

RADIATION LEUKEMIA IN C57BL/6 MICE
III. Correlation of Altered Expression of Terminal
Deoxynucleotidyl Transferase to Induction of Leukemia*

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Whole-body irradiation of a variety of inbred strains of mice has been shown to induce thymic lymphomas (1-3). Although considerable research has been directed at the etiology of radiation-induced leukemia in strains of mice such as C57BL, the factors involved are still problematical. Initially it was proposed that primary tumors in irradiated mice were induced by the activation of a latent endogenous C-type virus, based on the observation that leukemogenic viruses could be obtained by continued passage of cell-free extracts (4-6). However, detailed serological and virological studies have demonstrated that there is little correlation between endogenous ecotropic virus expression and induction of leukemia by irradiation in C57BL/6 mice (7-9). Moreover, irradiation induces leukemia in mice such as the NIH Swiss (10) and C57L (11) which genetically lack an endogenous ecotropic C-type virus. Serological and structural analyses of the virus isolated from radiation-induced leukemia indicate that this virus may be a recombinant virus which may have developed after the primary event of radiation-induced leukemia (12). Thus, it appears that the primary etiological factor in radiation-induced leukemia does not involve complete endogenous ecotropic C-type virus expression. Therefore, this disease is quite distinct from the spontaneous leukemias in AKR mice, which are more clearly associated with active replication of endogenous ecotropic C-type viruses (13, 14).

In spite of the lack of a consistent etiological factor associated with radiation-induced leukemia, a number of biological phenomena have been described for radiation-induced leukemias which bear directly on the possible mechanisms of induction. First, the regimen of irradiation has been shown to be extremely important (3), in that a single irradiation of 350 rads is only weakly leukemogenic, whereas four weekly doses of 175 rads is highly leukemogenic. This observation argues against a simple mechanism of somatic mutation and supports a more complex inductive mechanism (6). A second observation is that the primary leukemogenic effects are in the bone marrow. This is illustrated by the ability to block the leukemogenic effect of whole-body irradiation by shielding the legs (15) and by the observation that early after irradiation, preleukemic cells can be transferred to appropriate recipients by bone marrow cells but not by thymocytes (16). This effect is also evident by the ability to induce leukemia in thymectomized mice which have received a normal thymus

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transplant after irradiation (17, 18). A third observation is that, although the primary effects of irradiation are in the bone marrow, the thymus is required for ultimate expression of the neoplastic state (6, 17). Lastly, a striking effect in radiation leukemia is the ability of normal bone marrow reconstitution after irradiation to abrogate leukemia induction (19). This effect provides a strong argument against the concept of an infectious process and suggests a primary effect on bone marrow stem cell populations. Taken together, these observations suggest that radiation-induced leukemia is associated with specific alterations in the early stages of thymocyte differentiation occurring in the bone marrow.

In an effort to study the changes induced by irradiation in early thymocyte differentiation, we have studied the distribution and regulation of the enzyme terminal deoxynucleotidyl transferase (TdT)¹ in mice. This enzyme has been purified and its *in vitro* activity characterized (20–22). TdT has been found only in thymocytes and bone marrow in a variety of species. In the thymus, TdT is restricted to the major thymocyte subpopulation characterized by cortisone sensitivity and moderate density (23–25). In the bone marrow, TdT is associated with a minor subpopulation separable on bovine serum albumin (BSA) discontinuous gradients (26). It has been suggested that the expression of TdT in the bone marrow is associated with a prothymocyte stem cell, based on the observation that treatment of bone marrow cells with thymopietin induces the expression of theta on TdT-containing cells (27). Similarly, the expression of TdT in this bone marrow subpopulation is thymic-dependent, because only low levels of TdT are found in nude or thymectomized mice but can be induced both *in vivo* and *in vitro* with thymosin fraction V, a thymic hormone preparation (28, 29). These results have therefore strongly suggested a proposed pathway of differentiation involving the thymic hormone-dependent, induction of TdT, followed by the induction of θ and presumably subsequent migration to the thymus. In the present studies we have utilized this information to assess the changes that occur in these populations which are specific to leukemogenic doses of irradiation. The results demonstrate that very striking changes are induced which correlate with the ultimate induction of leukemia.

