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Cre-loxP–Mediated Recombination between the *SIL* and *SCL* Genes Leads to a Block in T-Cell Development at the $CD4^-CD8^-$ to $CD4^+CD8^+$ Transition[1]

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Abstract

In the most common form of stem cell leukemia (*SCL*) gene rearrangement, an interstitial deletion of 82 kb brings *SCL* under the control of regulatory elements that normally govern expression of the ubiquitously expressed *SCL* interrupting locus (*SIL*) gene, which is located directly upstream of *SCL*. To investigate the effect of this fusion in a mouse model, a bacterial artificial chromosome (BAC) clone containing both human *SIL* and *SCL* genes was isolated, and loxP sites were inserted into intron 1 of both the *SIL* and *SCL* genes, corresponding to the sites at which recombination occurs in human T-cell acute lymphocytic leukemia patients. This BAC clone was used to generate transgenic *SILloxloxSCL* mice. These transgenic mice were subsequently bred to *Lck-Cre* mice that express the Cre recombinase specifically in the thymus. The BAC transgene was recombined between the two loxP sites in over 50% of the thymocytes from *SILloxloxSCL/Cre* double-transgenic mice, bringing the *SCL* gene under the direct control of *SIL* regulatory elements. Aberrant *SCL* gene expression in the thymus was verified by reverse transcription–polymerase chain reaction. Using FACS analysis, we found that mice carrying both *SILloxloxSCL* and *Cre* transgenes have increased $CD4^-/CD8^-$ thymocytes compared with transgene-negative mice. In the spleen, these transgenic mice show a marked reduction in the number of mature $CD4^+$ or $CD8^+$ cells. These results demonstrate that conditional activation of *SCL* under control of *SIL* regulatory elements can impair normal T-cell development.

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Keywords: *SCL*, *SIL*, T-cell development, Cre-LoxP, T-ALL.

Introduction

Activation of the stem cell leukemia (*SCL*) (also known as T-cell acute lymphoblastic leukemia 1 (*TAL-1* or *TCL5*) transcription factor is a frequent gain-of-function mutation in pediatric T-cell acute lymphocytic leukemia (T-ALL) [1,2]. *SCL* is normally expressed in hematopoietic cells, endothelium, and the central nervous system, and has been shown to be important for blood vessel formation, endothelial development, and the control of normal hematopoiesis [3–6]. Mice with an *SCL* null genotype are nonviable, but can be rescued by the human *SCL* locus [7].

Normally, expression of *SCL* in the thymus is restricted to the DN1–DN2 subset of immature $CD4^-/CD8^-$ thymocytes [8]. However, in the context of T-ALL, chromosomal translocations involving the *SCL* gene and chromosomes 3, 5, 7, and 14 have been associated with T-ALL and lead to unscheduled *SCL* expression [5,9–11]. In addition to the aforementioned chromosomal translocations, the most common event that deregulates *SCL* expression in T-ALL patients is an 82-kb interstitial deletion that occurs in approximately 25% of patients with T-ALL [2,5,12] and replaces *SCL* 5′ regulatory sequences with those of an upstream gene, known as *SCL*-interrupting locus (*SIL*) [1,2].

Attempts to model human T-ALL in mice have met with variable success. In two independent studies, expression of a full-length *SCL* gene under the control of the *Lck* promoter led to T-ALL at an advanced age in a fraction at *Lck-SCL* transgenic mice [13,14]. In contrast, other studies showed that deregulated *SCL* expression alone did not cause T-cell tumors [15–17]. The reasons for varying results among different studies are not clear, but may be due to differences in mouse strains, promoters used, or integration sites. The collective observations that T-ALL occurred at an advanced age, with incomplete penetrance, suggested that additional events were required to produce T-ALL in these mice. Indeed, several reports have indicated that aberrant *SCL* expression, in collaboration with activation of *CKIIα*, *LMO1*, or *LMO2*, leads to the development of aggressive T-cell malignancies in transgenic mice at an early age with a high degree of penetrance [14,16–19].

Transgenic mice that express either a full-length or an amino-terminal truncated SCL driven by a *SIL* promoter showed bony abnormalities and growth retardation, but did not develop

Abbreviations: *SCL*, stem cell leukemia; *SIL*, *SCL*-interrupting locus; BAC, bacterial artificial chromosome; T-ALL, T-cell acute lymphocytic leukemia

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T-cell malignancies [17].However, when crossed with mice that overexpress *LMO1* in the thymus, these double-transgenic mice formed aggressive T-cell malignancies with a high degree of penetrance at an early age [17,19]. Interestingly, abnormal T-cell development was noted in *SCL/LMO1* double-transgenic mice before the onset of malignancy, whereas no abnormal immunophenotype for T-cell development was detected in mice transgenic only for *SCL* or *LMO1* [8,19]. Consistent with these results, other groups also failed to detect a premalignant perturbation of T-cell differentiation in *Lck-SCL* or *CD2-SCL* mice before development of leukemia [13,14,16].

