Leukemia in the mouse, whether induced by chemical or physical agents or occurring spontaneously, frequently arises in the thymus (1). This discrete origin of the disease facilitates study of preleukemic changes that precede overt tumor development. Recent work has shown striking and characteristic changes in the pattern of cell surface antigens expressed by thymocytes during the preleukemic period in AKR mice, a widely studied strain developing a high incidence of spontaneous leukemia. By 6 mo of age, the surface phenotype of thymocytes from nonleukemic AKR mice comes to resemble the phenotype of thymic leukemia cells, i.e., low level of Thy-1 alloantigen, high level of H-2 alloantigens (2, 3), and increased expression of a range of surface antigens related to murine leukemia virus (MuLV) (2, 4). Parallel assays of infectious MuLV revealed increased levels of xenotropic MuLV (but not of ecotropic MuLV) in preleukemic thymus and tests on individual mice showed a close correlation between thymic levels of xenotropic virus and increased expression of MuLV antigens (4). Further analysis of viral isolates from preleukemic and leukemic AKR mice has led to the recognition of a new class of MuLV having both ecotropic and xenotropic properties as well as other unusual features (5), and this finding may be important in defining more precisely the nature of the transforming virus in AKR mice.

Just as AKR mice have been a favorite model for analyzing spontaneous leukemogenesis, most of our knowledge about radiation leukemogenesis has come from the study of C57BL mice (6). Mice of this strain develop thymic lymphomas with high frequency after exposure to fractionated doses of X-ray. The causative role of virus in X-ray leukemogenesis is still unclear. Although leukemogenic virus has been isolated from leukemic C57BL mice (7, 8), most radiation leukemias in this strain lack demonstrable MuLV or MuLV-related antigens (9-12). Cell surface antigens belonging to the TL (thymus-leukemia) system, however, are frequently found on X-ray-induced C57BL/6 leukemias (13). TL antigens are specified by the Tla locus which is closely linked to the H-2 complex on chromosome 17 (14). In normal mice, TL antigens behave as thymocyte-specific alloantigens, being found in some strains (TL+ strains) and not in others (TL− strains), such as C57BL/6 mice (13, 14). The unique feature of this system is that TL antigens may be anomalously expressed on leukemias occurring in mice of TL− strains (13, 14). This has been explained on the basis that all mice possess the structural gene for TL, but differ in whether they express TL

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genetic information or not. According to this view, the appearance of TL+ leukemias in TL- mice, such as occur in C57BL/6 mice, would result from activation of the normally silent Tla locus as a consequence of leukemogenesis (15-17).

Our assumption to date has been that anomalous TL expression coincides with the transformation event and the emergence of leukemia cells. However, the preleukemic changes in alloantigens and MuLV expression in AKR thymus have prompted us to examine this point directly in regard to X-ray leukemogenesis. What we have asked is whether TL can be detected in the thymus of X-irradiated C57BL/6 mice before the development of overt leukemia.

Materials and Methods

Mice. C57BL/6 (C57BL) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine.

X-Irradiation. 6-wk-old female mice received 150 R whole body irradiation (General Electric Maxitron, General Electric Company, Waterford, N. Y.) once weekly for 5 successive wk.

Antisera. The following typing sera were used: (a) A strain anti-EL4 (anti-H-2b), (b) (A-Thy-1.1 × AKR-H-2b)F1 anti-ASL1 (anti-Thy-1.2), (c) (C57BL-TL- × C57BL)F1 anti-ASL1 (anti-TL.1,2), and (d) C57BL-TL+ anti-ERLD (anti-TL.4). The polyvalent antiserum to MuLV is (W/Fu x BN)F1 rat anti-W/Fu leukemia (C58NT)D. This antiserum, which has been extensively studied, detects a broad range of MuLV-related antigens (2, 4, 10, 11, 18, 19).

Serological Techniques. For details of the cytotoxic test and absorption procedures, see previous publications from this laboratory (9, 11, 19).

Results

From past experience with the schedule of irradiation used in these experiments, >95% C57BL female mice (6 wk old at the time of initial X ray) develop leukemia, predominantly of thymic origin. In a recent series of 40 mice, the average latent period for overt leukemia development is 153 days (range 90-225) after the last course of X ray.

