Abnormal Thymic Development, Impaired Immune Function and γδ T Cell Lymphomas in a TL Transgenic Mouse Strain
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Summary
During derivation of transgenic mouse strains with various TL and TL/H-2 chimeric genes, one strain, TgTla'-3-1, introduced with a TL gene (Tla'-3), was found to have an abnormal thymic T cell population and to develop a high incidence of T cell lymphomas. To investigate the etiology of the thymic abnormalities and of the lymphomas, the development of lymphoid organs in transgenic mice was studied. The thymus of these mice goes through three unusual successive events: perturbation of thymic development during embryogenesis, disappearance of thymocytes between day 14 and day 21 after birth, and subsequent proliferation of large blast-like cells. These events are associated with the abolishment of T cell receptor (TCR) αβ lineage of the T cell differentiation, leading to preponderance of cells belonging to the TCR γδ L3T4- Lyt-2- double negative (DN) lineage. Bone marrow transplantation and thymic graft experiments demonstrate that the abnormality resides in the bone marrow stem cells rather than in the thymic environment. The expression of TL antigen in the transgenic mice is greatly increased and TL is expressed in a wide range of T cells, including normally TL- DN cells and L3T4+ Lyt-2- and L3T4- Lyt-2- single positive cells. These quantitative and qualitative abnormalities in TL expression most likely cause the abnormal T cell differentiation.

The γδ DN cells migrate into peripheral lymphoid organs and constitute nearly 50% of peripheral T cells. Immune function of the transgenic mice is severely impaired, as T cell function is defective in antibody production to sheep red blood cells, in mixed lymphocyte culture reaction to allogenic spleen cells and also in stimulation with concanavalin A. These results indicate that the γδ cells are incapable of participating in these reactions.

Molecular and serological analysis of T cell lymphomas reveal that they belong to the γδ lineage, suggesting that the γδ DN cells in this strain are susceptible to leukemic transformation. Based on cell surface phenotype and TCR expression of the DN thymocytes and T cell lymphomas, a map of the sequential steps involved in the differentiation of γδ DN cells is proposed. Tg.Tla'-3-1 mice should be useful in defining the role of TL in normal and abnormal T cell differentiation as well as in the development of T cell lymphomas, and further they should facilitate studies on the differentiation and function of γδ T cells.

1 Abbreviations used in this paper: B6, C57BL/6; DN, L3T4- Lyt-2- double negative; DP, L3T4+ Lyt-2+ double positive; HSA, heat-stable antigen; PFC, plaque-forming cell; Pgp-1, phagocytic glycoprotein 1; SP, L3T4+ Lyt-2- or L3T4- Lyt-2- single positive.

Mouse TL antigens belong to the family of MHC class I antigens. However, the expression of TL is distinct from other MHC class I antigens, being restricted to normal thymocytes and T cell lymphomas. Not all strains of mice express TL in the normal thymus, but even in such TL- strains an anomalous TL expression is found on T cell lymphomas (1, 2). To understand the structural basis for TL expression, we and others have isolated and characterized TL genes (3–6), and also analyzed TL expression in vitro by transfecting TL and TL/H-2 chimeric genes into L cells (7). These studies showed that TL genes resemble other MHC class I genes in their overall structure but have unique 5' regulatory elements. To investigate this further, we have recently derived several transgenic mouse strains in C3H/He (TL-) background with two TL genes, Tla'-3 isolated from the A-strain mouse (Tla', TL+), and T3b from TL+ leukemia arising in a C3H/He (B6)1 mouse (Tla', TL+), and with
two TL/H-2 chimeric genes (8). Analysis of the transgene expression showed that even TL− thymocytes of C3H/He provide conditions for the transcriptional activation of TLα-3, and that TLα-3 contains necessary elements for correct tissue-specific expression in normal thymocytes, whereas TLb and the endogenous TL genes of C3H/He lack these elements. During the course of the study, we found that one of the transgenic mouse strains, TgTLα-3-1, expresses large amounts of TL antigen in thymocytes, and has a small thymus consisting mainly of L3T4 (CD4)−Lyt-2 (CD8)− double negative (DN) cells. Furthermore, this strain develops a high incidence of lymphoid neoplasms.