We previously demonstrated that SCL could inhibit E2A activity in a dose-dependent fashion *in vitro* [8,19]. We considered the possibility that misexpression of *SCL* under the control of *SIL* regulatory elements [17] did not lead to T-ALL in mice because the levels of SCL were insufficient to inhibit E2A (or the closely related HEB protein). Because high levels of SCL expression under the control of *SIL* regulatory elements seemed to be toxic to the developing embryo (reference 17 and unpublished data), we used Cre-loxP technology to generate mice that would conditionally express *SCL* under the control of *SIL* regulatory elements. Moreover, we reason that including additional *SIL* regulatory sequences might lead to higher levels of SCL expression compared to the 2.3 kb of *SIL* promoter sequence used for the pSIL/SCL mice [19].

Materials and Methods

Generation of Bacterial Artificial Chromosome Clone and SILloxloxSCL *Transgenic Mice*

We isolated a bacterial artificial chromosome (BAC) clone containing both human *SIL* and *SCL* genes as well as 47 kb of sequence immediately 5′ of *SIL*, and used "recombineering" [20] to introduce loxP sites into intron 1 of both *SIL* (nucleotide 47491426 of human chromosome 1; National Center for Biotechnology Information [NCBI] build 35 by the International Human Genome Sequencing Consortium, May 2004) and *SCL* (nucleotide 47409602), corresponding to the sites at which recombination occurs in human T-ALL patients. This BAC clone was linearized with PI-*Sce*I and injected into fertilized FVB/N embryos to generate transgenic *SILloxloxSCL* mice.

Genomic DNA Polymerase Chain Reaction, Southern Blotting, and DNA Sequencing

All polymerase chain reaction (PCR) amplifications, unless otherwise indicated, were performed by using PCR SuperMix High Fidelity enzyme and buffers (Invitrogen, Carlsbad, CA). PCR primer sequences and annealing temperature are listed in Table 1. Mouse tail DNA was extracted for PCR analyses as follows. Two millimeters of tail was cut and added to 600 μl of 50 mmol/l NaOH, heated to 95°C for 1 hour, and neutralized with 50 μl of 1 mol/l Tris (pH 8). One microliter of this crude DNA preparation was used for mouse genotype analysis. For the human *SIL* gene, the primers were SIL5′F and 3′-SILR; for the human *SCL* gene, the primers were 3′SCLF and 3′SCLR. *Cre*-positive and -negative mice were identified by PCR using primers cre1083F and cre1085R. To identify *SIL-SCL* recombination at the LoxP site, sequences in the first intron of the *SIL* gene (SIL5′F) and the first intron of the *SCL* gene (SCLR-TARG) were used for PCR primers. To verify DNA quality, we amplified the mouse *Scid* locus by using primers SCIDA and SCIDB.

Table 1. PCR Primers.

Name	Sequence (5′ > 3′)	Annealing Temperature (°C)
SIL5′F (forward)	GCCCTGTAGTGGGTTCCGCCC	60
3′-SILR (reverse)	TCCAGTCAAACTGAACTACTTGC	60
3′of SCLF (forward)	GGCCTGGTGGGGAGGAGACAGC	60
3′of SCLR (reverse)	GATACATACAACTGTCCCAGCC	60
cre1083F (forward)	GCGGCATGGTGCAAGTTGAATAA	55
cre1085R (reverse)	GTGAAACAGCATTGCTGTCACTT	55
SCLR-TARG (reverse)	GACACCACCCAAACACAGTCGC	56
SCIDA (forward)	GGAAGAGTTTTGAGCAGACAATG	54
SCIDB (reverse)	CATCACAAGTTATAACAGCTGGG	54
SILexon1 (forward)	GCTCCTACCCTGCAAACAGA	60
SCLexon3 (reverse)	GGCATATTTAGAGAGACCG	60
Tal1 (forward)	ATGGTGCAGCTGAGTCCTCC	52
Tal1 (reverse)	TCTCATTCTTGCTGAGCTTC	52
β-Actin F (forward)	GTGGGCCGCTCTAGGCACCAA	58
β-Actin R (reverse)	CTCTTTGATGTCACGCACGATTTC	58

PCR products were cloned into the pGEM-T easy vector (Promega, Madison, WI) and transformed into DH-5α cells. Plasmid DNA was extracted (Qiagen, Valencia, CA) and sequenced (Napcore, The Children's Hospital of Philadelphia). All procedures were performed following the manufacturers' protocols.

