Brief Definitive Report

GENETICALLY DETERMINED RESISTANCE TO 3-METHYLCHOLANTHRENE-INDUCED LYMPHOMA IS EXPRESSED AT THE LEVEL OF BONE MARROW-DERIVED CELLS

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Mice of inbred laboratory strains differ in their susceptibility to lymphoma induction by viruses, chemical carcinogens, and ionizing radiation (1–5). In the case of retrovirus-induced lymphoma, a variety of genetically determined traits can confer relative resistance to lymphomagenesis; these factors include *H-2* type (reviewed in reference 6), endogenous expression of viral envelope proteins (7), and the as yet unidentified product of the *Fv-1* locus (8, 9). The genetic factors affecting resistance to lymphoma induction by percutaneous treatment with the hydrocarbon carcinogen, 3-methylcholanthrene (MCA), are less well understood. One such factor is inducibility for the enzyme aryl hydrocarbon hydroxylase (AHH) (10). In AHH-inducible mice, MCA treatment gives rise to skin tumors but not to lymphomas; by contrast, AHH-noninducible mice show a very weak skin tumor response to the treatment, but they vary widely in a strain-specific manner in their lymphoma response (2). In genetic studies of AHH-noninducible mouse strains, F_1 crosses of lymphoma-resistant ×-susceptible strains show that resistance to MCA-induced lymphoma is the dominant phenotype, and (F_1 × -susceptible) backcross populations show incidences of MCA-induced lymphoma suggestive of single-gene determination of resistance (11).

We now report the results of experiments designed to show whether this AHH-independent resistance mechanism acts at the level of bone marrow precursors that ultimately give rise to the lymphoma cells, or if the environment in which the marrow cells develop is the determinant of resistance. For this purpose we have observed the response to MCA of radiation chimeras produced by replacement of the bone marrow of susceptible or resistant hosts with that from donors of the opposite phenotype. Our findings indicate that resistance to MCA lymphomagenesis is largely a phenotype of the bone marrow cells themselves rather than of the host environment.

Materials and Methods

Mice. RF/J mice ($H\text{-}2^k$, Thy-1.1) were obtained from The Jackson Laboratory, Bar Harbor, ME. ST/bN mice ($H\text{-}2^k$, Thy-1.2) and (ST × RF)F_1 mice were bred in our colony using ST/bN breeders obtained from the National Institutes of Health, Bethesda, MD.

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Chimeras. Chimeras were produced by irradiating 4–6-wk-old female recipients with 950 rad in a cesium irradiator. Donor mice were 6–16 wk old. Both male and female mice were used as donors, although marrow for any single experiment was derived from mice of one sex. The sex of marrow donors was found not to affect the lymphoma incidence observed in the recipients (data not shown). Marrow was removed from donor femurs by flushing the marrow cavity with balanced salt solution (BSS) containing 2% FCS. The marrow was treated with either anti-Thy-1.1 (in the form of serum from a nude mouse subcutaneously injected with the hybridoma 19E12, courtesy of Dr. M. Lostrom, Genetics Systems, Seattle, WA), anti-Thy-1.2 (serum from a nude mouse bearing the hybridoma 6-68, obtained from Dr. U. Hammerling, Memorial Sloan-Kettering Cancer Center, New York), or a combination of the two when F_1 marrow was treated. The antiserum was diluted 1:500, and the marrow cells were incubated in it for 20 min on ice. After washing in BSS with 2% FCS, the cells were treated with a 1:10 dilution of normal rabbit serum as a source of complement (Accurate Chemical & Scientific Corp., Westbury, NY) for 45 min at 37°C. This antibody and complement treatment was repeated, the cells were washed twice in BSS with 2% FCS, suspended in PBS with 2% FCS, and injected intravenously into recipients. 5×10^6 to 1×10^7 cells were given to each recipient. Mice were kept in filter-top cages for 3 wk after marrow transfer, and in some cases were given tetracycline in their drinking water for 1 wk. Chimeras are designated by placing the donor strain before an arrow, and the recipient strain after it, e.g., RF → ST.

