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Advances in Brief

scid Thymocytes with TCRβ Gene Rearrangements Are Targets for the Oncogenic Effect of *SCL* and *LMO1* Transgenes[1]

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Abstract

***SCL* and *LMO1* were both discovered by virtue of their activation by chromosomal translocation in patients with T-cell acute lymphoblastic leukemia (T-ALL). Overexpression of *SCL* and *LMO1* in the thymus of transgenic mice leads to T-ALL at a young age. *scid* (severe combined immunodeficient) mice are unable to efficiently recombine antigen receptor genes and consequently display a developmental block at the CD4-CD8- to CD4+CD8+ transition. To test the hypothesis that this developmental block would protect *SCL*/*LMO1* transgenic mice from developing T-ALL, we crossed the *SCL* and *LMO1* transgenes onto a *scid* background. The age of onset for T-ALL in the *SCL*/*LMO1*/*scid* mice was significantly delayed ($P < 0.001$) compared with *SCL*/*LMO1*/*wild-type* mice. Intriguingly, all of the *SCL*/*LMO1*/*scid* malignancies displayed clonal, in-frame TCRβ gene rearrangements. Taken together, these findings suggest that the "leaky" *scid* thymocyte that undergoes a productive TCRβ gene rearrangement is susceptible to the oncogenic action of *SCL* and *LMO1* and additionally suggests that TCRβ gene rearrangements may be required for the oncogenic action of *SCL* and *LMO1*.**

Introduction

SCL[3] is the gene most commonly activated by chromosomal rearrangement in patients with T-ALL (1, 2). Several recent reports have demonstrated that unscheduled *SCL* expression directed to the thymus of transgenic mice infrequently leads to T-cell malignancies, relatively late in life (3, 4). However, in collaboration with either *LMO2* (5) or *LMO1* (6, 7), *SCL* activation leads to aggressive T-cell malignancies early in life (mean age of onset: 4 months), with almost complete penetrance, demonstrating that high-level expression of *SCL* and *LMO1* or *LMO2* in the thymus leads to T-cell malignancies. The notion of collaboration between SCL and LMO proteins is supported by observations that: (*a*) some human T-ALL patients have activated both *SCL* and *LMO1* or *LMO2* (8); (*b*) a subset of tumors derived from *LMO1* transgenic mice have activated the endogenous mouse *SCL*;[4] (*c*) *MSH2*-deficient mice develop T-cell malignancies that have activated *SCL* and *LMO2* (9); and (*d*) SCL and LMO proteins have been shown to form a multiprotein complex both *in vitro* and *in vivo* (8, 10).

Coexpression of *SCL* and *LMO1* leads to a number of consistent abnormalities in the developing thymus before the onset of a clinically detectable malignancy (6, 11). Paradoxically, thymi from young *SCL*/*LMO1* double transgenic mice aged 4–5 weeks are reduced in size and have a >3-fold reduction in the number of total thymocytes. This reduction is attributable, at least in part, to an increase in the number of apoptotic CD4+/CD8+ cells (6, 11). There is a prominent reduction in CD4+/CD8+ cells and a small absolute increase in the number of immature CD4-/CD8- cells. At the same time, expansion of an oligoclonal population of cells with clonal TCRβ gene rearrangements can be detected. However, this oligoclonal population of cells is not frankly malignant, as evidenced by their failure to generate tumors when injected into immunodeficient nude mice (6). The rate at which malignancies develop in *SCL*/*LMO1* mice, along with the aforementioned failure of thymocytes from 4–5 week-old mice to produce tumors in immunodeficient mice, suggests that additional genetic events are likely to be required before *SCL*/*LMO1* thymocytes become fully malignant. One hypothesis consistent with these observations is that activation of both *SCL* and *LMO1* in the developing thymus leads to expansion of a relatively limited number of clones, one of which undergoes additional mutation(s) and becomes fully malignant.

scid mice (12) are immunodeficient because of their inability to effectively recombine antigen receptor genes; the molecular basis for this inability is caused by a mutation in the gene coding for DNAPKcs (13). This inability leads to a block in thymocyte development at the CD4+/CD8+ stage, because of a lack of a functional TCRβ gene rearrangement, a process that is required for thymocytes to progress to the CD4+/CD8+ stage. However, the inability of *scid* mice to rearrange antigen receptor genes, although severe, is not complete, as evidenced by the appearance of rare, "leaky" *scid* lymphocytes with functional rearrangements of TCR or immunoglobulin genes (14). To determine whether the T-lymphocyte developmental block present in *scid* thymocytes would protect mice from the leukemogenic effect of *SCL* and *LMO1* transgenes, we crossed *SCL* and *LMO1* transgenes onto a homozygous *scid* background.