For Southern blot studies, 10 μg of genomic DNA isolated from mouse tissue by use of a proteinase K/SDS salting out procedure [21] was digested with restriction enzymes and size fractionated using a 0.8% agarose gel. The DNA fragments were transferred to nitrocellulose membranes and hybridized to ^{32}P-labeled probes. Probes used included a previously described human TCR Cβ2 probe [17], a human *SIL* exon 1 probe (nucleotides 47491736 to 47492065, human chromosome 1; NCBI build 35 by the International Human Genome Sequencing Consortium, May 2004) [22,23], and a human *SCL* probe from the 3′ untranslated region (929 bp, nucleotides 47394123 to 47393194, human chromosome 1; NCBI build 35 by the International Human Genome Sequencing Consortium, May 2004). Fragments were labeled with ^{32}P by using Ready-To-Go DNA labeling beads (Amersham Bioscience, Piscataway, NJ). Hybridization was performed as previously described [21]. The washing conditions were 0.1% SDS/2 × SSC at 42°C for 20 minutes and 0.1% SDS/0.1 × SSC at 52°C for 40 minutes.

Reverse Transcription–PCR and Semiquantitative Reverse Transcription-PCR

Total RNA was isolated using Trizol reagent and the manufacturer's (Invitrogen) recommended protocol. To

eliminate potential contamination of genomic DNA, all total RNA samples were treated with 2 U DNAse for 30 minutes (Ambion, Austin, TX). First-strand cDNA was synthesized from 1 μg of DNAse-treated RNA by using SuperScript First-Strand Synthesis System for reverse transcription (RT)-PCR (Invitrogen), following the manufacturer's protocol. Controls without reverse transcriptase were performed in all reactions to detect possible contamination of genomic DNA. PCR amplification of β-actin was used to verify that intact mRNA was present.

The primers for the *SIL-SCL* fusion mRNA were SILexon1 and SCLexon3. RT-PCR products were cloned into plasmid vectors and sequenced as described above. Semiquantitative RT-PCR was performed by serial 10-fold dilution of cDNA templates (1×, 0.1×, 0.01×, and 0.001× for each sample).

Immunophenotype Analysis

Cells from thymus and spleen were minced and homogenized using a loose-fitting ground-glass homogenizer as previously described [19] to generate a single-cell suspension in RPMI 1640 medium. The cell suspension was filtered through a cell strainer (BD, Bedford, MA) and treated with ACK lysing buffer (Biosource, Camarillo, CA) for 10 minutes. Viable cells (1×10^6) were used for subsequent antibody staining. The cells were incubated with 5 μl (0.5 mg/ml) of rat anti–mouse CD16/CD32 for 20 minutes as a blocking agent. The single-cell suspension was incubated with 5 μl (0.2 mg/ml) of fluorescein isothiocyanate– or phycoerythrin-labeled antibodies (murine CD4, CD8, CD25 or CD 44) for 30 minutes. All antibodies were purchased from BD Pharmingen (San Jose, CA). Ten thousand events per sample were scored using a FACSort flow cytometer (Becton-Dickinson, San Jose, CA).

Results

Generation of Mice Susceptible to SIL-SCL *Recombination*

Three founder lines (F1, I2, and H6) that had incorporated at least one copy of the *SILloxloxSCL* BAC clone (Figure 1*A*) were identified by PCR analyses. We used Southern blot analysis of the BAC clone integration site to identify a founder that had integrated a single copy of the BAC clone (Figure 1*B*) and verified that this line had retained all of the *SIL* and *SCL* genomic sequences by PCR. To determine whether Cre-loxP–mediated *SIL-SCL* recombination could be achieved *in vivo*, we crossed offspring of the H6 founder, which had integrated a single copy of the *SILloxloxSCL* BAC clone, to mice that expressed the Cre recombinase under the control of the *Lck* promoter to produce *SILloxloxSCL*$^+$/*Cre*$^+$ double-transgenic mice.

We used genomic DNA PCR to verify that recombination between the SIL and SCL loci had taken place in *SILloxloxSCL*$^+$/*Cre*$^+$ mice. A PCR product of 543 bp could be identified in thymus, and to a lesser extent in spleen, of the *SILloxloxSCL*$^+$/*Cre*$^+$ mice, but not the control genotypes, indicating a precise Cre-LoxP–mediated excision of 82 kb (Figure 1*C*).

