of (AKR × NZB)F_1 and C57BR mice with AKR bone marrow cells produced high levels of infectious centers in their thymuses, comparable to AKR → AKR chimeras, whereas we have found no infectious centers in thymuses of AKR → B10.K chimeras. Significant differences in both the donor cells and recipient mice used to establish radiation chimeras in the two systems studied may account for these divergent results. The donor cells used by Datta et al. (12) were bone marrow cells from mature AKR mice, which would be expected to contain a high number of virus-producing stem cells (8), whereas we used nonvirus-shedding donor AKR fetal liver cells to repopulate B10.K mice. The recipient (AKR × NZB)F_1 mice used by Datta et al. (12) do not express virus in the thymus but do express high levels of virus in other organs; (AKR × NZB)F_1 as well as C57BR recipients are susceptible to AKR virus by virtue of their *Fv-1*^{n} genotype (19). B10.K mice used as AKR stem-cell recipients in our experiments express no endogenous virus and are resistant to infection by AKR viruses on the basis of their *Fv-1*^{b} genotype. The absence of virus expression in AKR → B10.K chimeras by thymic lymphoid cells may, therefore, depend upon differentiation of virus-free, pre-T cell donor AKR cells in virus-free, recipient mice.

The finding that these AKR → B10.K chimeras have not yet developed malignant lymphomas is consistent with previous findings that production of endogenous ecotropic virus in AKR mice is prerequisite for lymphoma in this strain (2, 6, 8). Furthermore, AKR thymocytes are apparently not programmed to become malignant. It should be cautioned that the absence of virus in thymic lymphoid cells of AKR → B10.K chimeras may be quantitative, rather than qualitative, i.e., the thymus may be expressing virus at a level below the threshold for detection, rather than not at all. Nonetheless, these changes indicate significant differences in thymic biology that may be pertinent to the pathogenesis of spontaneous lymphoma in AKR mice.

Summary

AKR/J thymocytes derived from fetal liver cells do not produce virus when they differentiate in lethally irradiated B10.K mice, whereas spleen and bone marrow cells are virus producers. In contrast, B10.K thymocytes that differentiate in lethally irradiated AKR mice become virus producers. These results suggest that infection of the thymus in AKR mice is initiated in thymic stromal cells.

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