Materials and Methods

Generation of Transgenic/*scid* Mice. The A(5)3 *SCL* line and #11 lck-*LMO1* line have been characterized previously (7, 15) and were maintained on a hybrid C57Bl6 × C3H background. C.B-17 *scid*/*scid* mice were obtained from Dr. Richard Bankert (Roswell Park Cancer Institute, Buffalo, NY). *scid*/*scid* mice were crossed with mice carrying either the *LMO1* or *SCL* transgenes. Progeny were genotyped for *SCL* and *LMO1* by Southern blotting, as described previously (7), and for the *scid* defect by PCR (16). Briefly, genomic DNA isolated from tail biopsies was amplified with primers 5′-GGAAGAGTTTTGAGCAGACAATG-3′ and 5′-CATCACAAGTTATAACAGCTGGG-3′ using 1 cycle of 94°C for 3 min followed by 30 cycles of 94°C × 1 min, 54°C × 1 min, and 72°C × 1 min and concluding with a 10-min terminal extension at 72°C. Five μl of the PCR reaction mixture were digested with *Alu*I, and the fragments were analyzed on a 4% agarose gel (Nusieve 3:1 agarose; FMC Corp., Rockland, ME). The wt *scid* allele generated fragments of 15 and 130 bp compared with the mutant *scid* allele, which produced fragments of 15, 30, and 100 bp. F1 animals that were positive for *SCL* or *LMO1* transgenes were crossed, and F2 animals were genotyped and placed into one of eight possible cohorts based on transgene and *scid* defect status (*wt*, *scid*, *SCL/wt*, *SCL/scid*, *LMO1/wt*, *LMO1/scid*, *SCL/LMO1/wt*, and

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[3] The abbreviations used are: *SCL*, *SCL/tal*; TCR, T-cell receptor; T-ALL, T-cell acute lymphoblastic leukemia; wt, wild type; *scid*, severe combined immunodeficient; DN, CD4-CD8-.

[4] D. S. Chervinsky and P. D. Aplan, unpublished data.

SCL/LMO1/scid). Survival between groups was analyzed using the χ^2 test with one degree of freedom. Animals were maintained under standard conditions according to institutional animal care and use guidelines.

Nucleic Acid Manipulations. Genomic DNA isolation and Southern blots were performed as described previously (7). Probes used in this study included a 1.2-kb *Hin*dIII-*Xba*I human *SCL* cDNA fragment (67HX, Ref. 7), a PCR-amplified human *LMO1* cDNA fragment (nucleotides 544–957 of GenBank accession no. M26682), PCR-amplified murine pre-Tα (nucleotides 218–622 of GenBank accession no. NM 011195), and TCR Jδ1, Cβ2, and Cα (all gifts of Dr. Ilan Kirsch, National Cancer Institute).

Sample Isolation and Immunophenotype. Thymus and spleen or tumor masses were removed from mice and placed on ice in RPMI 1640 containing 10% fetal bovine serum, 5 μg/ml penicillin, and 5 μg/ml streptomycin. Single cell suspensions were made using a loose fitting ground glass homogenizer. Debris was removed by gravity sedimentation, and the single cell suspension was used for subsequent studies. Cells were immunophenotyped using conjugated monoclonal antibodies and standard techniques as described previously (6). The conjugates used were CD4 FITC, CD8 R-PE, CD25 R-PE, CD44 FITC, TCR α/β R-PE (all from Caltag, Burlingame, CA), IgM FITC (PharMingen, San Diego, CA), and CD45 R-PE (Life Technologies, Inc., Gaithersburg, MD).