Materials and Methods

Mice. C57BL/6 and NIH Swiss nu/nu specific pathogen-free mice used in these experiments were obtained from the Frederick Cancer Research Center's Animal Production Area (Frederick, Md.). Ages of different mice are given in Results.

Cell Fractionation. Preparation of the cells and subsequent fractionation on discontinuous BSA gradients have been described previously (26).

Enzyme Preparation. Preparation of crude cell-free extracts and enzyme purification on phosphocellulose pll columns (Whatman, Inc., Clifton, N. J.) have been described previously (25).

TdT Assay. The TdT assay, adapted from Kung et al. (23), has been previously described (25). 1 U of enzyme activity is defined as the amount catalyzing the incorporation of 1 pmol of deoxyguanosine 5'-triphosphate (dGTP) into acid-insoluble material per hour. Specific activity was calculated from the total enzyme activity recovered from phosphocellulose per 10⁸ nucleated viable cells. Under the conditions of the assay, the lowest activity detectable is ≈ 0.05 pmol of dGTP incorporated.

¹ Abbreviations used in this paper: BSA, bovine serum albumin; dGTP, deoxyguanosine 5'-triphosphate; TdT, terminal deoxynucleotidyl transferase.

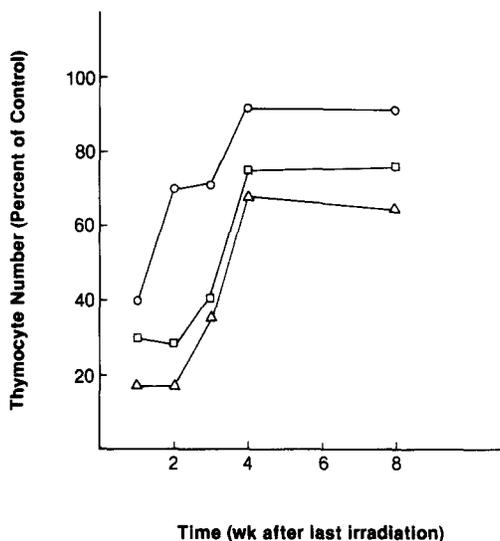


FIG. 1. The effect of irradiation on the number of thymus cells. C57BL/6 mice, 4-wk-old, were irradiated at weekly intervals with 175 rads either once (O), three times (□), or four times (Δ). Thymocyte numbers were determined as described in Materials and Methods and are expressed as the percentage of the number of thymocytes found in unirradiated, control mice. Percentages are the average from three to five individual mice at each point.

Thymectomy. 3-wk-old C57BL/6 mice were thymectomized according to the procedure of Sjodin et al. (30).

In Vitro Induction. For induction experiments, cells were washed three times after BSA gradient fractionation in Ham's F-12 medium containing 100 U/ml penicillin and 100 μ g/ml streptomycin. After being washed, cell concentrations were adjusted to 1×10^8 cells/ml in the same medium containing antibiotics, 20% fetal bovine serum (Flow Laboratories, Inc., Rockville, Md.), and different concentrations of thymosin or spleen fraction V, and β_3 , a thymosin component. After incubation for 12 h, cells were washed twice with Ham's F-12, and extracted as described previously for TdT activity (28).

Radiation. Mice were irradiated with the doses and at the ages indicated in Results by the method previously described (8). The source was a Phillips MG-301 X-ray therapy unit (Phillips Electronic Instruments, Mount Vernon, N. Y.) with a 0.2-mm³ filter operated at 10 mA and 300 kV with a dose rate of 175 rads/min.

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.