Carcinogen Treatment. 12-wk-old mice were treated percutaneously with a 1% solution of MCA (Fluka, Ronkonkoma, NY) in benzene (3). Their backs and flanks were shaved before the first treatment; five daily treatments were given. Control mice were painted with benzene alone. Lymphomatous animals were diagnosed by enlarged lymph nodes or hunched posture and labored breathing, and were killed and autopsied. They were examined particularly for enlargement of the spleen, lymph nodes, and thymus.

Immunofluorescence. Thymocytes were assayed for their Thy-1 phenotype by immunofluorescence. Thymic biopsy was performed on 8–9-wk-old chimeras by sedating them with sodium pentobarbital and removing a portion of the thymus by suction into a glass pipette. In other cases the thymus was dissected out on autopsy. Cells were obtained by teasing the tissue into a solution of PBS with 2% FCS. Aliquots of 3×10^6 cells were stained according to published technique (12). The anti-Thy-1 reagents described above were used at a dilution of 1:1,000, with fluoresceinated goat anti-mouse Ig (Cooper Biomedical, Malvern, PA) used as the second-step reagent.

Results and Discussion

In initial experiments we performed reciprocal bone marrow transfers using the RF and ST/b strains; 80–90% of RF mice develop lymphoma by 6 mo after carcinogen treatment, while ST/b show only a 15% frequency in the same time span. These strains have the further advantage of being identical at the *H-2* locus, which minimizes problems of histocompatibility in making chimeras. Finally, they differ at the *Thy-1* locus, so T cells in chimeras can be typed to ascertain whether marrow engraftment was successful and whether lymphomas derived from donor or host cells. However, preliminary work showed that chimeras made using ST/b recipients, while surviving marrow transfer, could not withstand treatment with MCA. For this reason (ST × RF)F_1 animals were used instead of pure strain ST/b; as resistance to MCA leukemogenesis is dominant, these mice show only a 30% incidence of lymphoma after MCA treatment, and the same *H-2* and *Thy-1*-related advantages apply. Results are shown, however, for the viable ST → RF chimeras made in early experiments.

Table I summarizes the survival of chimeras before and after MCA treatment. The overall rate of survival within the 2 mo after marrow transfer was 73%, with RF recipients being somewhat more fragile than their (ST × RF)F_1 coun-

TABLE I
Chimera Viability and Thy-1 Phenotypes

Statistic	Chimera type*					
	RF → RF	RF → (ST × RF)	(ST × RF) → RF	(ST × RF) → (ST × RF)	ST → RF	Total
Number made	67	52	62	28	51	260
Number surviving‡	44	43	40	28	34	189
Percent survival	66	83	65	100	67	73
Number dying of causes other than lymphoma:						
MCA-treated	5/22	6/27	2/26	0/18	7/23	20/116
Benzene-treated	1/14	0/9	1/7	0/6	0/6	2/42
Control	0/8	0/7	2/7	0/4	1/3	3/29
Number with donor Thy-1 phenotype:						
Before treatment‖	—	8/8	—	—	4/4	12/12
After treatment:¶						
With lymphoma	—	9/10	10/12	—	1/4	20/26
Without lymphoma	—	5/5	7/8	—	5/6	17/19

* Chimeras are designated by placing the name of the bone marrow donor strain before the arrow, and the name of the recipient strain after the arrow.
‡ The number of mice surviving at the time of MCA treatment, or 2 mo after marrow transfer.
‖ Thymic biopsy was performed, and immunofluorescence was used to stain the cells obtained.
¶ Immunofluorescence was performed on thymoctyes obtained at the time of killing at 270 d of age, or 6 months after MCA treatment.

terparts. Animals treated with MCA, as opposed to benzene-treated or untreated controls, did show increased mortality from causes other than lymphoma, but only 17% of MCA-treated animals fell into this category and were excluded from our data. Thus the animals included in the study do not constitute an unusually sturdy minority population.