Analysis of TCRβ Gene Rearrangements. TCR gene rearrangements were assayed by Southern blot hybridization to TCR Cα, Cβ2, or Jδ1 probes as described (6). For a subset of samples, TCRβ gene rearrangements were amplified for sequence analysis using a modification of a previously published protocol (17). First strand cDNA was synthesized from 1 μg of total RNA using Superscript II reverse transcriptase (Life Technologies, Inc.); the quality of the cDNA was assessed by amplification of β-actin, using sense (5′-GTGGGCCGCTCTAGGCACCAA-3′) and antisense (5′-CTCTTTGATGTCACGCACGATTTC-3′) primers. TCRβ mRNA was amplified using a degenerate Vβ region primer (5′-TAAGCGGCCGCATGSLYTGGTAYWXXCAG-3′; S = G or T, L = A, G, or T, Y = C or T, W = A or C, X = A or G) and a Cβ primer (5′-CCCACCAGCTCAGCTCCACGTGG-3′), the sequence of which is identical for both Cβ1 and Cβ2. The cycling parameters were 40 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, followed by a terminal extension of 10 min at 72°C. The PCR products were gel purified and subcloned into pGEM T-Easy (Promega, Madison WI). Individual plasmid clones were isolated for sequence analysis.

Results and Discussion

Prolonged Survival of *LMO1/SCL/scid* Mice Compared with *LMO1/SCL/wt* Mice. Mice transgenic for *SCL* or *LMO1* were crossed to C.B-17 *scid*/*scid* mice. F1 mice carrying the *SCL* or *LMO1* transgenes were crossed as described in "Materials and Methods." The F2 offspring were genotyped; mice with one or two normal copies of the *scid* gene product were designated *wt* with respect to the *scid* locus, and mice with two mutant copies of the *scid* gene product were designated *scid*. The mice were placed into one of eight cohorts, *wt*, *scid*, *SCL/wt*, *SCL/scid*, *LMO1/wt*, *LMO1/scid*, *SCL/LMO1/wt*, and *SCL/LMO1/scid*, and 20 animals with each genotype were observed for a period of 1 year. The overall survival for each cohort of mice is shown in Fig. 1. Most of the dead mice showed evidence of leukemia; however, one *LMO1*/*wt*, one *LMO1*/*scid*, and one *SCL*/*LMO1*/*scid* had no clear evidence of malignancy at necropsy. There was a clear survival advantage for the *SCL*/*LMO1*/*scid* mice compared with the *SCL*/*LMO1*/*wt* mice at 6 months of age (65% *versus* 0%; $P < 0.001$); however, with time, the difference between these two groups became less marked as more of the *SCL*/*LMO1*/*scid* mice developed leukemia. Although none of the *SCL*/*wt* or *LMO1*/*wt* mice developed leukemia by 6 months of age in our previous studies (6, 7), one *LMO1*/*wt* and one *LMO1*/*scid* mouse each developed leukemia before 6 months of age in this cohort. At 1 year of age, 10–20% of the *SCL*/*wt* or *SCL*/*scid* mice had developed leukemia, and 55–60% of the *LMO1*/*wt* or *LMO1*/*scid* mice had developed leukemia. However, at no point was the difference in survival between *SCL*/*wt* and *SCL*/*scid* or *LMO1*/*wt* and *LMO1*/*scid* significant ($P > 0.05$).

The clinical presentation of leukemia in the *SCL*/*LMO1*/*scid* mice was similar to that reported previously for *SCL*/*LMO1*/*wt* mice, with peripheral lymphadenopathy, massive thymic enlargement, bone marrow infiltration, and widespread organ involvement. The immunophenotype of the tumor samples was heterogeneous. Almost all of the samples were CD8+, but CD4 expression was variable, with samples being negative, strongly positive, or heterogeneous. Several representative FACS analyses are shown in Fig. 1.