Results

The effects of 1, 3, or 4 whole-body weekly irradiations with 175 rads on the thymus cell number are shown in Fig. 1. Each regimen of irradiation results in an initial loss of thymocytes of between 60 and 80% at 1 wk post-irradiation. With 1×175 rads there is a subsequent repopulation such that at 3–4 wk postirradiation 90% of the initial cell number is present. With either 3 or 4×175 rads, repopulation of the thymus after the last irradiation occurs somewhat slower and recovery at 4 wk is only ~60–80% of the cell number found in unirradiated controls. As previously reported (8), the leukemia incidences in C57BL/6 mice given 1, 3, or 4 weekly irradiations with 175 rads are ~0, 4, and 70%, respectively. The peak incidence of leukemia occurs at 4–5 mo after the last irradiation in mice treated with 4×175 rads.

The effect of irradiation on TdT activity in the thymus and in fractionated bone

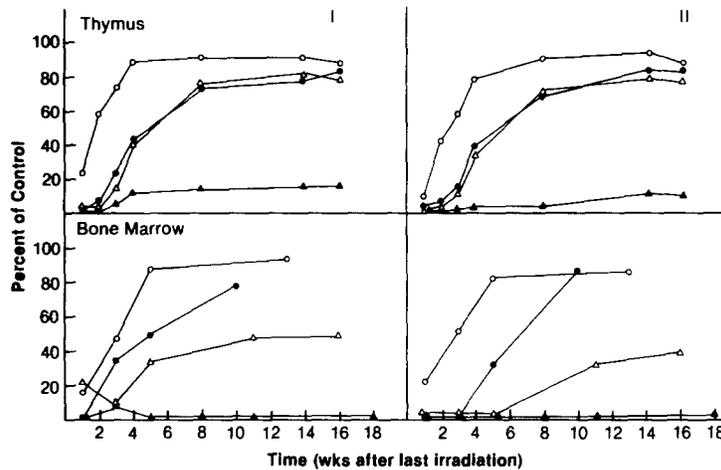


FIG. 2. The effect of irradiation on TdT activity in thymus and bone marrow. C57BL/6 mice at 4 wk of age were irradiated (either once [O], twice [●], three times [Δ], or four times [▲]) at weekly intervals with 175 rads. The specific activity of TdT peaks I and II was determined after phosphocellulose p11 chromatography as described in Materials and Methods. TdT activity associated with thymocytes is shown in the top panel and TdT associated with fraction A cells of BSA gradient fractionated bone marrow cells is shown in the bottom panel. Each point represents the activity found in three to five pooled thymuses or the pooled bone marrow cells from 20–30 mice. The data are expressed as the percentage of the specific activity found in thymocytes or bone marrow cells of unirradiated control mice.

marrow cells of mice irradiated with 1, 2, 3, or 4 weekly doses of 175 rads is shown in Fig. 2. To compensate for the changes in total cell numbers, the data are presented as the specific activity (enzyme activity per 10^8 cells) expressed as the percentage of nonirradiated controls. Because TdT activity is associated with two enzymatic peaks of activity separable on phosphocellulose (23, 25), peak I and II activities are shown separately in Fig. 2. The TdT activity in the bone marrow has been shown to be associated with a minor subpopulation of cells separable on BSA gradients (26) and the results presented here represent the specific activity in bone marrow fraction A cells. In the thymus and bone marrow, TdT peak I and II activities were dramatically reduced 1 wk after a single irradiation with 175 rads. Subsequently, the specific activity of peak I and II activities in the thymus and bone marrow increased such that by 4 wk postirradiation the specific activity of TdT was comparable to untreated controls. With either two or three weekly doses of 175 rads, the specific activity of TdT was similarly reduced and recovered with time, although recovery took considerably longer and the specific activity of TdT in the bone marrow returned to only ~50% of the control values. With four weekly irradiations of 175 rads, the specific activity of TdT was initially reduced to undetectable levels in both the thymus and bone marrow fraction A cells. In the bone marrow, TdT activity remained below detectable levels for at least 18 wk after the last irradiation. In the thymus, TdT activity remained at levels of <20% of that found in control unirradiated mice. These results demonstrate that one consequence of the leukemogenic dose of irradiation (4×175 rads) is the striking reduction or elimination of TdT activity in both the thymus and bone marrow. Because the results are expressed as the specific activity and, as shown in Fig. 1, cellular reconstitution of the thymus occurs at 4 wk after the last irradiation, the reduction of TdT activity indicates the specific loss of a subpop-