Fig. 1 summarizes the incidences of lymphoma in different groups of chimeras. Fig. 1 *a* shows lymphoma-susceptible RF marrow developing under three separate circumstances: under normal conditions in an unmanipulated RF mouse, after transfer into a syngeneic RF animal, and after transfer into a lymphoma-resistant (ST × RF)F_1 animal. It is clear that the rate of lymphoma development after MCA treatment was similar regardless of the milieu in which the marrow matured and was exposed to carcinogen. In the case of the RF marrow, there was a roughly 20% "background" of lymphoma occurring in untreated chimeras, which increased to 50% in benzene-treated chimeras after a protracted latent period. This may be due to accelerated development of the spontaneous T cell lymphomas seen in normal RF mice after 9 mo of age (2). The comparatively early spontaneous cases may result from the stresses of transfer and repopulation on the marrow. In any case, it is important to note that even these background rates were similar whether the RF cells matured in a lymphoma-sensitive or -resistant stroma.

Fig. 1 *b* shows the reciprocal transfer of resistant (ST × RF)F_1 marrow into susceptible RF mice. Once again, the incidence of lymphoma was not significantly altered by the environment in which the marrow developed and was exposed to carcinogen. Fig. 1 *c* presents similar data for ST → RF chimeras. Like the resistant (ST × RF)F_1 marrow, pure strain ST/b marrow did not show increased susceptibility to lymphoma when it matured in an RF animal.