***SCL/LMO1/scid* Mice Show Clonal TCRβ Gene Rearrangements.** Interestingly, all *SCL*/*LMO1*/*scid* tumor samples ($n = 11$) that were analyzed by Southern blot showed clonal or oligoclonal TCRβ gene rearrangements and, in at least 8 of the 11 cases, biallelic TCRβ gene rearrangements (Fig. 2). We used a reverse transcriptase-PCR approach to sequence the TCRβ coding junctions from six samples (Table 1). The TCRβ PCR products were subcloned into plasmid vectors for sequence analysis. In five of the six cases, we recovered

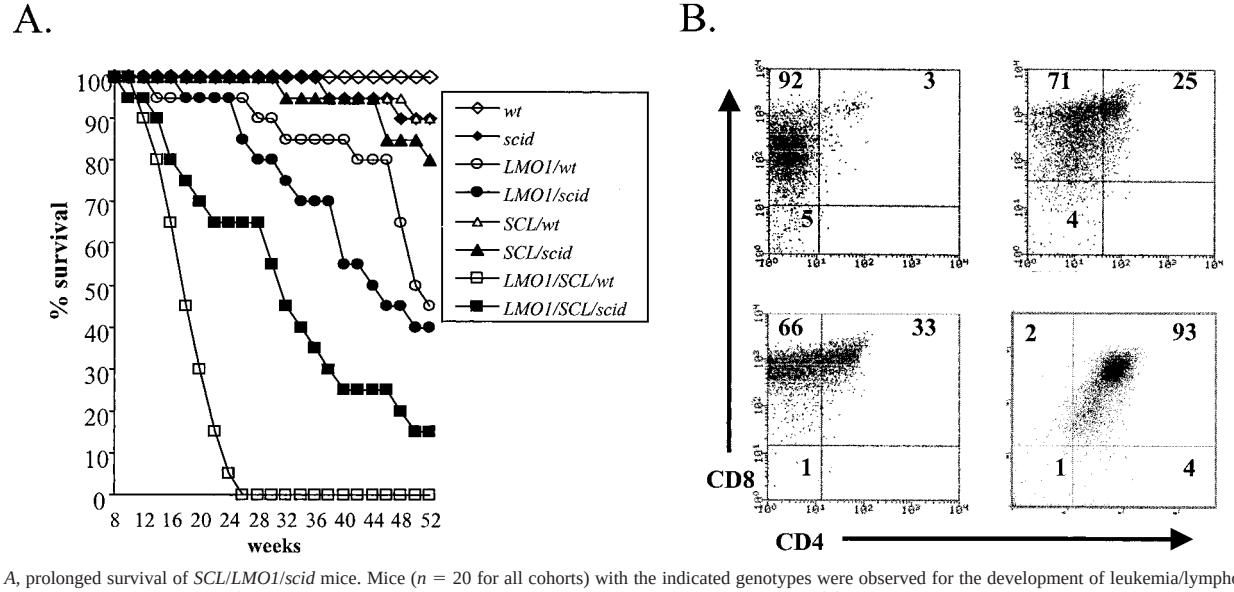

Fig. 1. *A*, prolonged survival of *SCL*/*LMO1*/*scid* mice. Mice ($n = 20$ for all cohorts) with the indicated genotypes were observed for the development of leukemia/lymphoma over a 1-year period. *B*, immunophenotype of leukemia/lymphoma from *SCL*/*LMO1*/*scid* mice. The immunophenotype of four representative *SCL*/*LMO1*/*scid* tumor samples is shown. Percentage of positive cells for each quadrant is indicated.