The differentiation pathway of T cells bearing αβ TCR in the thymus has been extensively studied, and it has been conclusively demonstrated that the interaction between αβ TCR and MHC antigens plays a critical role in the development of thymic T cells (for review, see references 9–12). The maturation of αβ cells in the thymus has been staged using various cell surface markers (13, 14). The differentiation pathway of the other lineage, γδ cells, has also been vigorously investigated, and many aspects of γδ cells, including function, localization of different subsets, thymic selection of certain subsets and putative ligands, have been reported (for review, see references 15–18). γδ cells, particularly those localized in epithelial structures, are speculated to function in the primary defense of the body against infection and other insults (19). However, because of the limited number of γδ cells in lymphoid organs (20–22), analysis of their function and staging of their developmental pathway have been difficult.

In the present study, we have investigated the development of the immune system in TgTLα-3-1 mice to elucidate the role of the transgene in abnormal thymic differentiation. We found that T cell differentiation in the transgenic mice is forced to deviate to the γδ lineage through at least three unusual events in the thymus and that consequently γδ cells become abundant rather than αβ cells. To delineate the function and the maturation steps involved in the differentiation of γδ cells, we characterize DN thymocytes, γδ T cell lymphomas and immune responses of TgTLα-3-1 mice.

Materials and Methods

Mice. Derivation of the TL transgenic mouse strain, TgTLα-3-1, has been described elsewhere (8). Briefly, a DNA fragment containing TLα-3 isolated from an A-strain genomic library was injected into male pronuclei of fertilized eggs of C3H/He mice (TL−, Td−) (8, 23). The founder mouse of the TgTLα-3-1 strain contained seven copies of TLα-3 transgenes in a single chromosomal site. The offspring of the founder were interbred to produce homozygous stock, which then maintained by brother-sister mating. C3H/He mice were purchased from Japan SLC Inc. (Hamamatsu, Japan), and other mice were from our breeding colony or from the National Institute of Genetics (Mishima, Japan).

Histology. Tissues were fixed with Bouin’s solution and 5 μm sections were stained with hematoxyline-eosin.

Serological Analysis. Two-color analysis of cell surface antigens was performed with a fluorescence-activated cell sorter (FACStar; Becton Dickinson and Co., Mountain View, CA) using mAbs to TL (H168, reference 3), L3T4 (GK1.5, reference 24), Lyt-2 (53-7, reference 25), TCRαβ (H57-597, reference 26), TCRγδ (3A10, reference 22), CD3 (145-2C11, reference 27), heat-stable antigen (HSA) (11d, reference 28), phagocytic glycoprotein 1 (Pgp-1, Ly24) (NUS-50; provided by Dr. E. Nakayama, Nagasaki Univ., Nagasaki, Japan), IL-2 receptor α chain (IL2Rα) (7D4, reference 29), and Thy-1.1 and Thy-1.2 (purchased from Beckton Dickinson and Co.). Most antibodies were directly conjugated with fluorescein or biotin.

The secondary reagents, PE streptavidin (Biomed, Foster City, CA), FITC-labeled anti–hamster Ig (Caltag, South San Francisco, CA), anti–rat Ig (Tago Inc., Burlingame, CA) or anti-mouse Ig (Dako, Glostrup, Denmark) were used as appropriate.

Bone Marrow Transplantation and Thymic Graft. Recipient mice were lethally irradiated (10 Gy, Hitachi MBR-1520R; Hitachi, Tokyo, Japan) and 1 d later injected intravenously with 107 bone marrow cells from donor mice. After a few weeks, the lymphoid system of the mice was totally reconstituted by cells of the donor origin as determined by the expression of cell surface antigens (Thy-1.1 or Thy-1.2, and TL− or TL+ of the donor origin). For the thymic graft, a lobe of thymus was inserted under the renal capsule of the host mice. The lymphoid cells in the grafted thymus were gradually replaced by cells of host origin, which was monitored by the analysis of cell surface antigens as in the bone marrow transplantation experiments.

