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Transforming Growth Factor-β Pathway Serves as a Primary Tumor Suppressor in CD8$^+$ T Cell Tumorigenesis

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ABSTRACT

Tumorigenesis in rodents, as well as in humans, has been shown to be a multistep process, with each step reflecting an altered gene product or gene regulatory process leading to autonomy of cell growth. Initial genetic mutations are often associated with dysfunctional growth regulation, as is demonstrated in several transgenic mouse models. These changes are often followed by alterations in tumor suppressor gene function, allowing unchecked cell cycle progression and, by genomic instability, additional genetic mutations responsible for tumor metastasis. Here we show that reduced transforming growth factor-β signaling in T lymphocytes leads to a rapid expansion of a CD8$^+$ memory T-cell population and a subsequent transformation to leukemia/lymphoma as shown by multiple criteria, including peripheral blood cell counts histology, T-cell receptor monoclonality, and host transferability. Furthermore, spectral karyotype analysis of the tumors shows that the tumors have various chromosomal aberrations. These results suggest that reduced transforming growth factor-β signaling acts as a primary carcinogenic event, allowing uncontrolled proliferation with consequent accumulation of genetic defects and leukemic transformation.

INTRODUCTION

Tumorigenesis is known to be a multistep process that involves independent serial genetic modifications (1). Primary carcinogenic signals generally involve signals needed to move quiescent cells into cell cycle and usually involve mimicry of growth factors or hormones. Many tumor cells acquire this ability through alterations of extracellular growth signals or intracellular transducers of these signals (2). Subsequent steps required for carcinogenesis most often include changes in inhibitory signals used to control growth factor signals. One of the most potent of these inhibitory signals involves the transforming growth factor-β (TGF-β) family.

TGF-β family members are a multifunctional group of secreted proteins that function to control growth, differentiation, and cell death (3). TGF-β signals are complex in nature and exert different effects depending on cell type, environment, and subsequent signaling pathways. When interacting with epithelial and hematopoietic cells, two cell types that give rise to many cancers, TGF-β signaling becomes inhibitory, thus acting as a tumor suppressor in the early stage of carcinogenesis.

Evidence that TGF-β is involved at multiple stages of human cancer is extensive (4). Initially, high frequency of TGF-β involvement with human gastric and colon cancers was identified with deletions (5) or somatic mutations in the type II receptor (5–8) and shown to be caused by microsatellite instability in the coding region of this receptor. These studies led to investigations into the status of the TGF-β pathway in other human cancers, which identified TGF-β pathway involvement in many different human tumors (*e.g.*, pancreas, liver, breast, prostate, and hematopoietic cells; ref. 9–14). These studies have led to the use of different animal models containing mutated, enhanced, or deficient TGF-β signaling pathway members, confirming its role as a tumor suppressor (14–18). In addition, these animal model data support the concept that defective TGF-β signaling complements a defective growth signal and is rarely tumorigenic in itself. Some studies reporting spontaneous tumor formation involving a defective TGF-β pathway have been shown to require environment factors (18), supporting a later involvement for TGF-β signaling in the progression of tumorigenesis. Also, TGF-β signaling has recently been shown to play an important role as an oncogene in late stages of tumorigenesis by its ability to modify extracellular matrix proteins enabling metastasis (19, 20).

The lymphoid system is unique in the ability of component cells to migrate throughout the body in its natural state, posing a potentially dangerous situation if mutations lead to autonomous growth. In addition, lymphocytes on activation have the potential to expand several hundred-fold in a short period, providing the potential for rapid expansion of a growth favoring mutation. To protect against the potential for uncontrolled growth, lymphocytes have mechanisms to limit cell growth, including many cell death pathways, resistance to antigen stimulation, and sensitivity to the antiproliferative effects of TGF-β (21–23), making them a primary target for TGF-β-mediated suppression. In fact, several tumor models support the concept that TGF-β secretion by tumors is responsible for suppression of immune surveillance (24, 25). Surprisingly, however, only a few scattered reports have identified a role for TGF-β signaling defects in leukemogenesis (10, 14, 26–28).