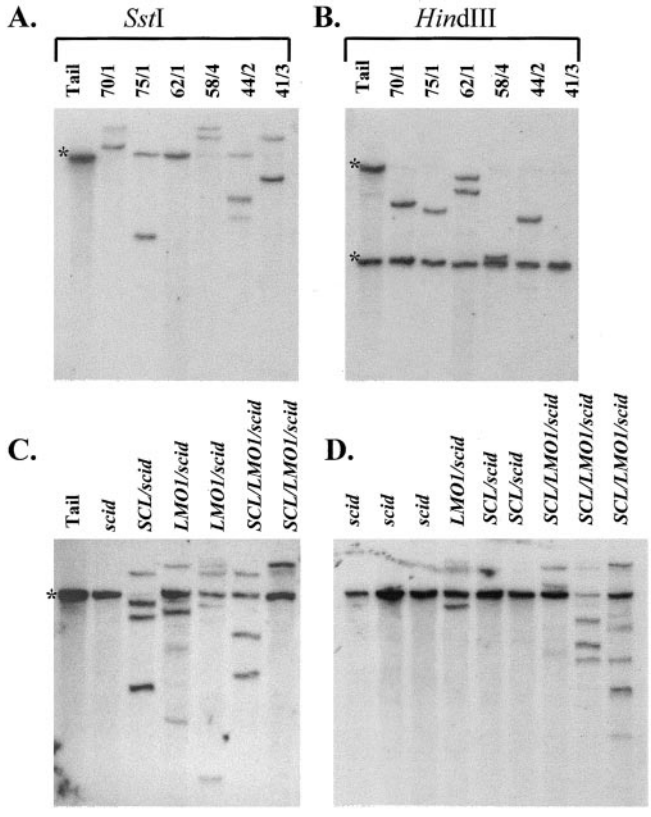

Fig. 2. Clonal TCRβ gene rearrangements. *A*, genomic DNA from six independent *SCL/LMO1* tumors was digested with *Sst*I, size fractionated, blotted, and hybridized to a Cβ2 probe. *, the germline Cβ2 fragment; at least one non-germline fragment is seen in each tumor sample. *B*, the same samples as in *A* digested with *Hin*dIII. *, two germline fragments (Cβ1 and Cβ2). The top (Cβ1) fragment is deleted in all samples, indicating bi-allelic rearrangements in all of the tumor samples. *C*, *Sst*I digested thymocyte DNA harvested from clinically well mice at 12 weeks of age, hybridized to the Cβ2 probe. Genotypes are as indicated; clonal, non-germline fragments are seen in all samples except for the *scid* only sample. *D*, *Sst*I digested thymocyte DNA from clinically well mice at 4 weeks of age, hybridized to Cβ2. Clonal rearranged bands are seen in the *LMO1*/*scid* and *SCL*/*LMO1*/*scid* samples.

the same unique rearrangement from multiple plasmid subclones; however, in sample 58/4, we recovered nine different rearrangements, although the Southern blot for this sample showed only two clear rearranged fragments. The V(D)J rearrangements that were observed in only a single plasmid subclone from tumor 58/4 may have been derived by ongoing rearrangements of a parental clone and/or contaminating nonmalignant T cells. In sum, 14 unique rearrangements were sequenced; all 14 were in frame. As reported previously for antigen receptor coding junctions from *scid* mice (18, 19), these junctions typically had P rather than N region nucleotide additions, some of which were exceptionally long (nine nucleotides). However, in contrast to other reports (19), we did not see abnormally large nucleotide deletions at the coding junctions; this may be attributable to a biological selection for cells that had undergone an in-frame, functional TCRβ rearrangement (see below). TCR rearrangement by inversion has been thought to be uncommon in *scid* thymocytes; however, we recovered a sample (62/1) that had rearranged TCRβ V14S1 and J1S5 by inversion. Two samples (from mice 58/4 and 70/1) had abnormal coding junctions that included J region heptameter signal sequence; one of these (58/4) joined a known J region heptameter sequence to V coding sequences similar to hybrid junctions described previously for *scid* mice (20).

Hybridization of *SCL*/*LMO1*/*scid* tumor samples to a TCRJδ1 probe revealed only germline TCRδ bands of the expected intensity (data not shown), indicating that there were no clonal TCRδ rearrangements and suggesting that there were no clonal TCRα rearrangements, because a TCRα rearrangement would delete TCRJδ1 sequences. Moreover, we were unable to detect expression of TCRα mRNA in these tumor samples. However, all six *SCL*/*LMO1*/*scid* tumor samples assayed expressed pre-T α mRNA (data not shown).

