Comparative Activity of Isologous versus Homologous Mouse Bone Marrow on Lymphoid Tumor Production Following Irradiation.* (24051)

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Much recent work has been focused on protection of mice against lethal doses of irradiation by bone marrow and spleen(1-7). In an earlier paper(8), using thymic weight regeneration after sublethal doses of x-irradiation as an assay endpoint, only isologous or compatible† marrow was effective. Previous work on another effect of marrow injection, namely the inhibition of radiation-induced lymphoid tumors, utilized only isologous marrow(9,10). It seemed necessary to determine whether this marrow effect is also strain-specific. For this purpose, 2 inbred strains of mice and their F_1 hybrid were employed, since the genetics of tissue transplantation(11) predict that marrow from an inbred strain should be accepted (a) under isologous conditions, in other mice of the same strain, and (b) in a limited homologous situation in their hybrids; whereas hybrid marrow should be rejected by hosts of either parental strain and accepted only by isologous hybrids.

Materials and methods. Strain C57BL mice and reciprocal (C57BL x BALB) F_1 hybrids of both sexes were randomized into groups and exposed to fractionated total body irradiation starting at 33 ± 3 days of age. The C57BL mice received 4 doses of 168 r at 7-day intervals, and the F_1 hybrids 4 doses of 225 r at 7-day intervals.‡ Immediately following last irradiation, 3 groups of each series were injected intravenously with a suspension of approximately 30-40 x 10^6 bone marrow cells (equivalent to contents of 2 femurs/0.5 ml Locke's solution/recipient) from C57BL, BALB, or F_1 hybrid donors, respectively, of the same sex and of approximately same age. An additional group in each series served as irradiated control, receiving no bone marrow. The animals were maintained under identical conditions, with free access to Purina laboratory chow and water. Any dead animal was routinely autopsied; representative viscera and lymphoid tissues were fixed for histological study, unless a typical lymphoma was grossly apparent. An attempt was made to watch particularly carefully those animals which had received homologous marrow. When labored breathing or enlargement of nodes or spleen indicated that a lymphoma was present, the animal was killed and autopsied; when possible, fragments of its tissue were transplanted subcutaneously into normal mice of the 3 genotypes, to determine whether repopulation of the irradiated host thymus by the injected bone marrow cells might have occurred.

Results. Results are presented in Fig. 1 and 2. The high incidence of lymphoid tumors arising in C57BL mice after this dose of irradiation alone (27 of 30 = 90%), was unaltered by injection of either BALB or F_1 hybrid homologous bone marrow (32 of 34, 34 of 36 = 94%), whereas isologous C57BL marrow exerted, as before, a powerful inhibitory influence (9 of 37 = 24%). By contrast, in F_1 hybrids, where 51 of 52 (98%) of irradiated controls developed lymphomas, both isologous hybrid marrow and compatible marrow from either parent were capable of inhibiting lymphoid tumor development (hybrid, 6 of 36 = 17%; C57BL, 4 of 29 = 14%, BALB, 11 of 38 = 29%).

Table I presents results of transplanting fragments of tumorous tissue, in most cases from the thymus, subcutaneously into normal animals of the 3 different genotypes. Transplantation of tumors arising in C57BL hosts

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† The term "isologous" as used here refers to marrow from animals of identical genotypes; "compatible" as used here refers to special case of parental marrow in a hybrid host, where, despite differences in genotype, the parental tissue can "take" without exciting an immune response from hybrid host.

‡ Physical factors were 120 Kvp, 7.5 ma, 0.25 mm Cu + 1.0 mm. Al added filter, 30 cm target-mouse distance, output 24 r/minute.

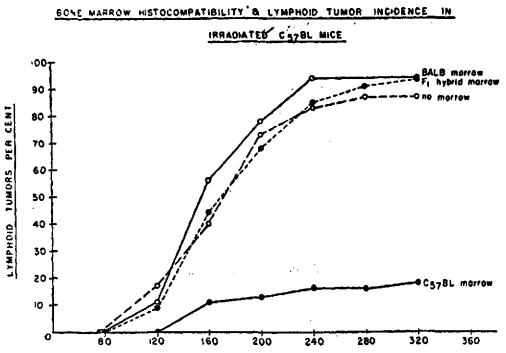

FIG. 1.