To obtain a more quantitative estimate of the relative proportion of *SILloxloxSCL* alleles in the rearranged versus nonrearranged configuration, we used *SIL* exon 1 as a probe to detect recombination in the transgenic mice through Southern blot analysis. In *SILloxloxSCL*$^+$/*Cre*$^+$ mice, the ratio of recombined to unrecombined products is approximately 1 as seen in Figure 1*D*. As anticipated from the PCR results, these recombination events occurred only in the thymus and spleen of *SILloxloxSCL*$^+$/*Cre*$^+$double-transgenic mice. The sequence of the PCR product from thymus shows a *SIL-SCL* genomic fusion, with intervening LoxP sequence (Figure 1*E*).

SILloxloxSCL$^+$/Cre$^+$ *Double-Transgenic Mice Do Not Develop T-Cell Tumors*

Using semiquantitative RT-PCR analysis, we demonstrated that *SILloxloxSCL*$^+$/*Cre*$^+$ mice that have undergone Cre-LoxP–mediated recombination in the thymus express ~10- to 100-fold higher levels of SCL expression than those of the pSIL/SCL transgenic mice that express an *SCL* cDNA from 2.3 kb of SIL 5′ regulatory sequence [19] (Figure 2). We followed a cohort of *SILloxloxSCL*$^+$/*Cre*$^+$ mice for 19 months and compared them with three control groups: positive for either the *SILloxloxSCL* or the *Cre* only and negative for both transgenes. Their survival curves were similar; none of 14 double-transgenic mice or 46 control mice from this cohort developed T-cell malignancy (Figure 3).

Mice Transgenic for SIL-SCL *and* Cre *Demonstrate Impaired T-Cell Differentiation*

Because *SILloxloxSCL*$^+$/*Cre*$^+$ double-transgenic mice expressed higher levels of *SIL-SCL* fusion mRNA in the thymus than did the pSIL/SCL mice (Figure 2), we searched for evidence that these higher levels of ectopically expressed *SCL* might affect T-cell growth and differentiation. To achieve this purpose, we performed T-cell subset analyses on the thymus and spleen of mice aged 6 to 15 months.

The *SILloxloxSCL*$^+$/*Cre*$^+$ mice showed a variable, and in some cases quite dramatic, increase in CD4$^-$/CD8$^-$ (DN) cells and a corresponding decrease in CD4$^+$/CD8$^+$ (DP) cells in the thymus (Figures 4 and 5*A*). On average, the *SILloxloxSCL*$^+$/*Cre*$^+$ mice had 30.3 ± 17.4% DN cells and 44.5 ± 19.9% DP cells, compared with control mice, which had 8.0 ± 2.5% DN and 69.8 ± 8.5% DP cells ($P < .001$). Consistent with the thymocyte data, mice with decreased DP cells in the thymus had decreased percentages of mature single positive (SP) CD4$^+$ ($P < .01$) and CD8$^+$ ($P < .05$) cells in the spleen, compared with wild-type controls (Figures 4 and 5*B*). In addition, we searched for immature CD44$^+$CD25$^-$ cells in the thymus of some *SILloxloxSCL*$^+$/*Cre*$^+$ mice. As shown in Figure 5*C*, *SILloxloxSCL*$^+$/*Cre*$^+$ mice had a marked increase in this population compare with wild-type controls.

To determine whether the *SILloxloxSCL*$^+$/*Cre*$^+$ mice had a clonal or oligoclonal expansion of thymocytes, genomic DNA from *SILloxloxSCL*$^+$/*Cre*$^+$ mice was digested with *Sst*I,