To better understand the role of TGF-β on immune surveillance and leukemogenesis, a transgenic mouse model was generated in which a dominant-negative TGF-β type II receptor was expressed on T lymphocytes using a CD2 promoter construct (29). T cells from these mice have a much reduced sensitivity to TGF-β family members, allowing *in vivo* functional analysis of the role of TGF-β signaling on naïve T-cell biology. Interestingly, TGF-β was shown to have a differential effect on CD8$^+$ *versus* CD4$^+$ T cells, with CD4$^+$ T cells remaining relatively unaffected, whereas CD8$^+$ T cells exhibited ongoing expansion with animals developing a lymphoproliferative disease (29). In this report, we characterize the CD8$^+$ T cells involved in this lymphoproliferation and determine that, at later time points, the expanded population of CD8$^+$ T cells undergoes a transition to leukemia. This suggests that the TGF-β signaling pathway can act as an early leukemogenic suppressor in murine CD8$^+$ T cells, and when this occurs, the resulting leukemias lack a common identifying translocation.

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MATERIALS AND METHODS

Mice. Dominant-negative TGF-β type II receptor transgenic mice were generated as described previously (29). C57BL/6 control mice were obtained from SAIC Frederick (Frederick, MD). All mice were generated, maintained on a C57BL/6 background strain, and housed according to NIH guidelines.

Cell Populations. Splenocytes and lymph node cells were prepared as described previously (30). T-cell populations were purified from splenocytes by either magnetic bead separation (Miltenyi Biotec, Auburn, CA) or by passage over a T-cell column (Biotex Laboratories, Edmonton, Alberta, Canada) followed by magnetic bead separation. Purity of the T-cell populations was established by flow cytometric analysis and was always >85%. Cells were maintained in complete culture medium: RPMI 1640 (Life Technologies, Inc., Gaithersburg, MD) with 1 mmol/L sodium pyruvate (Life Technologies, Inc.), 100 mmol/L nonessential amino acids (Life Technologies, Inc.), 100 units/ml penicillin +100 mg/ml streptomycin (Life Technologies, Inc.), 50 mmol/L 2-mercaptoethanol (Fisher Scientific, Pittsburgh, PA), 2 mmol/L L-glutamine (Life Technologies, Inc.), and 10% FCS (Hyclone, Logan, UT). Cells were incubated at 37°C with 5% CO2/95% air.

Flow Cytometric Analysis. Biotin-conjugated, FITC-conjugated, and phycoerythrin-conjugated antibodies against mouse CD4, CD8 (Becton Dickinson, San Jose, CA), TCR, CD25, CD122, and CD44 (PharMingen, San Diego, CA) were used to stain cells from lymph node and spleen. Biotin-conjugated (1 μg) Abs were incubated in a total volume of 20 μL for 20 minutes on ice, washed once in FACS media [Hank's balanced salt solution (Life Technologies, Inc.) plus 1% BSA (Sigma Chemical Company, St. Louis, MO) plus 1% azide (w/v; Sigma Chemical Company)]. A F_c-specific antibody, 2.4G2, was used to block F_c binding. Antibody stained cells were analyzed as reported previously (30). Data are presented on a log scale as either histogram or dot plot.

Histology. Five-micrometer paraffin sections of thymus, spleen, lymph node, bone marrow, kidney, and intestine were generated, stained with H&E, and visualized by light microscopy.

T-Cell Receptor Rearrangement Analysis. Genomic DNA (20 μg) from lymphocytes was digested with *Hind*III restriction enzyme (New England Biolabs, Beverly, MA) incubated overnight at 37°C. Cut DNA was phenol/chloroform extracted, EtOH precipitated, and resuspended in water for analysis. Samples were run on a 1% agarose gel, blotted onto nitrocellulose, and probed with T-cell receptor constant region probe (31) labeled with [^{32}P]dCTP-α (Amersham Biosciences, Piscataway, NJ).

Adoptive Transfer Experiments. Lymph node or splenic tumor cells were washed and resuspended at 50×10^6 cell/ml in PBS. Five million tumor cells were injected into recipient mice via tail vein using a 30-gauge needle. Mice were sacrificed 4 to 14 weeks later and analyzed by flow cytometric analysis, whole blood counts, and histology. Recipient mice were irradiated with 5 Gy before injection with tumor.

