Tal1 Transgenic Expression Reveals Absence of B Lymphocytes

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Abstract

***TAL1* oncogene encodes a helix-loop-helix transcription factor, Tal1, which is required for blood cell development, and its activation is a frequent event in T-cell acute lymphoblastic leukemia. Tal1 interacts and inhibits other helix-loop-helix factors such as E47 and HEB. To investigate the function of Tal1 in B cells, we generated Eμ-*TAL1* transgenic mouse line, expressing Tal1 in mouse B-cell lineage. Fluorescence-activated cell sorting (FACS) analysis of lymphocytes isolated from spleens of five out of five founders reveals complete absence of IgM- or CD19-expressing cells. Only 2% to 3% of these cells were $B220^+$ and 100% of $B220^+$ cells were $CD43^+$, indicating that these mice were able to make pro-B cells. Similarly, FACS analysis of bone marrow cells in Eμ-*TAL1* mice revealed complete absence of $B220^+IgM^+$ and $B220^+CD19^+$ cells. Analysis of the recombination status of *IgH* genes revealed the presence of D-J but absence or drastic reduction of V-D-J rearrangements. Our results suggest that Tal1 overexpression in B cells results in a phenotype similar to that of B cells of E47/E2A knockout animals. This represents first *in vivo* evidence that Tal1 can completely inhibit E47/E2A function.** (Cancer Res 2006; 66(12): 6014-7)

Introduction

Activation of *TAL1* gene (also known as *SCL* or *TCL5*) by chromosomal rearrangements at 1p32 is a common event in T-cell acute lymphoblastic leukemia (T-ALL; ref. 1). Whereas in normal T cells Tal1 is not expressed, it is activated in the majority of T-ALL (1). Transgenic expression of Tal1 in T cells causes the development of clonal T-cell leukemias (2, 3). The *TAL1* gene encodes a helix-loop-helix transcription factor (1). These factors often form homodimers and heterodimers that recognize specific DNA sequences (1). Although Tal1 cannot form homodimers, it interacts and inhibits other helix-loop-helix factors such as E47/E2A and HEB (1, 3). E47/E2A has been implicated as a gene with tumor suppressor activity because mice deficient for E2A succumb to T-cell lymphomas (4). A recent report showed that Tal1 induces T-ALL primarily by inhibiting transcriptional activity of E47 (3). *Tal1* gene knockout in mice resulted in midgestational lethality and complete absence of yolk sac erythropoiesis (5). Further analysis of chimeric mice showed that Tal1 is essential for development of all hematopoietic lineages (5). Mice lacking the *E2A* gene showed impaired lymphoid development with reduced amount of mature T cells and complete absence of B cells, and no recombination of *IgH* genes was detected (6). A transgenic mouse model expressing truncated form of human Tal1 under the control of ubiquitous SIL promoter showed 30% to 60% reduction of IgM^+ cells and no effect on V-D-J recombination of *IgH* genes was observed in these animals (7). A similar report described *TAL1* transgenic mouse model expressing *TAL1* under the control of lymphoid-specific *Ly-6E.1* promoter. These mice also showed ~50% reduction of $B220^+CD19^+$ cells in bone marrow (8). Recently, we reported that Tal1 is phosphorylated and regulated by Akt (9). To investigate the function of Tal1 in B cells, we generated Eμ-*TAL1* transgenic mouse line expressing Tal1 in mouse B-cell lineage. Here we report the phenotype of these mice.