Immune Response. (A) Plaque forming cell (PFC) assay: 106 SRBC were injected intravenously into the transgenic or control mice. 1 wk later, 106 spleen cells of the immunized mice were mixed with 2 × 106 SRBC and incubated for 2 h at 37°C in agarose in the presence of guinea pig complement. The number of plaques producing IgM antibody to SRBC was counted (direct assay), and the number of plaques producing IgG and IgM antibodies was counted by addition of anti–mouse Ig (indirect assay). (B) MLC: 106 spleen cells of the transgenic or C3H/He mice were mixed with irradiated (24 Gy, Hitachi MBR-1520R; Hitachi) spleen cells of BALB/c or B6 and incubated for 4 d at 37°C, followed by a 6-h incubation in the presence of 18.5 kBq [3H]thymidine. Cells were harvested on a filter by LABO MASH (Labo Science Inc., Tokyo, Japan) and precipitated with TCA. The amount of [3H]thymidine in the precipitates was counted with a scintillation counter (Beckman Instruments, Palo Alto, CA). (C) Con A stimulation. Total spleen cells or the T cell fraction of spleen purified by a T cell recovery column kit (Biotex, Alberta, Canada) were incubated in the presence of 2 μg/ml Con A for 3 d, followed by a 6-h incubation with the addition of 18.5 kBq [3H]thymidine. The uptake of [3H]thymidine was measured as in the MLC experiments. (D) LPS stimulation. The experimental procedure was essentially the same as the Con A stimulation, except that 20 μg/ml LPS (Sigma Chemical Co., St. Louis, MO) was added instead of Con A.

DNA and RNA Blot Analysis. Probes for Southern (30) and Northern (31) blot analyses were prepared either by nick-translation (32) or by the random priming method (33). The DNA rearrangement and RNA transcripts were analyzed with the following probes: TCRα with pclα (34), TCRβ with PL5 (provided by Dr. D. Y. Loh, Washington Univ., St. Louis, MO), TCRγ with p6/ 10-2711 (35), TCRδ with RAD11C (36), and TL with pTL1 (3). Conditions for hybridization and washing were described previously (3, 5).

Results

Abnormal Growth of the Thymus in TgTLα-3-1 Transgenic Mice. The growth of the thymus in TgTLα-3-1 mice was

352 γδ T Cells in TL Transgenic Mice
The mean cell number was calculated from data of at least three virgin female mice. For counting the cells in lymph nodes, axillary, brachial, inguinal, and mesenteric nodes were combined. The mean cell number was calculated from data of at least three virgin female mice. The data for C3H/He mice are consistent with published results (37). Thymus and lymph nodes of TgTlaA+3-1 consistently contain fewer cells than those of age-matched C3H/He mice. The low cell number of newborn thymus, sudden disappearance of thymocytes between day 14 and day 21, and gradual increase in the number of thymocytes after day 35 are characteristic of the transgenic mice.

Changes in the weight of the lymphoid organs correlate well with those in the cell number. The weight of the thymus of transgenic mice starts from 3.9 ± 0.3 mg at day 0, increases to 21.1 ± 2.7 mg at day 14, then reduces to 13.6 ± 2.5 mg at day 21 and recovery to 21.9 ± 6.2 mg at day 190. In C3H/He mice, the weight of the thymus is 4.8 ± 0.3 mg at day 0, 57.8 ± 6.9 mg at day 14, 64.1 ± 2.3 mg at day 21 and 36.9 ± 4.4 mg at day 190. The weight of lymph nodes of transgenic mice is consistently smaller than the control; 13.7 ± 2.5 mg versus 24.8 ± 2.5 mg at day 14 and 69.1 ± 11.5 mg versus 92.2 ± 16.2 mg at day 190. Spleen weight or total body weight do not differ significantly between the transgenic and the control mice throughout the observation period.