Spectral Karyotype Analysis. Lymph node and splenic cells were prepared and stimulated with either phorbol myristate acetate + ionomycin or phorbol myristate acetate + ionomycin + interleukin (IL)-15. Cultures were incubated 24 to 72 hours and prepared for spectral karyotype analysis as described previously (32).

RESULTS

Dominant-Negative TGF-β Type II Receptor Transgenic Mice Develop a Severe Lymphoproliferative Disease. CD44^{lo} to CD44^{hi} CD8$^+$ T-cell ratios are generally constant throughout the lifetime of mice, although the ratio does gradually decrease with age in antigen-unchallenged mice. The presence of the dominant-negative TGF-β type II receptor transgene, however, leads to a rapid decrease in this ratio. By 12 weeks of age, dominant-negative TGF-β type II receptor transgenic CD8$^+$ T-cell populations already contain a higher percentage of CD44^{hi} T cells than (older) control C57BL/6 mice at 60 weeks of age (Fig. 1, *top panel*). In concert with the increased proportion of cells with CD44^{hi} cell surface expression, an increased expression of the IL-2 receptor-β is observed (Fig. 1, *bottom panel*), indicating a possible cytokine supported expansion of the CD8$^+$ memory T cells. These data suggest that a decrease in TGF-β signaling present in dominant-negative TGF-β type II receptor CD8$^+$ memory T cells may give a proliferative advantage to these cells.

To establish whether the increase in percentage of T cells with CD8$^+$ memory phenotype reflects an expanding population of such cells, differential blood counts were performed on peripheral blood from dominant-negative TGF-β type II receptor transgenic mice that were either heterozygous (n = 48) or homozygous (n = 65) for the transgene. Control mice (n = 20) had a white blood cell count of 4,800 +/− 750 leukocytes/μL blood at various ages with memory CD8$^+$ T-cell populations consisting of 2 +/− 1% of the lymphocyte population. In contrast, dominant-negative TGF-β type II receptor transgenic mice had progressive increases in leukocyte counts with dramatic increases after week 30 (Fig. 2*A*). These increases in white blood cell counts were associated with increases in CD8$^+$ memory T cells as determined by flow cytometry (Fig. 2*B*). In parallel with these marked increases of CD8$^+$ memory T-cell populations, mice exhibited symptoms of decreased activity, loss of weight, and difficulty breathing. Homozygous dominant-negative TGF-β type II receptor transgenic mice became symptomatic 4 months before heterozygous dominant-negative TGF-β type II receptor transgenic mice, indicating a direct relationship between decreased TGF-β signaling and development of a disease state. Flow cytometric analysis of the peripheral blood from mice with leukocytosis determined that the increase in white blood cells was the result of an increase in CD8$^+$ T cells with memory phenotype (data not shown).

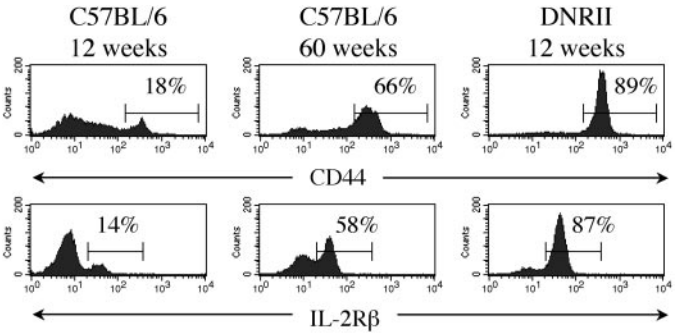

Fig. 1. Rapid T-cell conversion to memory CD8$^+$ phenotype in dominant-negative TGF-β type II receptor transgenic mice. Flow cytometric analysis of CD8$^+$ T lymphocytes from C57BL/6 (*left* and *center columns*) and dominant-negative TGF-β type II receptor transgenic (*right column*) mice. Memory T-cell surface markers CD44 (*top row*) and CD122 (IL-2Rβ; *bottom row*) used to distinguish memory CD8 T-cell populations over time. Phenotype consistent between five experiments. *Bars* represent cell gate. (*DNRII*, dominant-negative TGF-β type II receptor)