Materials and Methods

Eμ-*TAL1* transgenic mice. A 1.5-kb fragment encoding the entire open reading frame of human *TAL1* with 3′ HA tag was cloned into previously described pBSVE6BK (pEμ) plasmid containing a mouse V_H promoter (V186.2) and the IgH-μ enhancer (10). The construct, free from vector sequences, was injected into fertilized oocytes from C57Bl/6 and FVB/N animals. Transgenic mice were produced in Ohio State University transgenic mouse facility. Transgenic heterozygote mice were genotyped by PCR using the following primers: Tal3, AGATGACCTCCTGCAAGACGTGCTT; Tal4, GGTCGACCTAAGCGTAATCTGCAA. Control primers used in Figs. 1 and 2 amplify exon 5 of mouse *Fhit* gene: FhitD, CTTGAATCTAGGCTGCATTCTAGCGAG; FhitR, GATTCCTTGCTTACCTTTTGGGGATGG. PCR was carried out for 30 cycles at 94° for 30 minutes, 64° for 30 minutes, and 68° for 40 minutes using Advantage 2 polymerase (Clontech, Palo Alto, CA).

Western blot analysis. Cell proteins were extracted and Western blot analysis was carried out as previously described (11). Antibodies used were CD20 (H-170) and CD2 (TM2-5; Santa Cruz Biotechnology, Santa Cruz, CA), horseradish peroxidase-goat anti-mouse IgM (Zymed, South San Francisco, CA), and antitubulin (ab-1; Calbiochem, La Jolla, CA).

Fluorescence-activated cell sorting analysis. Spleen and bone marrow cells were isolated and analyzed by flow cytometry as previously described (12) using FACSCalibur (Becton Dickinson, Mountain View, CA). Antibodies were purchased from PharMingen (San Diego, CA): B220 (RA3-6B2), TCR β-chain (H57-597), IgM (R6-60.2), and CD43 (S7).

PCR analysis of D-J and V-D-J rearrangements. These experiments were carried out using previously described primers and conditions (6) except nested PCR was carried out using Advantage 2 polymerase. For the first PCR reaction, 20 cycles were used, whereas in the second PCR reaction, 15 cycles were used.

Results and Discussion

We generated transgenic mice in which the expression of human *TAL1* was under the control of a V_H promoter-IgH-Eμ enhancer of which the activity specifically targets expression of the transgene to immature and mature B cells (ref. 10; Fig. 1*A*). Five transgenic founders, one on C57Bl/6 background and four on FVB/N

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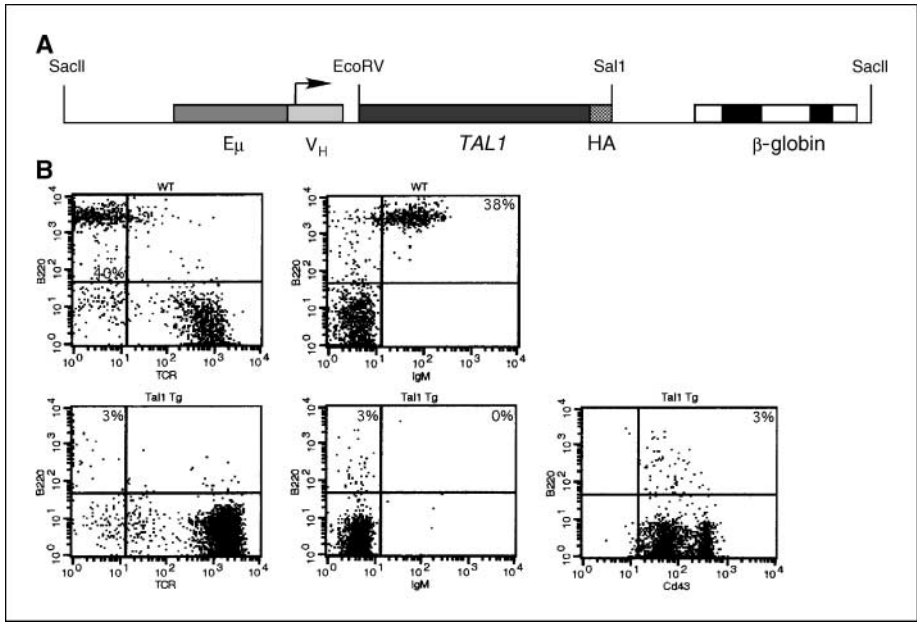