TABLE I
*Correlation of TdT Expression and Overt Leukemia in Thymocytes from Individual Mice After Irradiation**

Mouse No.	Wk Post-ir-radiation	Tumor status	sp act TdT	
			I	II
1	6	Normal	37	98
2	8	Thymoma‡	347	753
3	10	Normal	17	155
4	16	Thymoma	3,612	1,872
5	16	Normal	21	137
6	20	Normal	19	81
7	22	Thymoma	2,982	926
Unirradiated control	16	Normal	424	1,937

* C57BL/6 mice were irradiated with four weekly doses of 175 rads beginning at 1 mo of age. Individual mice were examined at the indicated times for the levels of TdT in thymocytes as described in Materials and Methods. Specific activity of TdT is the picomoles of dGTP incorporated per hour per 10^6 nucleated viable thymocytes. Normal mice were those showing no gross evidence of thymus enlargement indicative of the onset of leukemia or enlargement of peripheral lymphoid tissues.

‡ This particular thymoma was at a very early stage of development as judged by only a two- to threefold thymus enlargement and no involvement of peripheral sites. The other cases were overt thymomas with grossly enlarged thymuses and peripheral involvement.

ulation of cells which expresses TdT. It should also be noted that loss of TdT in the thymus or bone marrow was not associated with the appearance of TdT-positive cells in the spleen or lymph nodes and there was no expression of TdT in the other fractions of bone marrow cells (data not shown).

The relationship of TdT expression to the subsequent development of leukemia is shown in Table I. As previously demonstrated, radiation-induced thymic lymphomas of C57BL mice are TdT-positive (25). The thymic-localized cells express peaks I and II at levels comparable to age-matched nonirradiated controls, whereas leukemic thymocytes infiltrating peripheral lymphoid tissues generally have only peak I activity. As shown in Table I, high levels of TdT activity in the thymus were only detectable in overtly leukemic mice. Irrespective of the time after irradiation, mice that were not overtly leukemic had the characteristic low levels of TdT. Interestingly, mice which survived past the peak of tumor incidence (longer than 4 mo) similarly had low levels of TdT activity. These results suggest that the reappearance of high levels of TdT activity is correlated with the onset of the overtly leukemic phase.

To examine further the correlation of the loss of TdT with the ability to induce leukemia, we examined the effect of 350 rads and bone marrow reconstitution of 4×175 rads-treated mice on TdT expression. As previously described, a single irradiation of 350 rads induces incidences of leukemia of <5% compared with incidences of >70% with the fractionated regimen (8). As shown in Fig. 3, both regimens of irradiation initially eliminated detectable TdT activity; however, in mice treated with 350 rads, TdT activity was rapidly recovered, whereas no such recovery was seen in 4×175 rads-treated mice. The ability of bone marrow reconstitution to

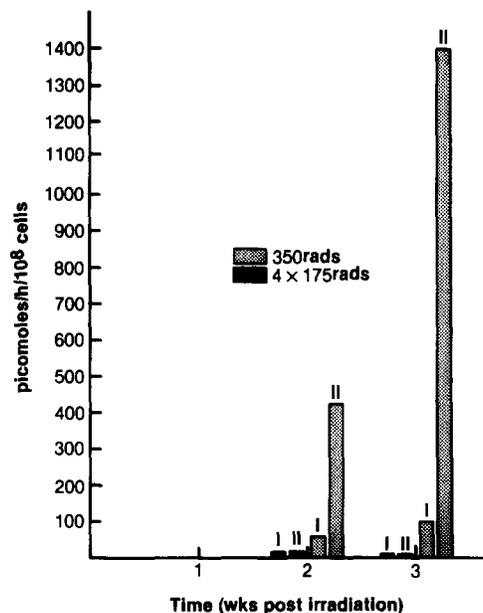


FIG. 3. Differential effects of 350 rads and 4×175 rads on thymus TdT activity. C57BL/6 mice at 4 wk of age were irradiated either once with 350 rads or were irradiated with four weekly doses of 175 rads. TdT activity in thymocytes was determined at 2 and 3 wk after the completion of irradiation as described in Materials and Methods. The specific activity was determined from pooled thymocytes from three to five mice in each case.