Infiltrating Lymphocytes in Dominant-Negative TGF-β Type II Receptor Transgenic Mice Determined to Be Transformed by Histologic Analysis. To investigate the markedly increased CD8$^+$ memory cell number, and to distinguish between a benign lymphoproliferative disorder and leukemia, histologic analysis was performed on lymphoid (Fig. 3, *A–D*) and nonlymphoid (Fig. 3, *E* and *F*) organs. Seven of 7 mice, determined to have markedly elevated leukocyte counts, were also found to have changes indicative of transformed infiltrating lymphocytes when H&E slides were read blindly. Lymphoid organs from symptomatic dominant-negative TGF-β type II receptor transgenic animals were greatly enlarged by infiltrating lymphocytes, leading to loss of architecture and organ integrity (Fig. 3, *B* and *D*). Nonlymphoid tissues were also affected with infiltrating lymphocytes accumulating at vascular junctions (Fig. 3*F*). Flow cytometric analysis as well as immunohistochemistry (data not shown) confirmed that the infiltrating cells were CD8$^+$ memory T cells.

CD8$^+$ T-Cell Expansion Is Clonal. We next examined the expanded populations of CD8$^+$ memory T cells for clonality by examining rearrangement of the T-cell receptor β locus. The analysis was performed using Southern blot analysis with a probe containing the constant region of the T-cell receptor β locus. In this assay, an unrearranged T-cell receptor β locus produces bands of 9.4 and 3.1 kb as a result of the binding of the probe to the two constant regions (Fig. 4*A*). On rearrangement, the 9.4-kb fragment shortens, whereas the 3.1-kb fragment remains constant, because the *Hind*III restriction

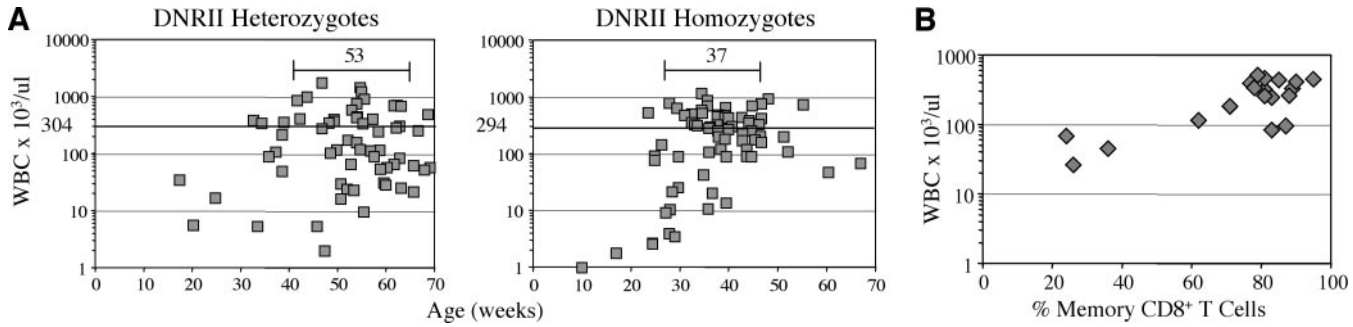

Fig. 2. Leukocyte count in peripheral blood of dominant-negative TGF-β type II receptor transgenic mice. *A*, Heterozygous (*left*) and homozygous (*right*) dominant-negative TGF-β type II receptor mice were bled at various ages and analyzed by a differential blood count procedure. White blood cell count was determined and plotted with respect to age at analysis. Control C57BL/6 bleeds at various ages were used as controls and were always $<5 \times 103$ white blood cell/μL at all age groups. Mean age located above data points; *bars*, ±SE. *Horizontal line*, mean white blood cell, which is labeled on left side of line. *B*, Lymphocytes from both heterozygous and homozygous dominant-negative TGF-β type II receptor transgenic mice ($n = 19$) were subsequently phenotyped for $CD8^+$ memory cell markers by flow cytometric analysis and plotted with respect to white blood cell counts. (*DNRII*, dominant-negative TGF-β type II receptor; *WBC*, white blood cell)

enzyme site is 3′ from the switch region (Fig. 4*A*). Results from a typical T-cell receptor β analysis are shown in Fig. 4*B*. The 9.4-kb region can be observed in both thymus (*left lane*) and spleen (*center lane*), but it is replaced in lymphocytes from one mouse with marked lymphocytosis by a single 7.0-kb band, demonstrating the presence of a clonal T-cell receptor β population.