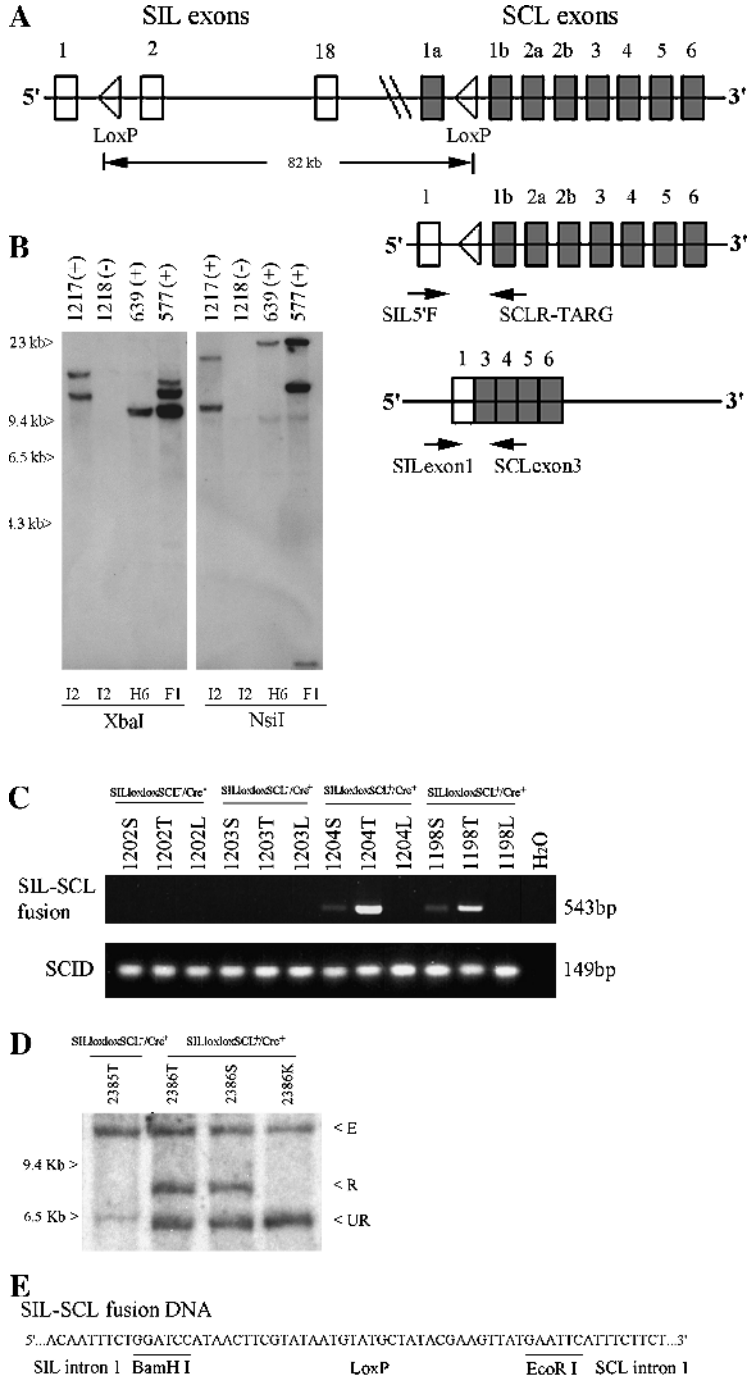

Figure 1. *Generation and characterization of* SILloxloxSCL *mice. (A) Top panel shows the human* SIL *(18 exons),* SCL *genes (8 exons), and the introduced Lox P sites in the regions of intron 1. Not all SIL exons are shown. Middle panel shows the fusion of genomic DNA. Two arrows indicate the primers used for a detection of fusion* SILloxSCL *DNA. Bottom panel shows the fusion mRNA that is generated between exon 1 of* SIL *and exon 3 of* SCL *in double-transgenic mice. Two arrows indicate the primers used for a detection of* SIL-SCL *fusion mRNA. (B) Detection of* SILloxloxSCL *BAC clone integration site(s) by Southern blot. Genomic DNA from F1 offspring of founders I2, H6, or F1 was digested with* Xba*I or* Nsi*I and hybridized to a probe from the* SCL *3′ untranslated region located at one terminal of the BAC clone. Variable-sized fragments representing unique integration sites can be seen. A cross-hybridizing band is seen in the* Nsi*I digest at 9.4 kb. Mouse numbers are indicated, as is transgene status (+ or −) (C)* SIL-SCL *genomic fusion can be detected in mice 1204 and 1198 by PCR. Mice 1202, 1203, and* H_2O *were negative controls. PCR amplification of the* scid *locus was used as a DNA quality control. T, thymus; S, spleen; L, liver. (D) Detection of* SIL-SCL *fusion by Southern blot analysis of* Sst*I-digested genomic DNA. 2385T, thymus from mouse without the* SILloxloxSCL *transgene. 2386T, 2386S, 2386K; thymus, spleen, and kidney from* SILloxloxSCL$^+$/Cre$^+$ *mouse. E, endogenous murine* SIL*; R, recombined* SIL-SCL *fusion; UR, unrearranged* SILloxloxSCL *transgene. (E) Nucleotide sequences of fusion* SILloxSCL *genomic DNA.*

blotted, and hybridized with a TCR Cβ2 probe. No clonal *TCRβ* gene rearrangements were detected in *SILloxloxSCL$^+$/Cre$^+$* transgenic mice nor in the control wild-type or single-transgenic mice (data not shown).