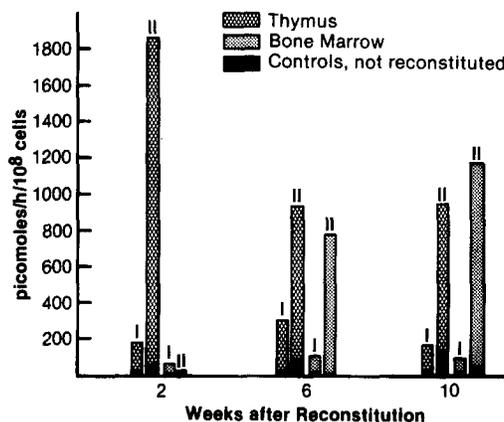


FIG. 4. The effect of bone marrow reconstitution on thymocyte TdT activity in mice given 4×175 rads. C57BL/6 mice at 4 wk of age were irradiated with four weekly doses of 175 rads. Immediately after the last irradiation, the experimental group was reconstituted with 1×10^7 normal bone marrow cells from 4-wk-old C57BL/6 mice. The levels of TdT were subsequently examined at 2, 6, and 10 wk as described in Materials and Methods. TdT activity in the thymus was determined from pooled thymuses of three to five mice. TdT activity in the bone marrow was determined using BSA gradient fractionated bone marrow cells from 20-30 mice. Bone marrow TdT activity is that associated with BSA gradient fraction A cells.

abrogate induction of leukemia by 4×175 rads has been described previously (8, 19). We therefore examined TdT levels in control and bone marrow-reconstituted mice. As shown in Fig. 4, bone marrow reconstitution of 4×175 rads-treated mice

TABLE II
Differential Effects of 4×175 rads on TdT Activity in Mice of Various Ages

Age at start of irradiation	TdT Activity (% of control)*	
	I	II
<i>mo</i>		
1	8.7	5.0
2	20.5	18.1
3	96.7	86.6

* C57BL/6 mice at 1, 2, or 3 mo of age were given four weekly doses of 4×175 rads. The thymocyte TdT activity was determined 1 mo after the last irradiation as described in the Materials and Methods. The results are presented as the percentage of activity found in unirradiated normal control mice.

resulted in a rapid reappearance of TdT in the thymus 1 wk after reconstitution. Interestingly, the specific activity of TdT in the thymus was approximately twice the control levels at 1 wk and decreased to normal levels by 6 wk. In contrast, the reappearance of TdT activity in the bone marrow was slower, although by 6 wk after reconstitution, normal levels were present. The above results therefore illustrate a striking correlation between the loss or recovery of TdT activity and the ultimate induction or lack of induction of thymic lymphomas.

To test further the correlation of the loss of TdT activity with the induction of leukemia, we examined the effect of 4×175 rads on mice of various ages. As previously demonstrated (8, 31), the ability of 4×175 rads to induce leukemia is highly age-dependent such that irradiation of mice at 1, 2, or 3 mo of age at the start of the regimen resulted in incidences of 75, 53, and 25%, respectively. As illustrated in Table II, there is also a striking age-dependent effect on the recovery of TdT activity when examined 1 mo after the last irradiation. As above, mice at 1 mo of age had <10% of the normal levels of TdT. Mice irradiated at 2 mo of age similarly had reduced levels of TdT, although these levels were ~30% of the normal. Mice irradiated at 3 mo of age had essentially normal levels of TdT by 1 mo postirradiation. Thus, as above, the ability to induce leukemia is strongly correlated with changes in TdT levels.