In some mice, both T-cell receptor α and T-cell receptor β phenotypic analysis could be detected by flow cytometry. An example of such analysis is shown in Fig. 4*C*, where a clonal T-cell population is defined by the expression of a single T-cell receptor α and β receptor. All of the dominant-negative TGF-β type II receptor transgenic mice determined to have developed leukemia by white blood cell or by histology had a predominant T-cell receptor β receptor rearrangement, as determined by Southern blot analysis or flow cytometry. This included mice from two different transgenic lines as well as mice containing one or two transgenic alleles, establishing that the expression, not the integration site, of the transgene was responsible for the leukemia.

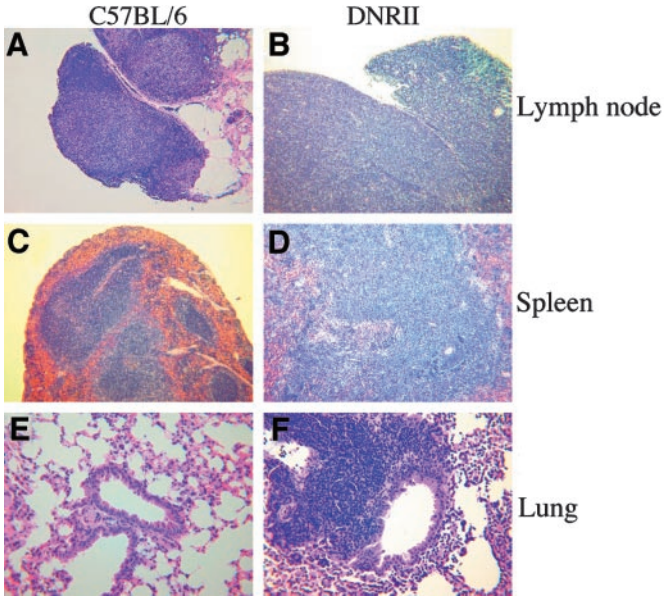

Fig. 3. Loss of architecture in dominant-negative TGF-β type II receptor lymphoid tissues. Age matched C57BL/6 (*left*), and dominant-negative TGF-β type II receptor transgenic (*right*) tissues were examined by H&E staining. *A*, Control mouse with normal lymph node architecture with visible separation of lymphoid areas. *B*, Lymph node from transgenic mouse, typically 10-fold larger than control lymph node, with no visible separation of lymphoid areas. *C*, Spleen from control mouse with distinct areas of *white* and *red pulp*. *D*, Spleen (increased 10-fold in size) from transgenic mouse with no distinct division of *red* and *white pulp*. *E*, Lung from control mouse with normal perivascular architecture. *F*, Transgenic mouse lung with extensive perivascular lymphoid infiltration. Magnification, ×100. (*DNRII*, dominant-negative TGF-β type II receptor)

Clonal $CD8^+$ Dominant-Negative TGF-β Type II Receptor-Derived Tumor Cells Can Be Transferred to Syngeneic Host. To additionally characterize the marked leukocytosis in dominant-negative TGF-β type II receptor transgenic mice as malignant, we examined the ability of these tumors to be transferred to syngeneic hosts. To determine transferability of the tumors, lymph node T cells from mice with palpable tumors were isolated and injected into nonlethal, irradiated (5 Gy) mice. Three of three different tumors injected into a total of 8 recipients were able to expand in irradiated hosts (Table 1) and develop tumors with associated symptoms and histologic changes indicative of neoplasia. In addition, all three tumors were successfully transferred from the first recipient to a second irradiated host with similar results. The ability to transfer tumors that developed in dominant-negative TGF-β type II receptor transgenic mice additionally demonstrates that the expanded monoclonal memory $CD8^+$ T cells in dominant-negative TGF-β type II receptor mice do indeed represent malignancy.