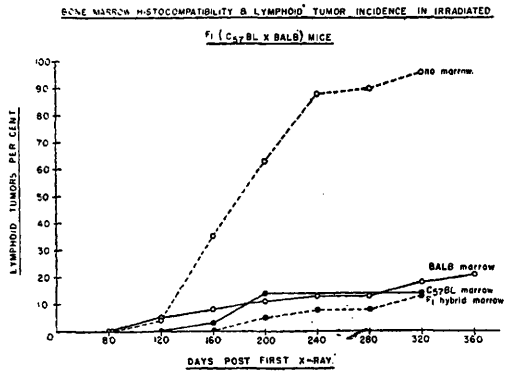

FIG. 2.

produced positive takes only in other C57BL mice, irrespective of marrow donor strain. However, similar tests of 9 tumors arising in F_1 hybrids after homologous parental marrow injection (1 C57BL, 8 BALB), led to positive takes in 3 instances in BALB mice, indicating that in these 3 cases repopulation of F_1 hybrid thymus by BALB marrow had occurred. Since it was not possible to test all such tumors, the frequency with which thymic repopulation by compatible homologous marrow may occur under these conditions is not established.

Discussion. It is clear that only isologous or compatible bone marrow is capable of inhibiting lymphoid tumor development at these radiation doses. This is, in fact, exactly the result obtained with respect to thymic weight regeneration after sublethal x-ray doses(8). The fact that sublethal radiation dose levels were employed is, however, an important limiting factor in interpretation of these data, since host immune mechanisms, though impaired after such doses, remain capable of destroying foreign cells(7). When this factor is taken into consideration, it becomes apparent that lymphoid tumor development was inhibited only in those donor-host combinations in which the injected marrow cells may be presumed to have remained viable.

It cannot properly be inferred from this, however, that direct repopulation of the thymus by injected marrow cells is necessarily the mechanism by which marrow facilitates thymic weight recovery or blocks lymphoma

TABLE I. Transplantation Behavior of Lymphoid Tumors Arising in Irradiated Marrow-Injected Mice.

Source of tumor	No. of tumors tested	Positive transplantation takes in C57BL	Hybrid	BALB	Remarks
C57BL + C57BL marrow	0				
" + hybrid "	14	28/35	16/35	0/14	1 tumor grew in no host
					4 *Idem* C57 only
					9 " hybrids also
" + BALB "	18	33/43	18/40	0/43	1 " no host
					6 " C57 only
					11 " hybrids also
" (no marrow)	4	4/11	2/11	0/ 6	1 " no host
					2 " C57 only
					1 " hybrids also
Hybrid + hybrid marrow	0				
" + C57BL "	1	0/ 3	0/ 3		
" + BALB "	8	0/13	17/26	7/28	5 " hybrids only
					3 " BALB also
" (no marrow)	6	0/15	3/16	0/16	4 " no host

development. Indeed, the 3 cited instances of apparent hybrid thymus repopulation by parental marrow cells represent *failures* of protective marrow action, since tumors did in fact develop in those hosts. Parenthetically, it may be pointed out that the cells of these 3 tumors, which behaved on transplantation like parental cells, had never been exposed to irradiation; this confirms and extends our previously reported evidence for an indirect mechanism in the induction of these tumors (12).

Recently, Gengozian *et al.*(13) reported regular occurrence in mice exposed to lethal doses of radiation, of thymic repopulation by injected rat marrow cells, as demonstrated by immunologic responses, chromosome counts and morphology. It was of particular interest that thymic weight failed to return to normal levels in these animals, whereas mice protected with isologous marrow cells showed the usual prompt recovery of thymic weight. Merwin and Congdon(14) also noted atrophy in thymuses of irradiated mice despite immunologic evidence that homologous cells derived from injected marrow had lodged in those thymuses. Thus it would seem that homologous cells may repopulate the irradiated thymus but do not support its recovery to normal weight and histologic appearance. Since our thymic weight regeneration assay has to date consistently yielded results with respect to marrow specificity which parallel those for lymphoid tumor induction, it seems likely that foreign marrow will also fail to inhibit lymphoid tumor development at higher radiation dose levels. To settle this point, however, another experiment at LD_{99} radiation dose levels will be needed, comparing lymphoid tumor incidence in mice protected with isologous versus homologous marrow. Such experiments are made difficult by occurrence of late deaths in homologous marrow-bearing animals, leaving inadequate numbers available over the entire tumor induction latent period.