Assessed by changes in weight, cell number and histology, and was compared with that of the control C3H/He mice (Fig. 1 and Fig. 2). Three unusual events were found to occur during the development of the transgenic thymus. First, at birth, the thymus of the transgenic mouse is already smaller and contains fewer cells than the control. The thymus has a thin cortex layer and the boundary of cortex and medulla is not as distinct as in control mice. Second and most strikingly, lymphoid cells abruptly disappear from the transgenic thymus between day 14 and day 21; the thymus loses 50% of its weight and 85% of its lymphoid cell population. Consequently, the thymus of 21-d and also 35-d mice has only a few percent of the thymocytes present in the control thymus. The thymus at day 21 lacks small lymphocytes in the cortex, and the distinction between cortex and medulla becomes ambiguous. Third, uniformly large blast-like cells with high mitotic activity repopulate and occupy the thymus of 35-d or older mice. With increase in this cell population, both thymus weight and cell number show partial recovery.

Abnormal Thymic T Cell Differentiation. As shown in Fig. 3, the antigenic profile of thymocytes of TgTlaA+3-1 mice also undergoes drastic changes, in parallel with the growth of the thymus described above. The thymus at each age (before day 14, at day 21 and after day 35) shows a distinct and abnormal representation of the four T cell subpopulations belonging to the different maturation stages, the least mature L3T4- Lyt-2- DN, the immature L3T4+ Lyt-2+ double positive (DP) and the mature L3T4+ Lyt-2- or L3T4- Lyt-2+ single positive (SP) cells. Thy-1 expression by the thymic cell population indicates their T cell origin. Until day 14, the thymus has a T cell profile similar to the control, but it contains an expanded population of DN cells and a relatively small population of DP cells. The sudden disappearance of cells from the transgenic thymus between day 14 and day 21 involves 90% of DP thymocytes as well as 85% of DN thymocytes. As a result, the thymus of day 21 mice has all four subpopulations in equal proportion. At day 35, DN cells become the major population, with 90% of thymocytes being DN and the remaining 10% Lyt-2- SP cells, while DP and L3T4+ SP cells become undetectable. This abnormal phenotype becomes stable after day 35, indicating that the differentiation pathway from DN to DP thymocytes has been lost, while the DN pathway remains active. As calculated from Fig. 1 and 3, the number of DP cells in transgenic thymus is <10^6 after day 35, while the control thymus contains 1.5 ± 10^6 DP cells at day 35 and 9 ± 10^7 at day 70. In contrast, the number of DN thymocytes increases after day 21 and the thymus at day 70 contains 8 × 10^6 DN thymocytes, twice as many as the age-matched control.

Studies of TCR expression show that DN cells in day 70 thymus express TCR γδ (Fig. 3, Row 4). However, this commitment of DN cells to TCR γδ becomes evident only at day 35, as the majority of DN cells are negative for TCR γδ expression before day 21. The differentiation pathway to TCR αβ+ SP cells is disturbed in the transgenic thymus (Fig. 3, Row 3), since there are very few TCR αβ+L3T4+ SP cells in 35-d or older mice. TCR αβ expression is detected on the relatively expanded SP thymocytes in day 21 mice and on Lyt-2- SP thymocytes in older mice. TCR αβ and γδ expression is always accompanied by CD3 expression (Fig. 3, Row 5), suggesting that both TCR αβ and γδ on the cell surface are functional.

A variable proportion of the DN thymocytes of young transgenic mice expresses IL-2 receptor α chain (IL-2Rα), HSA, and Pgp-1. For example, of the DN thymocytes of day 21 mice, 69% are IL-2Rα+, 30% HSA+ and 16% Pgp-1+. The DN cells of the control thymus are consistently heterogeneous in terms of the expression of these antigens. However, TCR γδ+ DN thymocytes of 70-d-old transgenic mice become homogeneously negative for these antigens (data not shown).