Chromosomal Aberrations Detected in Dominant-Negative TGF-β Type II Receptor-Derived Leukemia. To examine chromosome stability in tumors derived from dominant-negative TGF-β type II receptor transgenic mice, chromosomal analysis was performed using spectral karyotype analysis. Both heterozygous and homozygous symptomatic mice from two different transgenic founder lines were examined. Many of the mice exhibited chromosomal abnormalities, including aneuploidy, deletions, and translocations (Fig. 5). Chromosomal abnormalities were observed in tumors from both transgenic lines and did not seem to depend on transgenic copy number (Table 2). Some abnormalities appeared in multiple tumors, but no dominant abnormality could be detected. In addition, several mice had relatively normal chromosomal analysis (Table 2) despite possessing high white blood cell, a transformed histology, and a dominant T-cell receptor β positive population, suggesting smaller chromosomal alterations, undetectable by spectral karyotype analysis, may be occurring in these $CD8^+$ memory T cells.

DISCUSSION

TGF-β is a pleiotropic cytokine that, along with its receptors, is expressed by nearly all of the cells in the body. Studies on the role of TGF-β in carcinogenesis have identified it to be both a tumor suppressor and, later in the multistep process, an oncogene (4, 16). Because of these complexities, the role of TGF-β as an immunologic tumor suppressor has been difficult to study. To isolate the function of TGF-β on T-cell biology, we generated a transgenic mouse overex-

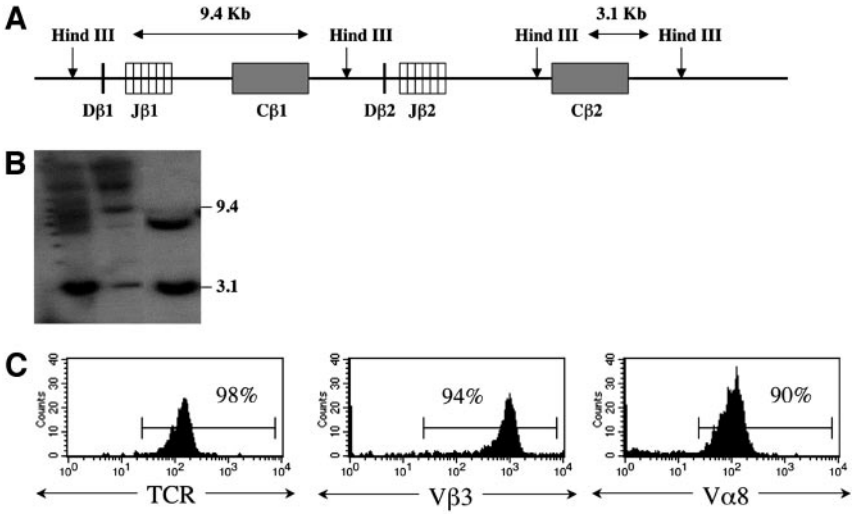

Fig. 4. Limited T-cell receptor repertoire of expanded memory T cells. *A*, Schematic representation of the T-cell receptor β chromosomal region used to detect receptor rearrangement. Hind, *Hind*III restriction enzyme site. *B*, Southern blot analysis of T-cell receptor β chromosomal region of thymus (*left lane*) and spleen (*center lane*) from a C57BL/6 age-matched control mouse, and tumor (*right lane*) from a dominant-negative TGF-β type II receptor transgenic mouse. *C*, Flow cytometric analysis of T-cell receptor α and β chain surface expression on lymphocytes from a symptomatic dominant-negative TGF-β type II receptor transgenic mouse. (*TCR*, T-cell receptor)

Table 1 *Transfer of tumors from dominant-negative TGF-β type II receptor transgenic mice to irradiated C57BL/6 hosts*

ID no.*	Age (wks)	Days after injection	Results
2069	29	45	Tumor
2069	29	75	Tumor
2069	29	45	Tumor†
2169	35	35	Tumor
2169	35	35	Tumor
2169	35	30	Tumor†
2303	31	35	Tumor
2303	31	30	Tumor†

* Eight of 8 mice from 3 different donor mice developed tumors.
† Tumor passed from first host to new recipient.

pressing a dominant-negative TGF-β type II receptor on all of the T cells (29). Our results demonstrate that the TGF-β family of cytokines is a major inhibitor of $CD8^+$ memory T-cell growth, and their removal initiates an expansion of this T-cell subset, leading to a fatal leukemia.

T lymphocytes have been shown to be influenced by TGF-β in their growth, differentiation, apoptosis, and activation, with each response different, depending on location and state of activation or differentiation (23). Most early data focused on TGF-β inhibition of activation-induced proliferation, which was later shown to involve up-regulation of cell cycle inhibitors (33–35). Other studies have shown that TGF-β plays an important role in differentiation of $CD4^+$ T-helper cells into Th-1 or Th-2 phenotype by altering lymphokine secretion pathways (36–39). Little data, however, are present on the effects of TGF-β on early $CD8^+$ T cells (40).