Figure 1. Absence of pro-B cells and mature B cells in spleen lymphocytes from Eμ-*TAL1* transgenic mice. *A,* the construct used in the transgenic mouse production. *B,* FACS analysis of lymphocytes from spleens of wild-type (*top*) or Eμ-*TAL1* transgenic mice (*bottom*).

background, were generated and bred to establish five transgenic lines. Pathologic examination of 1- to 2-month-old transgenic mice did not reveal any abnormalities, except spleens of transgenic animals were, on average, half the size and weight of those of their nontransgenic littermates. Because it was previously reported that transgenic expression of the truncated form of human Tal1 under the control of ubiquitous SIL promoter showed 30% to 60% reduction of IgM^+ cells (7), we used fluorescence-activated cell sorting (FACS) analysis to investigate lymphocyte lineages in spleens of Eμ-*TAL1* transgenic animals. FACS analysis of lymphocytes isolated from transgenic spleens of five out of five transgenic lines reveals complete absence of IgM-expressing (Fig. 1*B*) or CD19-expressing (not shown) cells. Almost all (97-98%) transgenic lymphocytes were T cells and only 2% to 3% of these cells were $B220^+$ whereas 40% of $B220^+$ cells were present in spleen lymphocytes isolated from wild-type mice (Fig. 1*B*). This indicates that Eμ-*TAL1* transgenic animals are unable to make pre-B cells and mature B cells. Figure 1*B* also shows that 100% of $B220^+$ cells were $CD43^+$, indicating that pro-B cells are present in the spleens of Eμ-*TAL1* transgenics. These results were consistent in all five transgenic lines. To confirm our findings, we analyzed the expression of B-cell and T-cell markers by Western blot of total protein extracted from spleens of 2-month-old mice (Fig. 2). Our results showed that IgM and CD20 (B-cell–specific markers) are not expressed in spleens of transgenic animals whereas CD2 (T-cell–specific marker) was expressed at normal levels (Fig. 2, *lanes 2-4* versus *lane 1*). These results were also consistent in all five transgenic lines. Consequently, transgenic expression of Tal1 was not detected in Western blot analysis using antihemagglutinin TAG antibody, thus confirming complete absence of pre-B cells and mature B cells in Eμ-*TAL1* transgenics. To confirm our findings, we analyzed bone marrow cells of Eμ-*TAL1* transgenics by FACS analysis. Figure 3 shows that bone marrow of Eμ-*TAL1* mice did not contain any $B220^+IgM^+$ or $B220^+CD19^+$ cells whereas bone marrow of their wild-type littermates contained 25% of $B220^+IgM^+$ and 62% of $B220^+CD19^+$ cells. All 21% $B220^+$ cells in Eμ-*TAL1* mice were also $CD43^+$, indicating the presence of pro-B cells but not of pre-B cells or mature B cells.

Because Tal1 functions as an inhibitor of E47 encoded by the *E2A* tumor suppressor gene (3) and *E2A* knockout mice show the absence of B cells and the absence of D-J and V-D-J rearrangements of *IgH* genes (6), we proceeded to determine the