T Cell Receptor (TCR) γδ+ Double Negative (DN) T Cells in Peripheral Lymphoid Organs. Lymph nodes of TgTlaA+3-1 are consistently smaller in size and contain fewer cells than...
Tg.Tla<sup>a</sup>-3-1

Day 0

Day 14

Day 21

Day 70

C3H/He

Day 0

Day 70

γδ T Cells in TL Transgenic Mice
those of C3H/He mice (Fig. 1), with only one fifth to one half the numbers of T cells (data not shown). The spleen of transgenic mice does not differ significantly in weight or in cell number from age-matched controls, but contains about one half of the T cells present in control mice (14.1 ± 1.3% versus 28.3 ± 1.5% Thy-1+ spleen T cells, data not shown). Spleen and lymph nodes of Tg.Tlaa-3-1 showed no obvious histological abnormality, but the white pulp in the lymph nodes and spleen of Tg.Tlaa-3-1 is smaller than in the control (data not shown).

Figure 3. Surface antigenic profile of thymocytes. Thymocytes of Tg.Tlaa-3-1, C3H/He and B6-Tla+ were examined by two-color FACS analysis using various combinations of mAbs. To identify DN cells easily, a mixture of L3T4 and Lyt-2 antibodies was used in certain experiments; both DP and SP cells were positive with the mixture whereas DN cells were negative. The percentage of each population is shown in the four corners of each panel. The antigen profiles of day 3 and 7 thymocytes of transgenic mice were essentially identical to that of day 14 and are not shown in the figure. As the profile of C3H/He and B6-Tla+ remained consistent with ages, only those of 21-d-old mice are shown in the figure. The T cell population in C3H/He and B6-Tla+ thymus was comparable with published results (13, 14): 80–85% of thymocytes are DP cells, 3–9% L3T4+ SP cells, 3–6% Lyt-2+ SP cells, and 3–5% DN cells.
Figure 4. Thymic population of bone marrow chimera between Tg.Tlaa-3-1 and AKR mice. Lethally irradiated AKR mice were transplanted with bone marrow cells of 90-d-old Tg.Tlaa-3-1 mice, and the thymocytes were analyzed 28 d later. The thymocytes from AKR and Tg.Tlaa-3-1 of comparable age were tested as controls. The difference in Thy-1 allo-specificity between AKR (Thy-1.1) and Tg.Tlaa-3-1 (Thy-1.2) enabled the origin of thymocytes to be determined. TL expression also distinguishes the host (AKR, TL-) and the donor (Tg.Tlaa-3.1, TL+). Almost all thymocytes of the chimeric mice have the phenotype of Thy-1.1- Thy-1.2'TL+, indicating that they are derived from Tg.Tlaa-3-1 bone marrow cells. The thymic population in chimeric mice is almost identical to that of transgenic mice, except for the absence of Lyt-2' SP cells in the chimeric thymus.

The antigenic profile of T cells in peripheral nodes also undergoes changes characteristic of Tg.Tlaa-3-1 mice (data not shown). At day 21, the T cell population in the lymph nodes of transgenic mice is similar to that in control mice except the expression of TL; 95% of T cells are TCR γδ+ CD3+TL+ SP cells and only 5% are TCR γδ+ CD3+ TL+ DN cells. However, as transgenic mice age, the proportion of TCR γδ+ CD3+ TL+ DN cells gradually increases, occupying 30% at day 70 and reaching nearly 50% at day 190. These results indicate that lymph nodes are first populated by TCR αβ+ SP cells prior to day 21, and then by increasing numbers of TCR γδ+ DN cells after day 21.

Expression of the Transgene in Tg.Tlaa-3-1 Thymus. The product of the Tlaa-3 transgene, is expressed in Tg.Tlaa-3-1 thymus in a much larger quantity (about 10 times) than in the thymus of any inbred TL+ strain, including the A-strain from which the transgene was isolated (see reference 8). Further, TL antigen is detected in almost all thymic T cells of Tg.Tlaa-3-1, including DN and SP cells, although a fraction of DN cells in younger mice is TL- (Fig. 3, Row 2). The proportion of TL+ DP cells decreases with aging, while TL+ DN cells increase and become the major population of the thymus. In B6-Tlaa, only DP thymocytes express TL antigens, while DN and SP thymocytes are TL-. TL antigen is expressed in almost all T cells in the lymph nodes of transgenic mice, including TCR γδ+ DN and TCR αβ+ SP cells. This contrasts with the situation in other TL+ mouse strains, where peripheral T cells do not express TL antigen.