The loss of TdT-positive cells after leukemogenic doses of irradiation could be due to the absence of the requisite factors for differentiation. Because it has been demonstrated that thymosin fraction V can induce the expression of TdT in bone marrow cells from athymic mice both in vivo and in vitro (28, 29), we examined cells from various tissues of 4×175 rads-treated mice for their responsiveness to thymosin fraction V in vitro. As shown in Table III, no inducible cell populations were observed in 4×175 rads-irradiated mice. These results suggest that the absence of TdT is not simply due to the absence of the requisite factors for induction but rather appears to be due to a more complex effect on the responsive stem cell population.

To examine the effect of 4×175 rads on presumptive TdT-positive stem cells, we examined the effect of irradiation on thymectomized mice. As previously described (28), thymectomy results in a loss of TdT activity in the bone marrow and the accumulation of a presumptive stem cell, which under the influence of thymosin

TABLE III
Lack of Induction of TdT Activity in Thymus, Spleen, and Lymph Nodes from 4×175 rads-Irradiated Mice

Wk Post-irradiation	Peak	Thymus		Spleen		Lymph nodes	
		Control	Thymosin	Control	Thymosin	Control	Thymosin
1	I	ND	ND	3.8	4.9	ND	ND
	II	ND	ND	5.2	3.0	ND	ND
2	I	ND	ND	<0.1	1.5	ND	ND
	II	ND	ND	<0.1	4.0	ND	ND
4	I	11	17	1	<0.1	ND	ND
	II	29	41	<0.1	<0.1	ND	ND
8	I	12	19	7.8	7.3	ND	ND
	II	31	20	6.4	4.1	ND	ND
16	I	21	27	4.3	5.4	8.7	3.8
	II	35	48	6.8	7.9	<0.1	<0.1

C57BL/6 mice were irradiated beginning at 1 mo of age with four weekly doses of 175 rads. At the indicated times, various lymphoid tissues were obtained and cell suspensions prepared as indicated in Materials and Methods. In the case of spleen and lymph node cells, the cell suspensions were further fractionated on BSA gradients and fraction B cells were used. The cells were subsequently incubated in media containing 25 ng/ml thymosin fraction V or saline for controls. After 12 h, the cells were extracted and assayed for TdT activity as described in Materials and Methods.

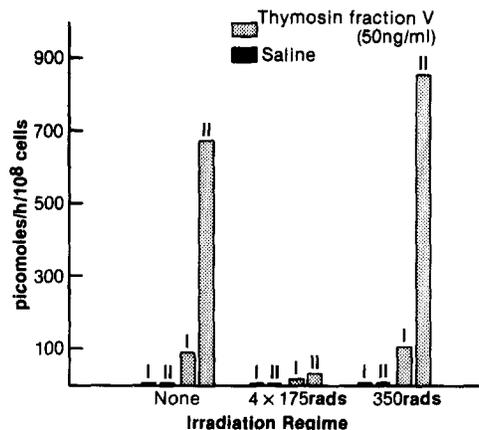


FIG. 5. In vitro induction of TdT in bone marrow cells from thymectomized irradiated mice. C57BL/6 mice at 4 wk of age were thymectomized and irradiated with four weekly doses of 175 rads beginning at 5 wk of age. For induction experiments, bone marrow cells were collected from 20–30 mice at 4 wk after the last irradiation and fractionated on BSA gradients. Fraction B cells were used as previously described (28) for induction of TdT. Bone marrow cells were incubated with either thymosin fraction V or saline at 5×10^6 cells/ml for 12 h at 37°C in Ham's F-12 as described in Materials and Methods. TdT activity was determined after phosphocellulose chromatography as described in Materials and Methods.

fraction V can be induced to express TdT. In the experiment shown in Fig. 5, C57BL/6 mice were thymectomized and then given either no irradiation, 1×350 rads, or 4×175 rads. As illustrated by the results, TdT activity was induced in bone marrow fraction B cells from nonirradiated mice or mice given the nonleukemogenic dose of irradiation, but was not inducible in cells from mice treated with 4×175 rads. These results indicate that leukemogenic doses of irradiation specifically elimi-

nate or alter the bone marrow stem cell population inducible for TdT. Furthermore, these results indicate that the loss of the TdT stem cell is thymus-independent, suggesting that the primary effect of irradiation is only in the bone marrow.