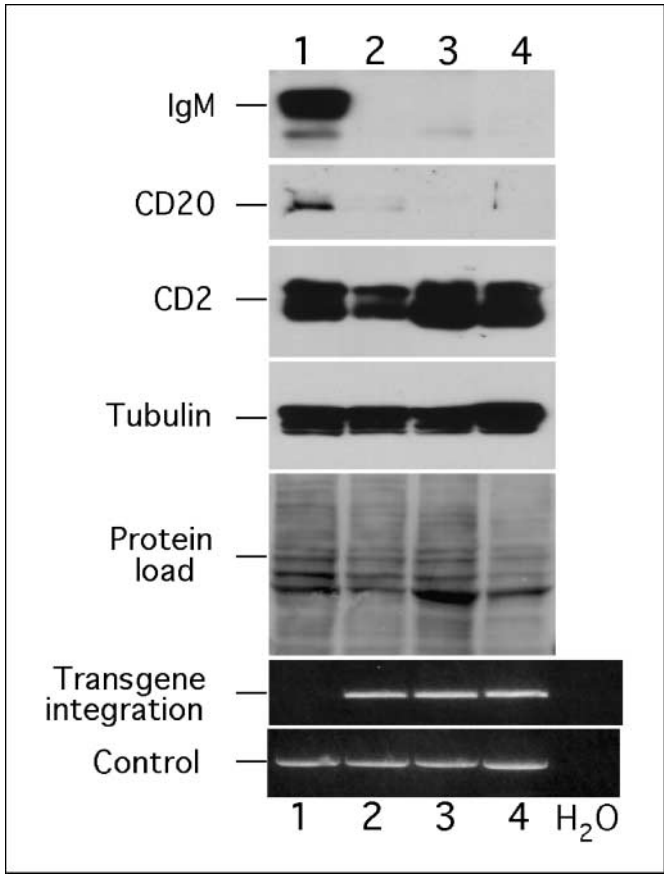

Figure 2. Western blot analysis of whole spleen lysates from Eμ-*TAL1* transgenics. Western blot analysis of whole spleen lysates from a wild-type mouse (*lane 1*) or transgenic Eμ-*TAL1* mice from three different lines (*lanes 2-4*). *Bottom,* PCR analysis confirming correct genotypes of transgenic animals.

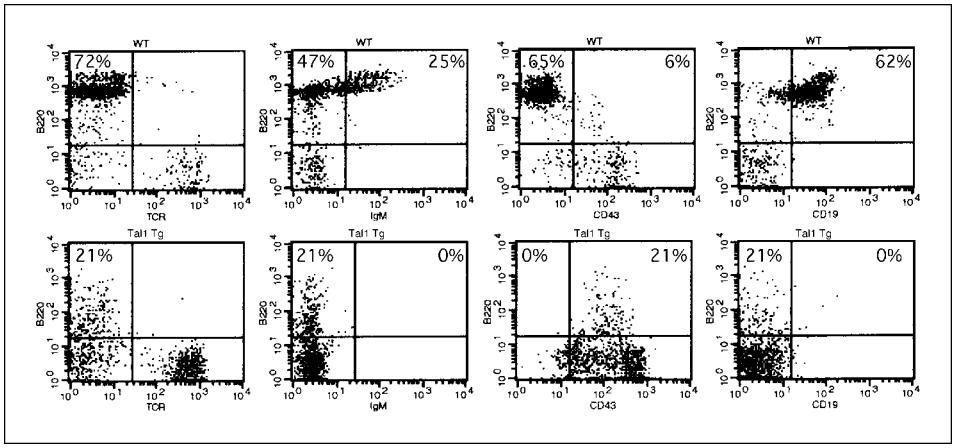

Figure 3. Defect of early B lymphopoiesis in bone marrow from Eμ-*TAL1* transgenic mice. FACS analysis of lymphocytes from bone marrow of wild-type (*top*) or Eμ-*TAL1* transgenic mice (*bottom*).

status of these rearrangements in bone marrow of Eμ-*TAL1* transgenics. We used the same primers used to analyze D-J and V-D-J rearrangements in *E2A* knockout mice (6). Figure 4 shows that D-J rearrangements are detectable in Eμ-*TAL1* transgenic B cells but no V-D-J rearrangements were observed. However, in some cases, the increase of cycle number in the nested PCR reaction (20-25 instead of 15) resulted in the appearance of faint rearranged bands (not shown).