Clonal Expansion of Thymocytes in Young *SCL/LMO1/scid* Mice. Thymocytes were harvested from *SCL*/*LMO1*/*scid*, *SCL*/*scid*, *LMO1*/*scid*, and *scid* mice at 4 and 12 weeks of age. In contrast to thymi from *scid* mice, which are typically atretic and consist of >85% DN cells, thymi from *SCL*/*LMO1*/*scid* mice invariably showed an increase in cellularity, a relative increase in CD4+CD8+ cells, a relative decrease in DN cells (Fig. 3), and oligoclonal TCRβ gene rearrangements at both 12 and 4 weeks of age (Fig. 2, *C* and *D*). Thymocytes from *LMO1*/*scid* mice often, but not invariably, showed a similar increase in CD8+ SP and CD4+8+ CD4+CD8+ cells, a decrease in DN cells, and oligoclonal TCRβ gene rearrangements at 4 or 12 weeks. Although thymocytes harvested from *SCL*/*scid* mice at 4 weeks of age were indistinguishable from *scid* thymocytes, thymocytes from two of five *SCL*/*scid* mice also showed an increase in CD4−8+ and CD4+8+ cells and clonal TCRβ gene rearrangements at 12 weeks of age. These findings stand in contrast with our prior studies (6) on thymocytes from clinically healthy, non-*scid SCL* and *LMO1* transgenic mice. In those studies, *SCL* transgenic mice did not display T-cell abnormalities in terms of immunophenotype or clonality at ages 4–12 weeks; *LMO1* transgenic mice also failed to show abnormalities in immunophenotype or clonality at 4 weeks of age, and only rarely (one of seven) showed evidence of clonal predominance at 12 weeks of age (6). A potential explanation for this difference is that these abnormalities (clonal predominance and increased number of DN thymocytes) might be masked by a population of normal, polyclonal thymocytes in the non-*scid* mouse that are not present in the thymocyte-deficient *scid* mouse.

We were able to amplify and sequence TCRβ gene rearrangements from *SCL*/*LMO1*/*scid* mice aged 4 weeks by reverse transcriptase-PCR; however, we were unable to amplify TCRβ gene rearrangements from *SCL*/*scid*, *LMO1*/*scid*, and *scid* littermates. We sequenced a total of 22 clones with four unique rearrangements from three thymus samples (Table 1). Similar to the TCRβ gene rearrangements described above for the *SCL*/*LMO1*/*scid* tumors, all four unique rearrangements were in frame and showed little evidence for N region nucleotide addition.

In this study, we have demonstrated that the onset of leukemia is significantly delayed in *SCL*/*LMO1*/*scid* mice compared with *SCL*/*LMO1*/*wt* mice. In fact, 3 of 20 *SCL*/*LMO1*/*scid* mice survived >1 year, whereas only 1 of >100 *SCL*/*LMO1* mice studied in our prior studies had survived for as long as 1 year without evidence of leukemia/lymphoma (6, 7).[4] Four groups have crossed p53-deficient mice, which are prone to developing B- and T-cell malignancies, with either *scid* (21–23) or RAG1- or RAG2-deficient mice (24, 25). In light of our results, it is somewhat surprising that these studies have generally shown that the inability to rearrange TCR genes does not delay the onset of disease and, in some cases, actually seems to accelerate the onset of T-cell leukemia/lymphoma (23–25). These differences suggest that the mechanism(s) underlying malignant transformation may be different between $p53^{-/-}$/*scid* and *SCL/LMO1/scid* mice. In the case of p53-deficient *scid* mice, it has been proposed that "broken" DNA ends, which are usually eliminated via a p53-dependent process, persist and serve as a potential source for oncogenic chromosomal translocations. However, in the case of *SCL/LMO1/scid* mice, the biological equivalent of a chromosomal translocation (*i.e.*, a mutation that leads to inappropriate activation of *SCL* and *LMO1*) is present in the germ line of the transgenic mice. The insertion of *SCL* and *LMO1* mutations in the mouse germ line serves to bypass the