Discussion

The fusion of the *SIL* gene promoter region with the downstream *SCL* gene is a common rearrangement detected in patients with T-ALL [2,12,22]. Surprisingly, pSIL/SCL mice

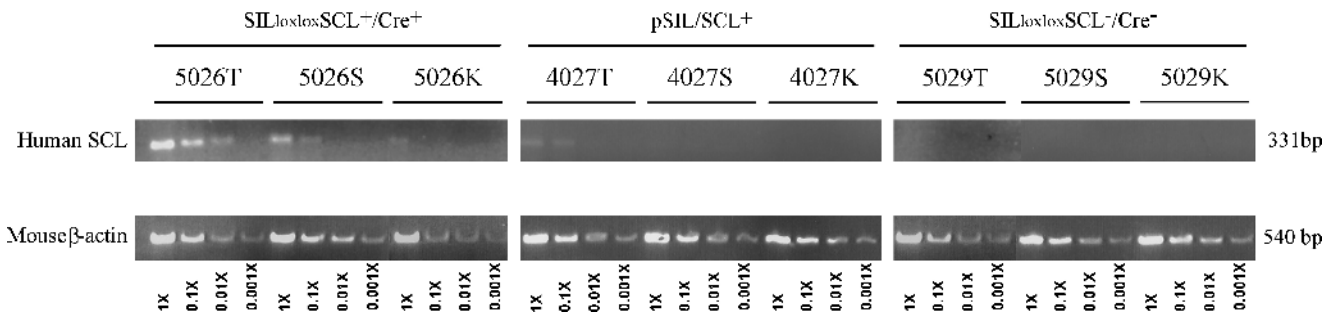

Figure 2. *Higher level* SCL *expression in* SILloxloxSCL$^+$/Cre$^+$ *mice than pSIL/SCL mice. Mouse 5026, positive for both* SILloxloxSCL *and* Cre *transgenes, has a higher level of* SCL *expression than a pSIL/SCL transgenic mouse (4027); mouse 5029 is negative for both* SILloxloxSCL *and* Cre *transgenes. T, S, and K are thymus, spleen, and kidney, respectively. The cDNA templates were diluted to 1×, 0.1×, 0.01×, and 0.001× times in PCR reactions.*

that express *SCL* under the control of *SIL* regulatory elements [19] did not develop T-ALL, nor did these mice show any abnormalities in T-cell differentiation. However, when crossed to transgenic mice that expressed *LMO1* in the thymus, the *SCL/LMO1* double-transgenic mice developed T-ALL preceded by abnormalities in thymocyte differentiation, including an oligoclonal expression of DN cells [17,19].

To determine if an *SCL* expression construct that more closely resembled the situation seen in human T-ALL patients could cause T-ALL, we isolated a human BAC clone that contained both the human *SIL* and *SCL* genes, and introduced LoxP sites into intron 1 of the *SIL* and *SCL* genes at the sites where recombination occurs in human T-ALL patients [12,24]. This clone was then used to generate transgenic *SILloxloxSCL* mice. After crossing the *SILloxloxSCL* mice to *Lck-Cre* mice, which expressed the Cre recombinase in the thymus, we were able to detect a *SILloxSCL* genomic fusion and a *SIL-SCL* fusion mRNA, thus mimicking the common form of *SCL* gene activation seen in T-ALL patients. Of note, although it is well established that the Cre recombinase efficiently catalyzes recombination between LoxP sites located less than 5 kb apart [25] and that the Cre recombinase can catalyze rare recombination between LoxP sites on different chromosomes [26], we show here that Cre-mediated recombination can efficiently act over a distance of 82 kb and cause recombination of approximately half of the *SILloxloxSCL* alleles.

In our previous studies, both T-ALL and abnormal T-cell development were detected in *SCL/LMO1* double-transgenic mice, but not in *pSIL/SCL* transgenic mice that expressed a full-length SCL protein [19]. Using a semiquantitative RT-PCR assay, we show that *SILloxloxSCL/Cre* transgenic mice have higher levels of *SCL* expression than those of *pSIL/SCL* transgenic mice. This difference may be due to incorporation of additional *SIL* regulatory elements (~47 kb of sequences 5′ of *SIL* exon 1), which may affect both timing and level of *SCL* expression in transgenic mice. Using *Lck* to direct *SCL* expression in the mouse thymus, two laboratories have induced T-ALL in mice but did not observe any evidence of a premalignant perturbation of T-cell development [13,14].

Four populations of developing T-cells can be identified through immunophenotype analysis: CD4$^-$CD8$^-$ (DN), the CD4$^+$CD8$^+$ (DP) and CD4$^-$CD8$^+$ (CD8 SP), or CD4$^+$CD8$^-$ (CD4 SP). The DN population can be further subdivided into CD44$^+$CD25$^-$ (DN1), CD44$^+$CD25$^+$ (DN2), CD44$^-$CD25$^+$ (DN3), and CD44$^-$CD25$^-$ (DN4) stages [27]. To determine whether expression of the *SIL-SCL* fusion generated in our current study affects T-cell development, we performed FACS analysis of thymocytes from *SILloxloxSCL$^+$/Cre$^+$* mice. The fraction of DN thymocytes in *SILloxloxSCL$^+$/Cre$^+$* mice was significantly increased compared with those of the control groups. Furthermore, we noted an increase in the percentage of immature DN1 cells in the thymus of *SILloxloxSCL$^+$/Cre$^+$* transgenic mice, which were not detected in either *SILloxloxSCL$^-$/Cre$^-$* or *SILloxloxSCL$^+$/Cre$^-$* mice, indicating impaired T-cell development at the DN1 stage. We also found that CD4 SP and CD8 SP cells were decreased in the spleen of *SILloxloxSCL$^+$/Cre$^+$* mice, consistent with the decrease in DP cells seen in *SILloxloxSCL$^+$/Cre$^+$* mice thymus.