Our data show that TGF-β family cytokines play an important role in early $CD8^+$ T-cell homeostasis, which normally is characterized by a relatively constant ratio of $CD44^{hi}$ to $CD44^{lo}$ $CD8^+$ T cells (41, 42). The absence of TGF-β signaling allows the preferential expansion of the $CD44^{hi}$ population over the $CD44^{lo}$ population. This regulatory pathway would allow expansion of memory T cells in the presence of an activating signal, such as one generated by a virus or bacterial infection (43, 44). In these mice, we observe a significant increase in the $CD44^{hi}$, $CD122^{hi}$, $CD8^+$ population, indicating that the expansion is driven by an IL-2 receptor cytokine. Interestingly, CD44 is known to bind to metalloproteinase-9, which in turn localizes to the cell

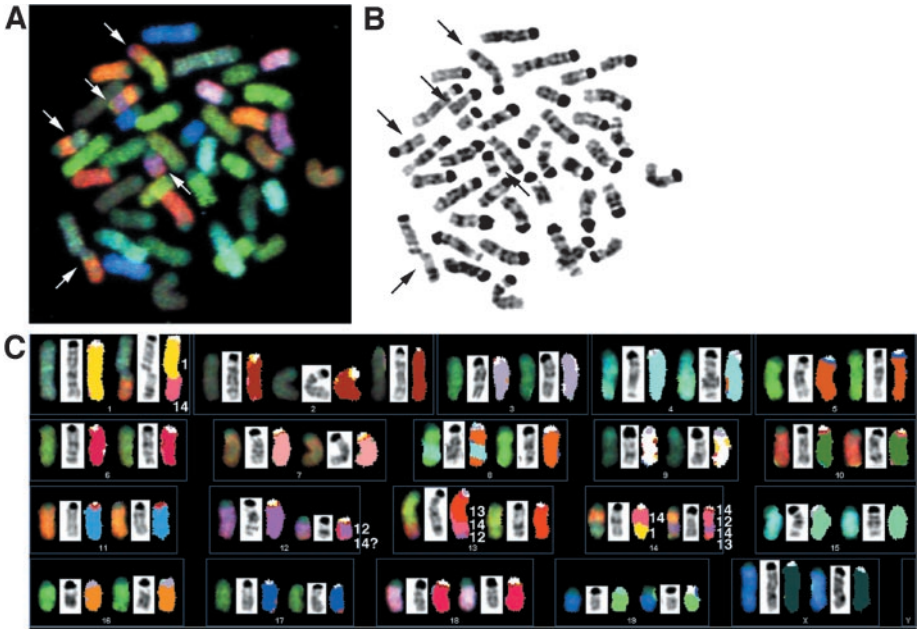

Fig. 5. Multiple translocations identified using spectral karyotype analysis. *A*, Labeled metaphase spread of 33-week-old dominant-negative TGF-β type II receptor transgenic mouse. *Arrows* indicate multiple translocation detected in chromosomes. *B*, Inverted 4′,6-diamidino-2-phenylindole images of the same metaphase spread. *C*, Computer generated spectral karyotype classification of labeled metaphase chromosomes arranged by chromosome number. Translocated chromosomes are labeled with corresponding chromosome number.

Table 2 *Chromosomal aberrations found by spectral karyotype analysis in tumors from dominant-negative TGF-β type II receptor transgenic mice*