Table 1 *TCRβ gene rearrangements from SCL/LMO1/scid mice*

Clones[a]	V	Seq	N/P	D	N/P	Seq	J	Open reading frame?
41/3								
6	V8S3	CC	TCTC	GGACTGGGGGG		TATGAACA	J2S7	Yes
44/2								
4	V8S2	CCAGCGGTGATG				CAGAAACG	J2S3	Yes
58/4								
1	V8S3	CCAGCAG				CTCCTATGAACA	J2S7	Yes
12	V8S3	CCAGCAGTGATG				ACACC	J2S5	Yes
1	V8S3	CCAGCAGTGATG	**CATCA**[b]	GG		CACC	J2S5	Yes
1	V8S3	CCAGCAGT	A	GGGACTGG		CAAGACACC	J2S5	Yes
1	V8S3	CCAGCAGTGATG	**CATCACTTGCT**	GGGACT		AGTCAAAACACC	J2S4	Yes
1	V8S3	CCAGCAGTGATG	**CATC**		GG	CCGGGC	J2S2	Yes
3	V5S1	GCCAGCTCTCTC	**GA**		GGCTGTGAGTG-CAGAAACG		J2S3	Yes
1	V5S2	GCCAGCTCTCTC	**GAGA**		**CT**	AGTGCAGAAACG	J2S3	Yes
1	V2S1	C				C	J2S5	Yes
62/1								
7	V14S1[c]	TGCCTGGAGTCT	**AG**			ACCAGGC	J1S5	Yes
70/1								
5	V8S3	CCAGCAGTGATG	CCCTGTATCAGGAAAGA-GGAGGTTTGTGCCAGC-ATTTCCAGGACTGTG				J2S2+	Yes
75/1								
5	V11S1	AAGCAGCTTAGA	**TC**	GGGACAGGGG		CTCCTATGAACA	J2S7	Yes
100/2								
6	V8S2	CCAGCGGTGATG				CAGAAACG	J2S3	Yes
1	V8S2	CCAGCGGTGATG				ACACC	J2S5	Yes
101/1								
8	V6S1	CCAGCAGTAT		CTGGGGGG	T	CCAAGACACC	J2S5	Yes
102/2								
7	V12S1	CAGCAG	**CC**	GGGACAGG	**AA**	TTCTGGAAATAC	J1S3	Yes

[a] Samples 41/3, 44/2, 58/4, 62/1, 70/1, and 75/1 were obtained from tumor tissue at necropsy; samples 100/2, 101/1, and 102/2 were obtained from the thymus of clinically healthy 4-week-old mice.
[b] Potential "P" region nucleotides are indicated in bold type; J region heptamer sequences incorporated into coding joints are underlined.
[c] Recombination by inversion (V14S1); + indicates coding joint includes 48 nucleotides immediately 5′ of J2S2.