Expression of H-2, Thy-1, TL, and MuLV Antigens on Thymocytes of Irradiated Mice. Fig. 1 shows cytotoxic tests with thymocytes from individual C57BL mice 28-38 days after the last course of X ray. For comparison, thymocytes from normal C57BL mice (phenotype H-2b, Thy-1.2, TL-, Gα+) of similar age are included. The trend toward a higher level of H-2 alloantigens and a reduced level of Thy-1.2 alloantigen is consistent with the preleukemic changes previously reported for AKR mice and irradiated C57BL mice (2, 3). TL+ thymocytes were detected in a high proportion of the irradiated mice, with the number of TL+ cells in individual thymuses ranging from 20 to 95%. Fig. 2 summarizes the results of TL tests over the 53-day postirradiation period and shows that TL antigen was found in the thymus of 30 of 49 mice. Typing with anti-TL.4 or with anti-TL.1,2 antisera gave similar results, indicating concomitant preleukemic expression of TL specificities characteristically found on C57BL leukemias. Figs. 1 and 2 also show reactions with the polyvalent anti-MuLV antiserum. According to these tests, low to moderate levels of MuLV antigen expression were found on thymocytes of 20 of 42 irradiated mice.

No sign of overt leukemia was noted in irradiated mice during the time interval of this study. The size of TL+ cells did not differ from the predominant cell type found in normal thymus and transplantation tests and histological
section of selected thymuses revealed no evidence for the presence of leukemia cells. No correlation was noted between thymic weight and TL or MuLV antigen expression.

Absorption Tests for TL.1 and Gx (MuLV) Antigens. To confirm the specificity of TL and MuLV reactions, absorption tests were performed for TL.1 antigen (14) and for Gx, a type-specific antigen of the gp70 envelope glycoprotein of MuLV (11, 18, 20). There was a clear parallel between the results of direct cytotoxic tests and the absorption capacity of the same cell population.

Relation Between TL and MuLV Antigen Expression. The results of typing
FIG. 2. Expression of TL and MuLV antigens on C57BL thymocytes at various intervals after last exposure to X ray.

FIG. 3. Relation between TL and MuLV antigen expression on preleukemic thymocytes. Thymocytes from individual C57BL mice (10–53 days after last exposure to X ray) were typed with anti-TL and anti-MuLV. No correlation between TL and MuLV antigen expression is evident.

thymocytes from individual mice with anti-TL and anti-MuLV are plotted in Fig. 3. No apparent correlation exists between the expression of TL and MuLV antigens.

Discussion

The results indicate that activation of the Tla locus occurs during the preleukemic phase of radiation leukemogenesis. Individual mice show considerable variability with regard to the time this genetic event takes place, and it should be possible, by using thymic biopsy, to determine whether early appearance of TL correlates with early development of leukemia. Experiments involving transfer of bone marrow to lethally irradiated recipients should also resolve whether the X-ray-induced TL⁻ → TL⁺ conversion is due to changes in the precursor cells of bone marrow origin that repopulate the thymus or to changes in the stromal cells of the thymus that provide the environment for T-cell
differentiation. Much work has been done by Kaplan (6) defining optimal conditions for leukemia induction by X ray and it will be interesting to see if TL induction by X ray follows the same rules. Chemical agents, such as urethan and carcinogenic polycyclic hydrocarbons, are also known to be leukemogenic in C57BL mice, and we are now testing these agents for TL-inducing activity.

The considerable number of mice showing MuLV antigen expression on thymocytes during the preleukemic phase is somewhat surprising. C57BL radiation-induced leukemias rarely express MuLV-related surface antigens (9-12); in a recent survey of 24 C57BL leukemias for the two major cell surface determinants specified by MuLV, GCSA (9), and GIX (11), 21 leukemias were GCSA-GIX, 2 were GCSA+GIX, and 1 was GCSA+GIX. To explain this difference between MuLV expression in preleukemic and leukemic cells, we can assume that MuLV is activated only transiently and at very low levels during the preleukemic period, and it would be at this time, and not after overt leukemia development, that infectious virus might be most easily isolated. The work of Haran-Ghera (8) and Haas (21) gives considerable support to this idea. A question that has been repeatedly asked since the discovery of TL concerns the role of MuLV in the anomalous expression of TL in leukemias. The possibility that the Tla locus may represent the integration site of a defective MuLV genome appears unlikely in view of the close structural similarities between the TL product and the products of the closely linked H-2 complex (22). Another possibility is that MuLV, directly or indirectly, activates the Tla locus. Since leukemias known to be MuLV-induced are frequently TL- (14), TL activation is clearly not an invariable consequence of MuLV infection, but would be restricted to a special class of MuLV. The lack of any direct correlation between TL and MuLV expression on preleukemic thymocytes tells against this possibility, although the association may be too complex to show up in this way.

Summary

Anomalous appearance of TL (thymus-leukemia) antigens is a characteristic feature of radiation-induced leukemias of C57BL/6 mice. We now report that thymocytes of irradiated C57BL/6 mice express TL antigens long before the development of overt leukemia. Thus, TL is a marker for preleukemic changes occurring during radiation leukemogenesis. Low levels of murine leukemia virus (MuLV)-related antigens are also detected on preleukemic thymocytes. Comparative tests on individual mice show no direct correlation between TL and MuLV antigen expression.

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