T Cell Abnormality Resides in Bone Marrow Stem Cells. To determine whether stem cells derived from the bone marrow or the thymic environment of Tg.Tlaa-3-1 are responsible for the abnormal differentiation of T cells, bone marrow transplantation and thymus grafting were carried out. The thymus of AKR mice (Thy-1.1' TL-) reconstituted with bone marrow cells of transgenic mice showed the characteristics of the transgenic thymus; the majority of thymocytes were large blast-like cells and the cortex-medulla distinction was unclear (data not shown). The thymocytes of chimeric mice were entirely replaced by Thy-1.2' TL+ cells of donor origin, and the major population was TCR γδ+ DN cells (Fig. 4). These results indicate that bone marrow stem cells rather than the thymic environment (including thymic epithelial cells) cause the abnormal thymic differentiation of Tg.Tlaa-3-1 mice. This conclusion is confirmed by experiments involving reciprocal thymic graft. C3H/He thymus transplanted under the capsule of Tg.Tlaa-3-1 kidney takes on the characteristics of the transgenic thymus. 5 wk after the grafting, the thymus becomes smaller with no clear cortex-medulla distinction and is constituted mainly of large lymphoid cells with a TL- TCR γδ+ DN phenotype. In contrast, Tg.Tlaa-3-1 thymus transplanted into C3H/He mice showed normal T cell population and morphology (data not shown).

Deficient T Cell Immune Function in Tg.Tlaa-3-1 Mice. Tg.Tlaa-3-1 mice of day 70 or older were examined for their immune capacity in four different assays. In PFC assays in the response to SRBC, Tg.Tlaa-3-1 mice yielded fewer IgM-
and IgG-producing cells than the control, as shown in Fig. 5 A. 

In vitro proliferative responses in MLC against allogenic BALB/c and B6 spleen cells showed that the transgenic mice were less reactive than the control (Fig. 5 B). Similarly, in the mitogenic response to Con A, transgenic spleen cells were less efficiently stimulated (Fig. 5 C). As the spleens of transgenic mice contain one half the T cells present in control spleens (see above), the same number of T cells isolated from Tg.Tla-3-1 and C3H/He spleens were compared in their response to Con A. Unfractionated spleen cells or purified splenic T cell fraction of the transgenic mice were less reactive than the controls.

Development of T Cell Lymphomas in Tg.Tla-3-1. Tg.Tla-3-1 mice of 8-15 mo-old often developed enlargement of spleen and lymph nodes. Liver, lung and kidney were frequently involved, while overt enlargement of the thymus was rare. The infiltrating cells were larger than normal lymphocytes, with more vesicular nuclei and with relatively abundant cytoplasm. The gross and microscopic characteristics were similar in all cases, and these neoplasms could be classified as "lymphocytic leukemia" according to Dunn (38). The incidence was over 60% (32/51 mice) and the tumors were transplantable in C3H/He mice. C3H/He, used for the derivation of the transgenic strain, has a low incidence of lymphocytic leukemia (39).

The cell surface phenotype of these lymphoma cells was examined (Fig. 6 and Table 1). Cells from 21 lymphomas were Thy-1+TL+L3T4-Lyt-2-TCRαβ-. Most cells expressed TCR γδ and CD3, while a few were negative for TCR γδ and CD3. The expression of IL-2Rα and HSA was generally not detected. The tumors were heterogeneous with regard to the expression of Pgp-1.