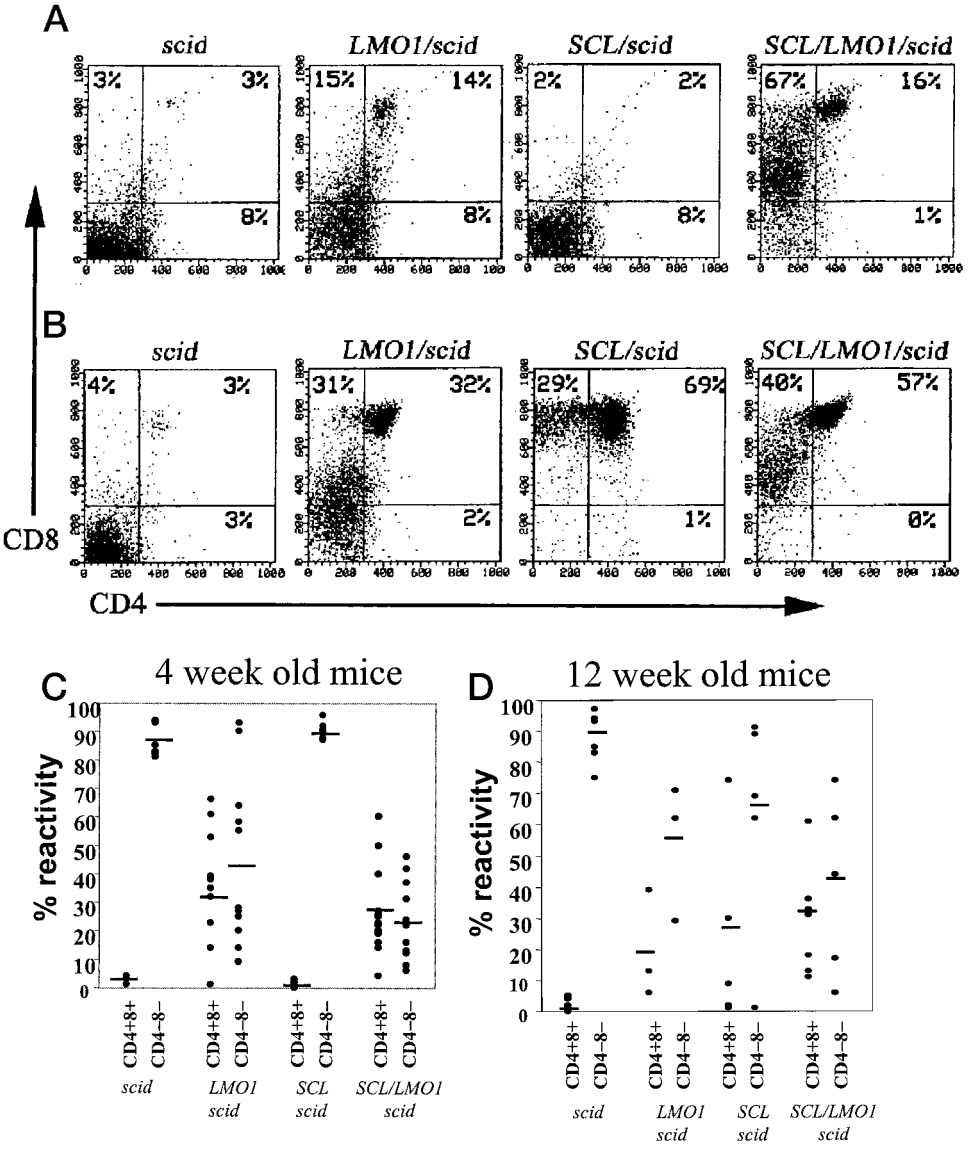

Fig. 3. Immunophenotype of thymocytes from clinically healthy mice. Representative CD4 and CD8 fluorescence-activated cell sorter profiles from mice of the indicated genotypes at 4 (*A*) and 12 (*B*) weeks of age. Percentage of reactivity is indicated. Scatter plot of thymocytes from 4- (*C*) and 12- (*D*) week-old mice analyzed for CD4 and CD8. Genotypes are as indicated; ●, values obtained from one mouse. Means are indicated with horizontal lines.

usual mechanism (chromosomal translocation joining *SCL* or *LMO1* with TCR genes) used to activate *SCL* and *LMO1*. Whereas it is likely that *SCL* and *LMO1* exert their leukemogenic effect, at least in part, through a functional inactivation of the E-proteins *E2A* and *HEB* (6, 7, 11), the downstream targets for chromosomal translocations in p53-deficient *scid* mice have not been identified.

The observation that all of the *SCL*/*LMO1*/*scid* tumors analyzed showed in-frame clonal (or oligoclonal) TCRβ gene rearrangements was unexpected, because the vast majority of *scid* thymocytes do not rearrange TCR genes. This finding suggests that only the rare *scid* thymocyte which undergoes an in-frame TCRβ gene rearrangement is able to develop to the point at which it becomes susceptible to the oncogenic effect of the *SCL* and *LMO1* transgenes. The observation that most (at least 8 of 11) of the tumors had both TCRβ alleles rearranged (Fig. 2 and data not shown) suggests that the malignant *scid* thymocytes are not necessarily impaired in the ability to rearrange TCRβ but rather in the ability to generate in-frame TCRβ rearrangements containing functional CDR3 coding sequences.

It would seem likely that TCRβ proteins from the *SCL*/*LMO1*/*scid* tumor cells pair with pre-Tα instead of TCRα, because we did not detect evidence of either TCRα mRNA expression or clonal TCRα rearrangements. The observation that pre-Tα mRNA was expressed, in light of a recent report (11) showing the combination of *SCL* and *LMO1* inhibits pre-Tα mRNA production, presents an apparent paradox. However, it is possible that the lack of effective TCRα recombination leads to a selection for relatively rare cells with TCRβ and pre-Tα expression as an alternative to TCR α/β cells.

The combination of *SCL* and *LMO1* transgenes provides a mouse model of T-ALL that is likely to be relevant to the human condition, because *SCL* and *LMO1* (or the closely related *LMO2*) are frequently activated in the leukemic cells of patients with T-ALL. This study demonstrates that the rare *scid* thymocyte with an in-frame TCRβ gene rearrangement is a preferred target for the oncogenic effect of *SCL* and *LMO1* and that the *scid* defect offers a relative protection against the oncogenic action of these two genes. These results provide a potential explanation for the clinical observation that *SCL* gene rearrangements are restricted to T-ALL samples of the α/β lineage (26, 27), as opposed to the γ/δ lineage, and support the hypothesis that in-frame TCRβ gene rearrangements are required for the oncogenic effect of *SCL* and *LMO1*.

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