ID no.	TG/Alleles	Aberration
2020	E1/1	40, XX [10/17]*//† 41, XX, T(1;14)(E2.3;C3),‡ +2,§T(14;1)(C3;H1) [3/17]// 41, XX, T(1;14)(E2.3;C3), +2, T(12;14)(C2;?), T(13;14;12)(D2.1;E1;F1) T(14;12;14;13)(C1;C1;D3;D1) [4/17]
675	E1/1	40, XX [2/8]// 40, XX, T(4;5)(C4;E2)[4/8]// 41, XX, T(4;5)(C4; E2), +T(4;5)(C3;F1) [2/8]
60	E1/1	40, XY [2/9]// 42, XXY, +5 [4/9]// 42, XXY, T(4;5)(D2.2;5G1.1), +5 [3/9]
26	E1/1	40, XY [5/7]// 40, XY, T(5;6)(C3.3;C3) [2/7]
5623	E1/1	40, XX [10]
5787	E1/2	40, XY [7/12]// 40, XY, T(4;5)(D2.2;D) [5/12]
5664	E1/2	40, XX [4]
5745	E1/2	40, XX [4/6]//39, XX, −2[2/6]
5820	E1/2	40, XY [9]
2569	E1/2	40, XX [6]
710	I2/1	40, XY[7/11]//41, XXY [4/11]
3073	I2/1	40, XX [7/12]//41, XXX [5/12]
5460	I2/1	40, XY [7]
532	I2/1	40, XY [4]
530	I2/1	40, XY [8]
711	I2/1	40, XY [5/10]//40, XY, T(4;7)(D2.2;?E1) [5/10]

* Number of chromosomes with aberration/total number of chromosomes studied.
† End of aberration.
‡ Translocation, with chromosome number involved listed in first set of parenthesis, and chromosome breakpoints listed in second set of parenthesis.
§ Addition (+) or deletion (−) of chromosome.

surface and activates latent TGF-β (45). This would colocalize both a stimulatory and inhibitory receptor on the same cell. In the absence of the inhibitory signal, constant cell growth could occur in the presence of antigen and cytokine.

In addition to changes in $CD44^{hi}$ to $CD44^{lo}$ ratios, significant increases in cell numbers also occur in dominant-negative TGF-β type II receptor transgenic mice. These increased cell numbers occur over a period of months and are dependent on the copy number of the transgene. This again suggests that the effect on $CD8^+$ T cells by TGF-β is normally an opposing effect that can be modified by the amount of TGF-β signaling present. These results are consistent with other TGF-β models that demonstrate that TGF-β can regulate differentially through the use of a TGF-β gradient (46). This would also suggest that the cytokine driving this proliferation is ubiquitous and not limiting, allowing for constant expansion over time, and that TGF-β signaling is therefore primarily responsible for maintaining homeostasis of memory $CD8^+$ T cells.

Cell division, usually a primary step in carcinogenesis, is not sufficient for transformation of an expanding population (1). TGF-β seems to be the primary signal preventing $CD8^+$ memory T-cell growth; however, additional events must occur to allow for the clonal expansion and tissue infiltration seen in dominant-negative TGF-β type II receptor transgenic mice. Several possibilities exist to explain the subsequent clonal nature of a $CD8^+$ memory T-cell expansion. One possibility is that TGF-β normally helps to maintain the integrity of the chromosome in $CD8^+$ T cells, and the removal of that signal allows for genetic instability, leading to growth altering mutations. There is some evidence that supports this hypothesis (47, 48); however, our chromosomal analysis of these clonal populations would indicate that gross chromosomal integrity for the most part is relatively stable. This does not rule out the possibility that mutations undetectable by metaphase spreads and spectral karyotype analysis, such as small duplication, deletions, and para- or pericentric inversions, could be occurring (49).

A second possibility is that the absence of TGF-β signaling is allowing cells to survive that would normally die by apoptosis. No evidence exists to support this possibility, and, in fact, some studies have shown that TGF-β signaling is known to have the opposite effect (50). A third possibility is that the stimulatory signal driving the $CD8^+$ memory T-cell expansion may be involved in suppressing apoptosis. One such candidate would be IL-15. IL-15 preferentially stimulates $CD8^+$ memory T cells, suppresses apoptosis, and has been shown to be able to cause leukemia in overexpressing transgenic mice (51). Together these data could explain both the increase in $CD8^+$ cell number and subsequent transformation.

In conclusion, we present a mouse model of $CD8^+$ T-cell leukemogenesis that is a direct consequence of a deficient tumor suppressor signal. Our model is unique in that it demonstrates the ability of TGF-β to act as a primary tumorigenic suppressor for $CD8^+$ T cells. This pathway may represent a common mechanism used to quickly expand $CD8^+$ memory T cells to eliminate a viral or bacterial pathogen and has implications in understanding clinical manifestations of $CD8^+$ T-cell leukemia.

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