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

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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 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, (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, thymus 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).

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 LMO1 or SCL 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′-GGAAGAATTTCGCGCAGAATGTG-3′ and 5′-CATCACAGTTTAAACACGCTGGG-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 HhaI, 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...
Survival between groups was analyzed using the χ² 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 HindIII-XbaI 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-TCR (nucleotides 218–622 of GenBank accession no. NM 011195), and TCR J61, JC2, 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 PE, 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 J61 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′-CTCTTGATGTCAACAGTATCC-3′) and antisense (5′-TCCTCTGCATGACCCAGATTC-3′) primers. TCRβ mRNA was amplified using a degenerate Vβ region primer (5′-TAAAGCGGCGCATGSLTGTAYXWXCX3′; S = G or T, L = A, G, or T, Y = C or T, W = A or C, X = A or G) and a CB primer (5′-CCCACCACTCATGCTCAAGTGC-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 LMO1/wt 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...
Clonal Expansion of Thymocytes in Young SCL/LMO1/scid Mice. Thymocytes were harvested from SCL/LMO1/scid, LMO1/scid, and scid mice at 4 and 12 weeks of age. In contrast to thymus from scid mice, which are typically atletic and consist of $>85\%$ DN cells, thymus from SCL/LMO1/scid mice invariably showed an increase in cellularity, a relative increase in CD4+CD8+ cells, and 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+ cells and 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+ 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). 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

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 malignant 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). TCRβ 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 heptamer signal sequence; one of these (58/4) joined a known J region heptamer sequence to V coding sequences similar to hybrid junctions described previously for scid mice (20).

Hybridization of SCL/LMO1/scid tumor samples to a TCRJ31 probe revealed only germline TCRβ bands of the expected intensity (data not shown), indicating that there were no clonal TCRβ rearrangements and suggesting that there were no clonal TCRα rearrangements, because a TCRα rearrangement would delete TCRJ81 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).
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* 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.

* Potential "P" region nucleotides are indicated in bold type; J region heptamer sequences incorporated into coding joints are underlined.

* Recombination by inversion (V14S1); + indicates coding joint includes 48 nucleotides immediately 5' of J2S2.
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) TCR\(_{\beta}\) 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 TCR\(_{\beta}\) 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 TCR\(_{\beta}\) alleles rearranged (Fig. 2 and data not shown) suggests that the malignant scid thymocytes are not necessarily impaired in the ability to rearrange TCR\(_{\beta}\) but rather in the ability to generate in-frame TCR\(_{\beta}\) rearrangements containing functional CDR3 coding sequences.

It would seem likely that TCR\(_{\beta}\) proteins from the SCL/LMO1/scid tumor cells pair with pre-\(\alpha\) instead of TCR\(_{\alpha}\), because we did not detect evidence of either TCR\(_{\alpha}\) mRNA expression or clonal TCR\(_{\alpha}\) rearrangements. The observation that pre-\(\alpha\) mRNA was expressed, in light of a recent report (11) showing the combination of SCL and LMO1 inhibits pre-\(\alpha\) mRNA production, presents an apparent paradox. However, it is possible that the lack of effective TCR\(_{\alpha}\) recombination leads to a selection for relatively rare cells with TCR\(_{\beta}\) and pre-\(\alpha\) expression as an alternative to TCR\(_{\alpha}/\beta\) 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 TCR\(_{\beta}\) 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 \(\alpha/\beta\) lineage (26, 27), as opposed to the \(\gamma/\delta\) lineage, and support the hypothesis that in-frame TCR\(_{\beta}\) gene rearrangements are required for the oncogenic effect of SCL and LMO1.

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