Discussion

The results demonstrate that leukemogenic doses of irradiation specifically alter the normal differentiation of TdT-positive cells in bone marrow and thymus. This is manifested by the lack of the repopulation of bone marrow and thymus with TdT-positive cells after recovery of an initial radiation-induced loss of cells. The correlation of this response to induction of thymic lymphomas was demonstrated in several ways. It has been previously demonstrated that the ability of whole-body irradiation to induce leukemia is highly dependent upon a fractionated regimen and that a striking increase in the incidence of leukemia is only seen after four weekly doses of 175 rads (3, 8). As shown here, the ability of irradiation to eliminate TdT-positive cells during thymic repopulation was similarly dependent in that in those mice given 1, 2, or 3 \times 175 rads, TdT activity was recovered, whereas little recovery of TdT activity was seen in mice given 4 \times 175 rads. Also, when mice were given the nonleukemogenic dose of a single treatment with 350 rads, TdT activity returned to normal levels with time after irradiation. When the leukemogenic effect of 4 \times 175 rads was abrogated by reconstitution with normal bone marrow immediately after the last irradiation, normal levels of TdT activity were obtained in both bone marrow and thymus within 6 wk after reconstitution. The age-dependent loss of susceptibility to induction of leukemia by 4 \times 175 rads was similarly correlated with the ability to recover TdT activity in the thymus. Thus, the phenotypic loss of TdT-positive cells is strongly correlated with the subsequent development of thymic lymphomas.

The molecular basis for the changes in TdT levels induced by leukemogenic doses of irradiation is not known. The most obvious explanation for the lack of a differentiation-specific enzyme such as TdT is the loss of the factors required for induction of differentiation. Because we have previously demonstrated that thymosin fraction V can induce TdT in a presumptive pre-T-cell from the prothymocyte bone marrow population (28, 29), we examined 4 \times 175 rads-treated mice for inducible cell populations. The results clearly demonstrate the lack of any such cells even in the bone marrow, suggesting that the lack of TdT expression is more complex than simply the absence of the appropriate factors required for induction. The complexity of the effect was even more evident in the results using irradiated, thymectomized mice. In particular, 4 \times 175 rads irradiation of thymectomized mice appears to have eliminated the presumptive stem cell which normally is inducible by thymosin fraction V to express TdT. Again, this effect was specific for leukemogenic doses of irradiation in that a comparable effect was not seen in thymectomized mice given 350 rads. These results also suggest that the initial effects observed on TdT expression are probably thymus-independent, the primary effect being in presumptive stem cell populations in the bone marrow. This result is consistent with a number of biological experiments which have demonstrated that the primary leukemogenic effects of irradiation are in the bone marrow and are thymus-independent (6, 16, 32).

In spite of the lack of recovery of TdT activity in the thymus after leukemogenic doses of irradiation, the thymus does become repopulated. Several questions therefore arise concerning the properties of these cells and their significance to leukemia. Our

results have demonstrated that the onset of leukemia is associated with and actually predictable by the reappearance of TdT. This would argue against the leukemic cell population emerging from the cell population found in the thymus after irradiation, although phenotypic changes in this population, perhaps associated with transformation, must be considered. More importantly, however, it has been demonstrated that during the preleukemic phase, transfer of bone marrow cells but not thymocytes can produce leukemia (16). This observation suggests that in fact the leukemogenic potential is not associated with the thymus-repopulating cells, but rather with an altered bone marrow stem cell population. The properties and the origin of the thymus-repopulating cells are largely unknown. Two possibilities include repopulation by cells of a lineage distinct from the lineage that expresses TdT or repopulation by cells of the appropriate lineage, but which because of somatic alterations or alterations in differentiation do not express TdT. Although we cannot presently differentiate between these possibilities, we have observed an increase in the specific activity of the enzyme 20- α -hydroxysteroid dehydrogenase in the repopulating thymocytes (unpublished data). Because this enzyme has been shown to be a specific marker for the minor thymocyte population (33), it appears that the repopulated thymus may have an altered distribution of the major and minor populations. Unfortunately, the relationship of the major and minor populations in differentiation has not been clarified to determine whether unique cell lineages are involved.