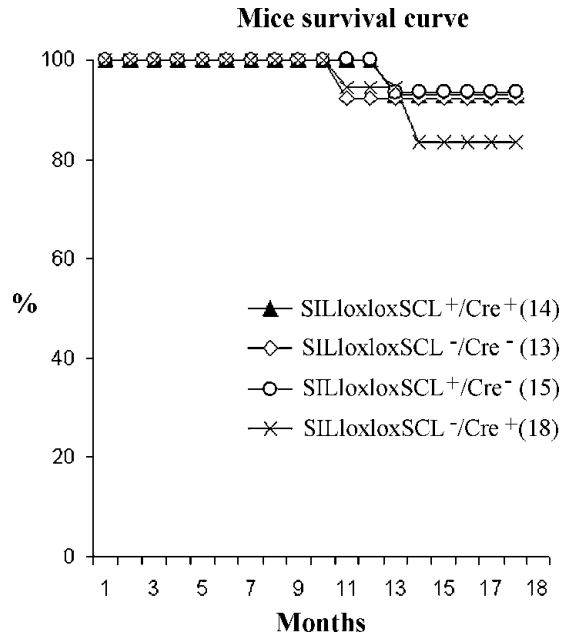

Figure 3. SILloxloxSCL$^+$/Cre$^+$ *mouse survival curve. Mice of the indicated genotypes were followed for 19 months and euthanized when morbid. There was no difference in survival for any group.*

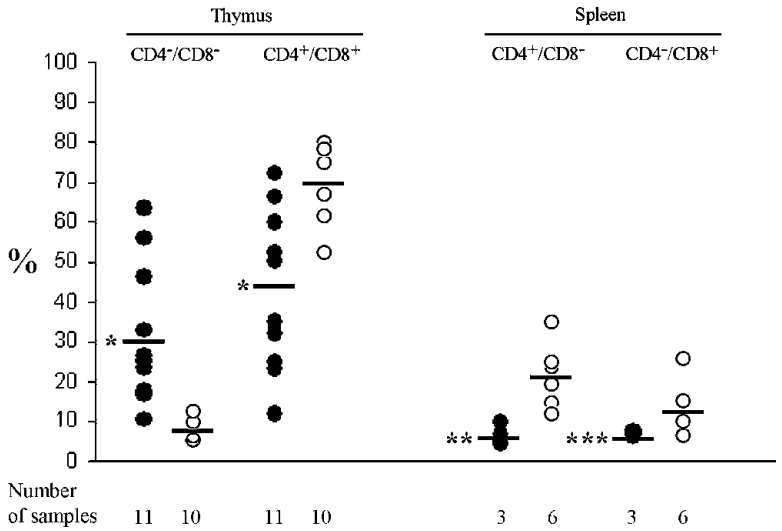

Figure 4. *Aberrant T-cell differentiation in* SILloxloxSCL$^+$/Cre$^+$ *mice. Thymocytes and splenocytes from* SILloxloxSCL$^+$/Cre$^+$ *(closed circles) or* SILloxloxSCL$^-$ *or* SILloxloxSCL$^+$/Cre$^-$ *mice (open circles) stained with CD4 + CD8. The number of samples analyzed is indicated. The means are indicated with a solid line. *P < .001; **P < .01; ***P < .05.*

Tumorigenesis is a multistep process [28]. Although the development of T cells was impaired in *SILloxloxSCL$^+$/Cre$^+$* mice, we did not detect any clonal *TCRβ* gene rearrangements in these mice, suggesting that there was no clonal or oligoclonal expansion. However, because *TCRβ* gene rearrangement typically occurs in the DN2 population, it is possible that clonal expansion, undetectable by *TCRβ* gene rearrangement, has occurred in the samples with a predominant DN1 population. None of the 14 mice with both the *SIL-SCL* and the *Cre* transgenes developed T-cell malignancies over a 19-month observation period. Despite the higher level of *SCL* expression and perturbations of thymocyte development observed in these mice, aberrant expression of *SCL* in the thymus was insufficient to induce leukemia.