In this report, we show that transgenic expression of Tal1 in mouse B cells results in the complete absence of pre-B cells and mature B cells. The function of Tal1 as an inhibitor of E2A/E47 is well established (1, 3). Because *E2A* knockout mice also show complete absence of B cells (6), our results represent first *in vivo* evidence that overexpression of Tal1 completely inhibits E47 function *in vivo*. The only difference between these two phenotypes is the detection of D-J rearrangements of the *IgH* genes in Eμ-*TAL1* transgenic B cells whereas these rearrangements were not detected in *E2A* knockout B cells. It is possible that our construct, which contains Eμ enhancer, starts expressing high levels of the transgene after the late pro-B-cell stage in which D-J rearrangements occur, explaining this difference in the phenotypes.

Two transgenic mouse models, expressing truncated or full-length form of human Tal1 under the control of ubiquitous SIL promoter, showing some expression in B cells or lymphoid-specific *Ly-6E.1* promoter, were previously reported (7, 8). These mice showed 2- to 3-fold reduction of IgM^+ cells with no effect on V-D-J recombination of *IgH* genes (7) or ~2-fold reduction of $B220^+CD19^+$ cells in bone marrow (8). These results suggested that Tal1 can only partially inhibit E2A function because E2A-deficient B cells did not show any pre-B cells or mature B cells (6). In contrast, Eμ-*TAL1* mice did not have any IgM^+ or $CD19^+$ cells and showed absence or drastic reduction of V-D-J rearrangements. The differences in phenotypes of Eμ-*TAL1* mice and two previously reported *TAL1* transgenic models can be explained by possible higher expression of the transgene in Eμ-*TAL1* mice or by the functional differences between the full-length and truncated forms of Tal1. A generation of a transgenic mouse model expressing the truncated form of Tal1 under the control of the Eμ enhancer would provide a more precise explanation of these differences in phenotypes.

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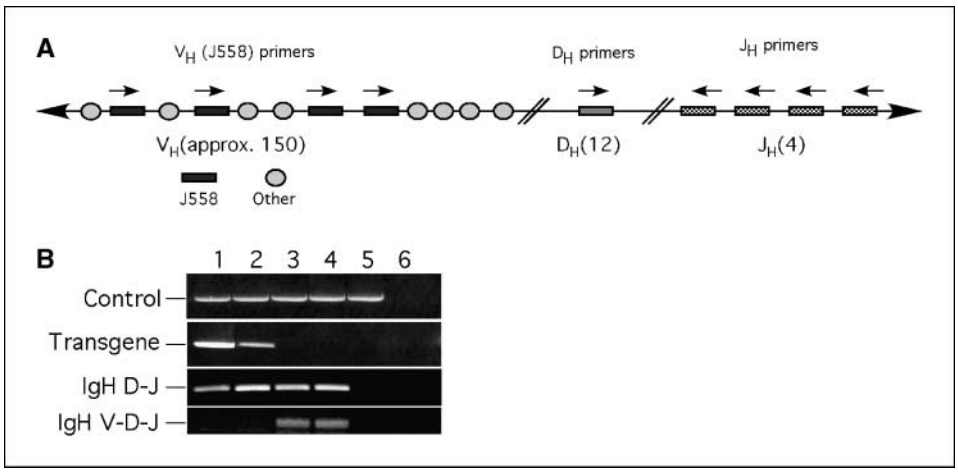

Figure 4. Status of D-J and V-D-J rearrangements of *IgH* genes in Eμ-*TAL1* transgenic mice. *A,* schematic representation of murine *IgH* locus. Of ~150 murine V_H genes close to half belongs to J558 family. *IgH* locus also contains 12 D_H genes and 4 J_H genes. *Arrows,* positions of the primers. The sizes of the PCR products are ~145 bp (D_{SP2}-J_H) and ~300 bp (J558-J_H). *B, lanes 1* to *4,* PCR using bone marrow genomic DNA isolated from two mice, each from a different transgenic line (*lanes 1* and *2*), and from two wild-type mice (*lanes 3* and *4*). *Lane 5,* DNA isolated from tails of wild-type mice was used in PCR. *Lane 6,* negative control with no DNA.

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