The results reported here as well as previous results suggest a possible mechanism for the induction of leukemia by irradiation. In particular it appears that an initial effect of leukemogenic doses of irradiation is the reduction or elimination of a bone marrow stem cell population which can be induced to express TdT. The second phase of leukemia induction then involves the repopulation of the thymus with cells of a lineage which we feel is distinct from the TdT-positive cell lineage. This population may be of the cortisone-resistant, TdT-negative, minor thymocyte population. As a consequence of this aberrant repopulation, we propose a third phase in which differentiation of TdT-positive cells may be actively suppressed. This is suggested by the time-dependent loss of the ability of normal bone marrow reconstitution to either abrogate leukemia (6, 19) or allow normal recovery of TdT-positive cells (unpublished data). This active suppression of differentiation of bone marrow stem cells could be mediated by thymic factors which antagonize the ability of the requisite factors to induce TdT-positive cells. Interestingly, we have recently demonstrated that the induction of TdT by thymosin fraction V is concentration-dependent and that at high concentrations there is a marked inhibition of induction; whereas the concentration curve for the appropriate peptide hormone, β_3 , purified from thymosin fraction V, shows no inhibition of induction at high concentrations (29). These results have been interpreted to suggest that thymosin fraction V contains a factor(s) which can suppress differentiation of TdT-positive cells, and thus a balance in appropriate factors may be essential to promote normal differentiation. The last phase in leukemia induction we envision involves an aberrant stem cell of the TdT-positive cell lineage which, because of the suppressive environment established by irradiation, emerges as a population lacking the ability to respond to normal controls in differentiation and thus resulting in uncontrolled proliferation. Such an aberrant population could arise by somatic mutation, phenotypic instability, changes induced by endogenous viruses, etc. Irrespective of the mechanism, the emergence of such cells is strongly implied by

the reappearance of TdT-positive cells as a prelude to overt leukemia and the lack of reappearance of such cells in "survivors." If correct, this hypothesis provides several opportunities for the regulation of the onset of leukemia. In particular it appears possible that elimination of specific factors, such as β_3 , which promote early steps of differentiation of TdT-positive cells may block the subsequent emergence of transformed cells. This is suggested by the observation that overtly transformed T cells do not arise in irradiated, thymectomized mice, although transfer of bone marrow cells to thymus-bearing recipients can transfer leukemia (16). Secondly, it may be possible to alter the cell populations found in the thymus postirradiation by thymic factors to allow a "normal" distribution of cell types. Although parts of this hypothesis are untested, it appears to be consistent with the known properties of radiation-induced leukemia. More importantly, however, the recent development of several new approaches to the study of T-cell differentiation and leukemia has provided the means to critically assess the hypothesis presented here.

Summary

The expression of terminal deoxynucleotidyl transferase (TdT) in the thymus and bone marrow of irradiated mice has been examined. Mice given the leukemogenic regimen of irradiation of four weekly doses of 175 rads starting at 1 mo of age show a long-term elimination of TdT activity in the bone marrow and a reduction of TdT activity in thymocytes. In such mice, the reappearance of normal levels of TdT in the thymus appears to only be associated with the onset of overt leukemia. This effect on TdT expression was shown to be uniquely associated with the leukemogenic regimen of irradiation in that nonleukemogenic irradiation or variations such as bone marrow reconstitution or age which reduce leukemias did not show the same phenotypic effects on TdT expression. The basis for the loss of TdT-positive cells was shown not to be due to the lack of the requisite factors involved in differentiation, but rather to the ability of leukemogenic doses of irradiation to reduce or eliminate an inducible bone marrow stem cell. These results are discussed with respect to the possible mechanisms involved in radiation-induced leukemias in mice.

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