The structure and expression of TCR genes were also examined and the results are summarized in Table 1. All except one lymphoma (A8) had rearranged TCR γ and δ genes and transcribed γ and δ mRNA of the functional sizes: A8 had germline TCR γ genes and no γ transcripts of the functional size. No tumor expressed both TCR α and β functional tran-
T cell differentiation in this strain is deviated almost exclusively toward the γδ lineage. Bone marrow transplantation and thymic graft experiments clearly demonstrate that the abnormality resides in the stem cells of bone marrow and not in the thymic environment including the thymic epithelial cells. The most striking event is a sudden disappearance of small lymphocytes from the thymus between day 14 and day 21. This event is distinct from the disappearance of thymocytes induced by stress, irradiation or administration of cortisone. The thymus of Tg.Tla^-3-1 mice shows neither a "moth-eaten" structure nor the accumulation of macrophages, eosinophils or necrotic cells in the cortex, which are associated with the disappearance of thymocytes by these other causes (40). The loss of thymocytes in transgenic mice between day 14 and day 21 is dependent on the genetic background, since a similar phenomenon occurs but much delayed in (C3H/
DNA fragmentation, a sign of apoptosis, in vitro organ culture was observed in day 14 or day 21 thymus, further study of lineage including all DP and some DN cells, while leaving possibility is the rapid removal of cells belonging to the c½ lineage with an abnormal deviation toward the yb lineage. The other possibility is the rapid removal of cells belonging to the c½ lineage including all DP and some DN cells, while leaving those in the yb lineage intact. Although no visible cell death was observed in day 14 or day 21 thymus, further study of this possibility is required, e.g., molecular analysis to detect DNA fragmentation, a sign of apoptosis, in vitro organ culture of the thymus, and determination of TCR repertoires of the yb DN thymocytes. The embryonic thymus of transgenic mice is not grossly abnormal, and the growth of the thymus after birth is close to normal until day 14. However, an event occurring during embryonic life may trigger the abnormal T cell differentiation, and a detailed analysis of thymic development during embryogenesis is now under way.

What is the role of the transgene in these abnormal events, and what is the function of TL in normal differentiation? It is certain that TL expression in the thymus of the transgenic mice is not a cause of these events, since conventional TL+ strains and the other transgenic strain, Tg.Tla-a-3-2, do not show the abnormality. We cannot exclude the possibility that the abnormality is a result of insertional activation or mutagenesis of genes unrelated to TL. However, this genetic alteration has to be under the control of the inserted TL transgene, since the abnormal phenotype is inherited as a dominant trait and is restricted to tissues that express the transgene. These requirements make unrelated insertional mechanisms unlikely. However, to eliminate this possibility, over 20 new transgenic mice with various TL and TL chimeric genes have been derived and are now being established. The possibility we feel best explains the results obtained thus far is that quantitative or qualitative abnormalities in TL expression in the transgenic mice above a certain threshold cause abnormal T cell differentiation in the transgenic mice. The facts are that (a) these mice express over 10 times the level of TL antigen found in conventional TL+ or Tg.Tla-a-3-2 mice, (b) TL is expressed on DN thymocytes which are negative in conventional TL+ mice, and (c) the abnormality is restricted to tissues that express TL. F1 mice of (C3H/He × Tg.Tla-a-3-1), (B6 × Tg.Tla-a-3-1) and (B6-Tla-a × Tg.Tla-a-3-1) crosses show the characteristic abnormalities of Tg.Tla-a-3-1 mice, but the onset of the abnormalities is delayed and not so pronounced as in parental homozygous Tg.Tla-a-3-1 mice (data not shown). These results indicate dominant inheritance of the abnormality with a gene dosage effect, suggesting that an amplified level of TL expression is causally related to abnormal thymic differentiation. If this assumption is correct, one can assume that TL plays a critical role in T cell development in the thymus and that thymocytes of TL- mice must express other MHC class I antigens that are functional equivalents to TL. It has been reported that some MHC class I genes mapping to the Tla region function as ligands for yb TCRs (15, 16, 41, 42). However, unlike TL genes, these other class I genes are expressed in a variety of tissues. Recently, the expression of TL in intestinal epithelium has been described and its relation to yb cells has been discussed (43, 44). It remains to be seen whether TL serves as ligands for certain yb TCRs, whether TL is involved in the selection process of yb cells, and whether altered expression of TL disturbs the normal differentiation of yb cells.