There are several possible explanations for the lack of leukemic transformation in the *SILloxloxSCL$^+$/Cre$^+$* mice that had undergone SIL-SCL recombination. First, the lack of leukemic transformation may be due to subtle, undetected

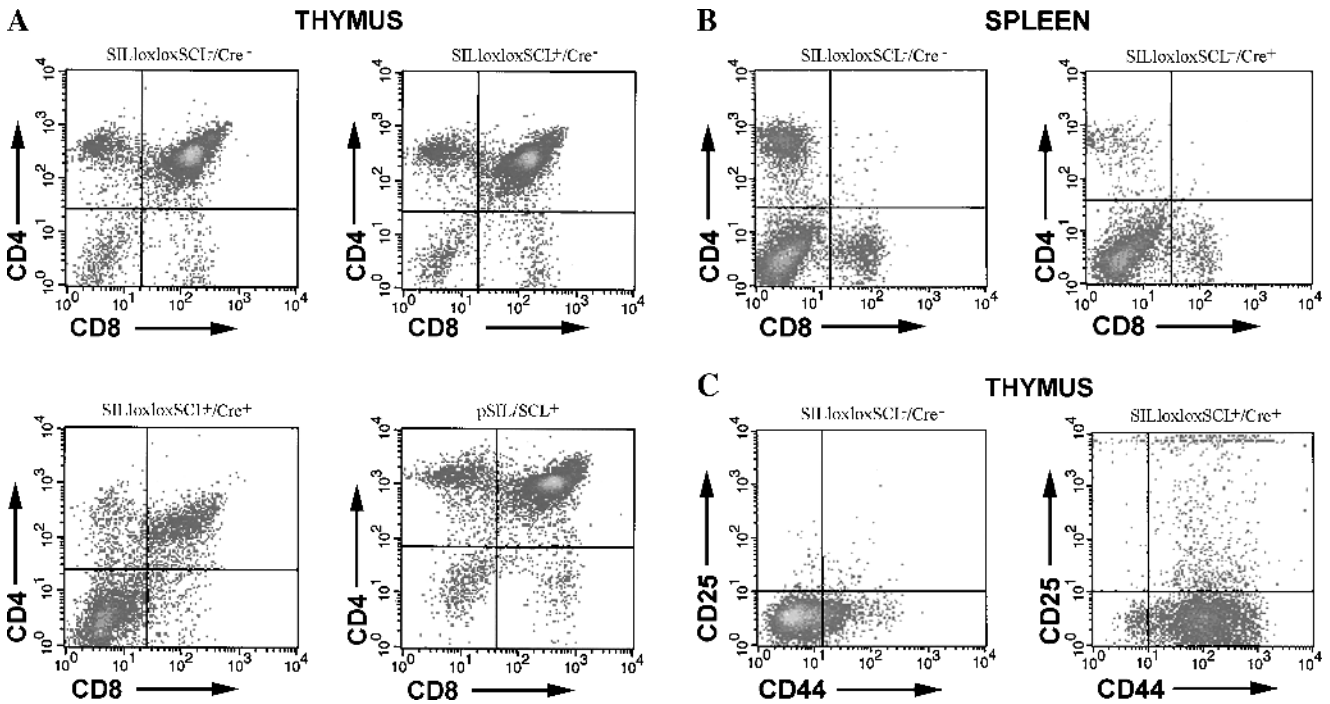

Figure 5. *FACS profiles demonstrating aberrant T-cell development. Thymocytes (A) and splenocytes (B) from clinically healthy mice with the indicated genotype were stained with CD4 and CD8. Note the increased DN population in the* SILloxloxSCL$^+$/Cre$^+$ *thymocytes, and decreased SP population in* SILloxloxSCL$^+$/Cre$^+$ *splenocytes. (C) Thymocytes from above mice were stained with CD44 and CD25; note increased CD44$^+$CD25$^-$ population in* SILloxloxSCL$^+$/Cre$^+$ *sample.*

mouse strain differences. Second, the *SCL* transgene may not be expressed at a "leukemogenic" point in thymocyte differentiation, as *SCL* expression is dependent on expression of the Cre recombinase under the control of *Lck* promoter. Lastly, it is possible that the *SIL-SCL* fusion is a primary oncogenic event and requires additional events for complete leukemic transformation [13,14].

In summary, we designed and generated a genetic event that faithfully recapitulates the interstitial deletion leading to *SIL-SCL* fusion gene seen in human T-ALL patients. We demonstrated that expression of *SCL*, under the control of *SIL* regulatory elements, can perturb normal T-cell development, which might provide an abnormal environment in which additional genetic events occur, and result in complete malignant transformation.

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