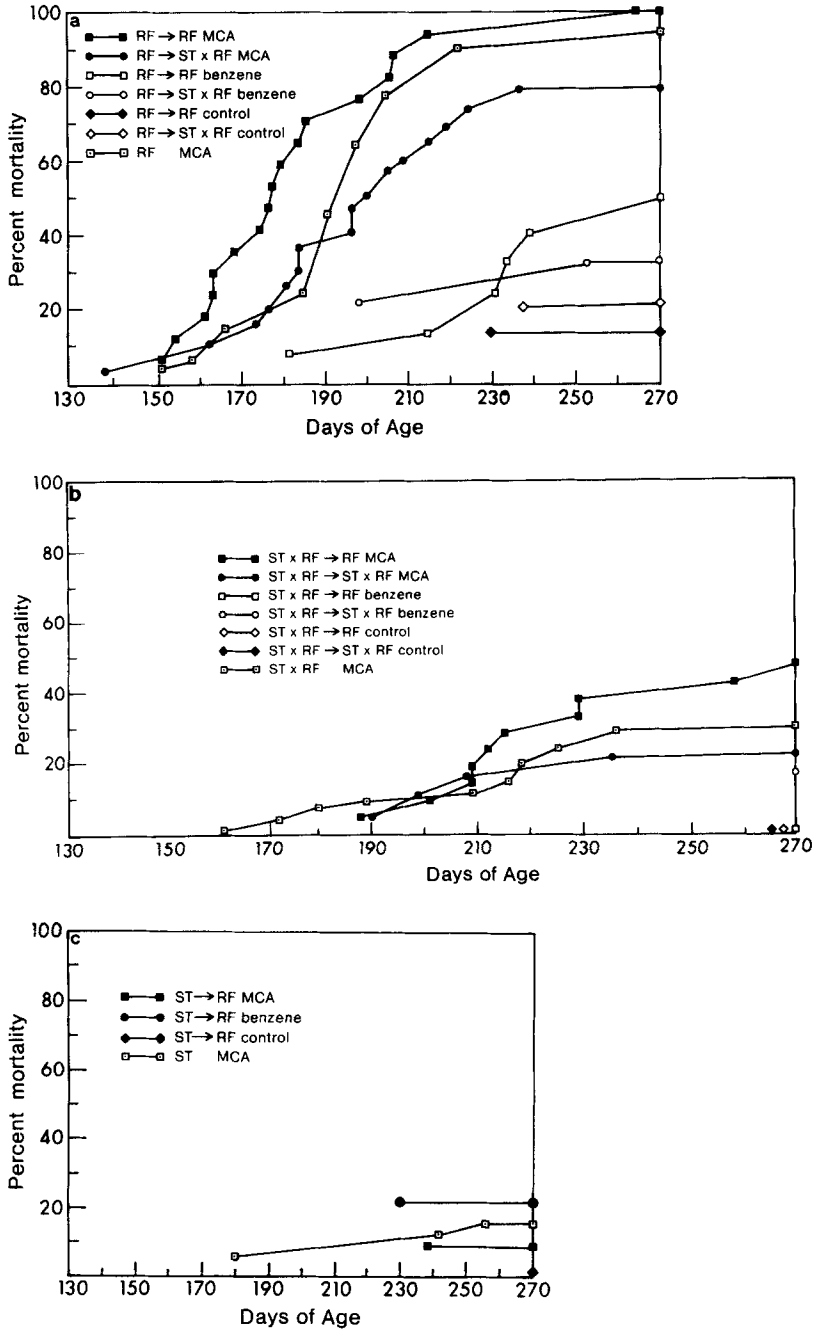

FIGURE 1. Incidences of lymphoma among MCA-treated chimeras. Chimeras were constructed and treated as described. Percent mortality refers only to animals dying of lymphoma; animals dying of other causes were excluded from these data. The number of chimeras in each experimental group is given in Table I. (*a*) RF (susceptible) marrow maturing in situ or in (ST × RF)F_1 or RF recipients. For MCA-treated RF mice, $n = 17$. (*b*) (ST × RF)F_1 (resistant) marrow maturing in situ or in RF or (ST × RF)F_1 recipients. For MCA-treated (ST × RF)F_1 mice, $n = 42$. (*c*) ST/b marrow maturing in situ or in RF recipients. For MCA-treated ST/b mice, $n = 25$.

To ensure that we were measuring true rates of lymphoma developing from donor marrow, a number of animals were tested for their Thy-1 phenotype upon sacrifice. The resulting data (Table I) show that most animals developed lymphomas of donor rather than host marrow origin. Only 14% of the animals tested showed an inappropriate lymphoma Thy-1 phenotype; a significant portion of these were ST → RF animals treated with MCA. This observation underscores the disparity in sensitivity to MCA lymphomagenesis of RF and ST/b bone marrow; although the vast majority of cells in an ST → RF thymus at 4 wk after transfer were of the ST/b Thy-1.2 phenotype, three of four lymphomas developing after MCA treatment originated from the minority RF population. This constitutes evidence that extracellular factors produced by lymphoma-resistant marrow-derived cells do not affect the susceptibility of marrow-derived cells from a sensitive strain present in the same chimera.

Our results indicate that, in this instance of genetically determined resistance to lymphoma, marrow does not alter its phenotype when it matures and is exposed to carcinogen in the stroma of a mouse of the opposite phenotype. This finding has a number of implications bearing on possible mechanisms of lymphomagenesis and lymphoma resistance. For example, one might hypothesize that RF and ST/b mice, although both AHH-noninducible, differ in the activity of some enzyme involved in MCA processing. Our data indicate that any such difference would have to reside in the marrow-derived target cell itself; resistance to lymphomagenesis cannot involve differential metabolism of carcinogen by stromal cells alone, since susceptible RF stroma does not confer susceptibility on resident (ST × RF)F_1 marrow.

An alternative hypothesis is that MCA treatment induces lymphoma by the intermediary of an endogenous lymphomagenic retrovirus, and these strains are not equally capable of generating such a virus after MCA treatment. The precedent for this possibility is set by the AKR strain that has a high incidence of spontaneous leukemia of viral etiology (13). To date no evidence of viral involvement in MCA-induced RF lymphoma has been found (14). However, if virus is responsible for MCA-induced lymphoma, the resistance of ST/b mice cannot be due to failure of stromal cells to release virus. It would instead have to be caused by the inability of a marrow-derived cell to release virus or to become reinfected. This finding differs from those on endogenous virus-induced spontaneous AKR lymphomas, where prelymphomatous changes in chimeric animals appeared to depend on stromal phenotype rather than marrow origin (15).

Finally, if the difference between susceptible and resistant strains lies in the immune response to tumor cells, it cannot be due to an aspect of immune response that would be altered by maturation in an allogeneic environment. Such an immune response gene would have to map outside the MHC, as resistance is not linked to the *H-2* complex in backcross mice (12). Any such difference would have to be a property intrinsic to the responding cells.

Summary

Reciprocal bone marrow tranfers were performed between mouse strains sensitive or resistant to 3-methylcholanthrene-induced thymic lymphoma. Sensi-

tivity and resistance are properties inherent in bone marrow, and cannot be altered by maturation of marrow in an environment of the opposite phenotype.

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