Much effort has been made to characterize yb cells and a vast amount of information has been accumulated (15-18). However, analysis of yb cells in the peripheral lymphoid organs has been difficult because of their minor representation (20-22). With the advantage of the relative abundance of yb cells and yb T cell lymphomas in Tg.Tla-a-3-1, we have attempted to stage the maturation of yb cells. From analysis of surface antigens, the following pathway for yb DN cells is proposed: the least mature stage 1, IL-2Rα+ HSA+ Pgp-1- TCR+; stage 2, IL-2Rα+ HSA+ Pgp-1+ TCR+; stage 3, IL-2Rα- HSA- Pgp-1+ TCR+ γδ+; and the most mature stage 4, IL-2Rα- HSA- Pgp-1- TCR+ γδ+. The appearance of surface TCR γδ accompanying CD3 expression occurs between stage 2 and 3. This proposed pathway is obviously not complete and may not be applicable to all subsets of yb cells, but this sequence of changes in the surface phenotype is similar to that proposed for TCR αβ lineage (13, 14). Determination of the V region usage (45) in the yb thymocytes and T cell lymphomas should reveal which subsets of yb cells belong to this pathway. Immunization using DN cells and T cell lymphomas from Tg.Tla-a-3-1 mice may produce reagents specific to the yb lineage and may identify counterparts of Lyt-2 and L3T4 on yb DN cells.

The immune response of Tg.Tla-a-3-1 mice is severely impaired, with T cells being inefficient in helper function for antibody production, in allogeneic MLC and in Con A stimulation. Since nearly one half of the peripheral T cells in mature Tg.Tla-a-3-1 mice are yb DN cells, the results strongly suggest that yb cells cannot participate in or are less active in these T cell dependent reactions. Further, the results indicate that the function of αβ cells in these transgenic mice is also affected, suggesting that only incompetent αβ cells are produced because of the abnormal thymic development. Alternatively, it is conceivable that the function of αβ cells is suppressed by yb cells in these transgenic mice. The function of yb cells is largely unknown and their reactivity to allo-antigens or to mitogens is still unclear. Accordingly, the use of isolated population of yb cells from Tg.Tla-a-3-1 mice in the analysis of other immune functions such as reactivity to mycobacterial antigens and generation of lymphokines, should help in dissecting the functions of yb cells and their relation to the functions of αβ cells.

Development of yb T cell lymphomas is another important feature of Tg.Tla-a-3-1 mice. A relatively long latent
period and clonality of these lymphomas clearly indicates that
tumor development requires other genetic events in addition
to the transgene, in a manner similar to lymphomagenesis
in c-myc transgenic mice (46, 47). Why are Tg.Tla'-3-1 mice
susceptible to lymphomas and why do all lymphomas originate
from the yδ lineage? An obvious reason for the yδ pheno-
type is that the preponderance of thymocytes in older trans-
genic mice are yδ cells, while αβ cells are a minor population.
There is no evidence to indicate that yδ cells are more sus-
ceptible to lymphomagenesis than αβ cells, as there have been
only a few reports on yδ T cell lymphomas in mice or in
human (48, 49). A higher mitotic rate of thymocytes in trans-
genic mice than in nontransgenic mice indicates that trans-
genic thymocytes are rapidly dividing, suggesting that one
of the events necessary for the ultimate development of ly-
phomas may have already occurred in these cells. To under-
stand the genetic basis for the sensitivity of yδ cells to ly-
phomagenesis and how this relates to abnormal T cell
differentiation, crosses between Tg.Tla'-3-1 mice and high or
low lymphoma strains have been made. These mice should
provide useful information on the role of TL in the develop-
ment of the yδ T cell lymphomas and also advance our un-
derstanding of the significance of TL expression in radiation-
induced lymphoma and prelymphoma cells (50–52).

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