The histocompatibility of homologous parental marrow in F_1 hybrid recipients is not a reciprocal relationship. Uphoff(15) has called attention to a phenomenon which she terms "the F_1 hybrid effect": whereas isologous hybrid marrow confers excellent protection on F_1 hybrids exposed to lethal doses of irradiation, injection of parental marrow into such irradiated hybrids often results in late deaths. She attributes these to a delayed immune response of injected marrow cells against the host, as postulated by Billingham, *et al.* (16) and Barnes and Loutit(17). This response occurs only when the 2 parental strains have different histocompatibility genes. We, too, have observed this effect in a few radiation mortality experiments, using strains C57BL, BALB, and their F_1 hybrids, where virtually none of the C57BL marrow-injected hybrids survived beyond 75 days (unpublished) and a similar result with injected spleen cells has recently been reported by Schwartz, *et al.*(18). In the present experiment, where hybrids received a sublethal but greater than usual dose of irradiation, 11 of 47 mice injected with C57BL marrow died after the 30-day period generally associated with radiation deaths. Irradiated hybrids receiving BALB or hybrid marrow did not manifest such late deaths.

Summary. 1. Lymphoid tumor development in irradiated C57BL mice was inhibited specifically by isologous marrow; homologous marrow, including F_1 hybrid marrow, was incapable of inhibiting the tumor process in C57BL mice after sublethal radiation exposures. 2. F_1 (C57BL x BALB) hybrid mice were protected against radiation-induced lymphomas by compatible marrow. The protection was essentially equal, whether the marrow injected was from F_1 hybrid or from either parental strain. The similarity between these results and those previously reported with respect to marrow-mediated thymic weight regeneration is discussed. Since host immune reactions to incompatible cells are not abolished at these radiation dose levels, it is inferred that these effects of marrow injection require persistent presence of viable marrow cells. 3. A number of lymphomas were tested for transplantability into various strains. Of those tested, none arising in C57BL mice injected with homologous marrow were found to be other than C57BL tumors, whereas 3 of 9 tumors which arose in F_1 hybrids despite marrow injections, grew in the parental mar-

row donor strain, indicating that repopulation must have occurred in these cases at least.

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Effect on Serum Cholesterol in Man of Mono-Ene Fatty Acid (Oleic Acid) in the Diet.* (24052)

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From analysis of data from 41 sets of dietary comparisons in controlled experiments on groups of 12 to 27 men, we derived the equation: 1) Δ Chol. $= 2.74\ \Delta S - 1.31\ \Delta P$, for average change in serum cholesterol concentration when diet is changed in fats 2 to 4 weeks, ΔS being the difference between percentages of total calories provided in the 2 diets from glycerides of saturated fatty acids and ΔP being corresponding difference in glycerides of poly-unsaturated fats(1,2). This equation satisfactorily predicted group average responses of men in calorie equilibrium with constant amounts of protein and vitamins but with different amounts and kinds of fats in the diet. The original approach to this solution included provision for mono-enes (M) in the diet in the equation form: 2) Δ Chol. $= b\Delta S + c\Delta M + d\Delta P$. However, in all analyses the value of coefficient c proved not significantly different from zero, *i.e.* no influence of mono-ene could be proved, so final analysis was made in the form of Equation 1), above. After Equation 1) appeared, Ahrens *et al.*(3) published results from formula feeding experiments and more recently(4) they agree that their data satisfactorily conform to Equation 1). But they state that Equation 1), "could have been expressed just as accurately, and less mysteriously, in terms of iodine value of dietary fat" (ref. 4, p. 253). Further, they question the lack of effect of dietary mono-ene (oleic acid) on serum cholesterol level in man. *Iodine Value* vs. *2S-L.* Ahrens *et al.*(4) propose, in effect, that Equation 1) be simplified by approximation: 3) Δ Chol./1.35 $= 2\Delta S - \Delta L$, rounding the coefficients and using L to refer to linoleic acid, since almost all the poly-unsaturated fatty acid in most ordinary food fats is actually linoleic acid. Further, they

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