We consider that in vitro studies are the most informative for identifying direct effects of Dr1 on individual polymerases. This is because the sustained overexpression of Dr1 in vivo will inevitably perturb all three polymerases, because each depends on the products of the others to maintain production of transcription factors and polymerase subunits. We speculate that the cellular role of Dr1 is in fine-tuning, allowing transitory adjustments in polymerase output in response to environmental changes.

We have established the potential of Dr1 to directly coregulate Pols II and III. In contrast, Pol I transcription appears immune to this factor. Dr1 may therefore serve to shift the overall balance of nuclear metabolism in favor of Pol I. This could be of considerable value when rRNA levels are limiting. Factors such as Dr1 that form a regulatory network to interlink the three transcriptional systems are likely to be of great importance to the overall cellular economy.

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Rescue of T Cell–Specific V(D)J Recombination in SCID Mice by DNA-Damaging Agents

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Assembly of antigen receptor V (variable), D (diversity), and J (joining) gene segments requires lymphocyte-specific genes and ubiquitous DNA repair activities. Severe combined immunodeficient (SCID) mice are defective in general double-strand (ds) DNA break repair and V(D)J coding joint formation, resulting in arrested lymphocyte development. A single treatment of newborn SCID mice with DNA-damaging agents restored functional, diverse, T cell receptor β chain coding joints, as well as development and expansion of thymocytes expressing both CD4 and CD8 coreceptors, but did not promote B cell development. Thymic lymphoma developed in all mice treated with DNA-damaging agents, suggesting an interrelation between V(D)J recombination, dsDNA break repair, and lymphomagenesis.

Lymphocyte development requires the participation of a site-specific recombinase system to somatically juxtapose widely dispersed V, D, and J gene segments that comprise the variable recognition domains of T and B cell antigen receptors. In V(D)J recombination-deficient mice resulting from natural mutation or created by gene-targeting techniques, precursor T (pre-T) and pre-B cell antigen receptors cannot be expressed by lymphocyte progenitors, which accounts for the absence of mature lymphocytes in these animals (*1*). The most well-defined components of the V(D)J recombinase machinery are developmentally regulated, lymphocyte-specific genes, such as the recombinase-activating genes *RAG-1* and *RAG-2* (*2*) and terminal deoxynucleotidyl transferase, which adds short stretches of nontemplated nucleotides (*3*). The murine *scid* gene defines a dsDNA break repair activity (*4*) that is also required for efficient joining of free V(D)J coding ends (*5–7*). Additional DNA repair activities may also participate in V(D)J recombination (*8*). In contrast to the lymphocyte-specific components of the recombinase machinery, genes encoding many of the general DNA repair activities have not been isolated and little is known about their regulation and precise functions. We now show that treatment of newborn SCID mice with DNA-damaging agents restored normal T cell receptor (TCR) β recombination and T cell maturation. However, all of these mice eventually developed thymic

lymphoma. These observations provide an in vivo model to elucidate the critical relation between the regulation of DNA repair, V(D)J recombination, and the molecular basis of lymphoid neoplasia.

T cell receptor β, which is rearranged and expressed before TCRα (9), regulates the proliferation and maturation of $CD4^+CD8^+$ double-positive (DP) thymocytes from interleukin-2 receptor α chain–positive ($IL\text{-}2R\alpha^+$) $CD4^-CD8^-$ (double-negative; DN) blast cell precursors (*10*, *11*). In SCID mice, defective V(D)J recombination arrests thymocyte development at the DN stage (*12*). Surprisingly, we detected a high frequency of DP thymocytes in newborn SCID mice treated with a low dose of radiation [100 centigrays (cGy)] (*13*). Therefore, we investigated the kinetics and mechanism of radiation-induced DP thymocyte development in SCID mice. Animals were given a single dose of radiation (100 cGy) within 72 hours of birth. One week later, irradiated newborn SCID (IRNB-SCID) mice had an average of 66% (range: 42 to 89%) DP thymocytes (Fig. 1A and Table 1). Immature transitional $CD4^{lo}CD8^+$ cells (*14*) were also seen (mean: 11%; range: 5 to 20%; Fig. 1A and Table 1). In addition, thymus cellularity in 1- to 2-week-old IRNB-SCID mice was 4 to 20 times greater than in age-matched SCID controls (Table 1). In wild-type mice, the DN to DP transition is accompanied by loss of IL-2Rα and a reduction in cell size that reflects exit of DP thymocytes from the cell cycle (*15*). These phenotypic transitions were also observed in IRNB-SCID mice within 1 to 2 weeks, concomitantly with the appearance of DP cells, which included large, cycling and small, postmitotic subsets (Fig. 1A). However, unlike wild-type DP thymocytes, radiation-induced DP cells in SCID mice did not express detectable surface TCRβ (Fig. 1A) or CD3ε (*16*). Several weeks after the radiation-induced appearance of DP cells in SCID mice, mature CD4 or CD8 single positive T cells were consistently found in the thymus and lymph nodes (LN) (Fig. 1A), which suggests that some rescued DP thymocytes can undergo further maturation. In contrast to DP thymocytes, mature thymocytes and peripheral T cells in IRNB-SCID mice always expressed a surface TCR, but at slightly lower density than that of peripheral T cells from wild-type mice (Fig. 1A).

To determine whether other DNA-damaging agents could rescue development of DP thymocytes, we injected newborn SCID mice with bleomycin, a drug that induces dsDNA breaks (*17*). Both 1 and 2 weeks later, we observed a predominant DP population, but the incidence of DP rescue was lower than that obtained with irradiation, and there was a less pronounced effect on thymus cellularity (Table 1), perhaps because of the toxicity of this drug. Thus, treatment of newborn SCID mice with low doses of irradiation or with bleomycin increased thymus cellularity to a similar extent as expression of a rearranged TCRβ transgene (*18*). Collectively, these results suggest that agents that induce dsDNA breaks can rescue the development and expansion of DP thymocytes in SCID mice.

Analagous to the transition from DN to DP in T cell development, maturation of large, $B220^+CD43^+$ progenitor B (pro-B) cell blasts into small, $B220^+CD43^-$ pre-B cells requires expression of the gene encoding immunoglobulin heavy chain (IgM) and a pre-B cell–specific surrogate light chain composed of $V_{pre\text{-}B}$ and λ5 (*19*). However, in contrast to the ability of low-dose irradiation to rescue development of DP thymocytes in SCID mice, we found no evidence for simultaneous rescue of B cell development. Surface IgM^+ ($sIgM^+$) B cells were undetectable in the bone marrow (BM), spleens, LN, and peritoneal cavities of IRNB-SCID mice at 1 to 16 weeks after irradiation (Fig. 1B, top) (*20*). In addition, the frequency of early B cell progenitors ($B220^+sIgM^-$) in BM and spleen was similar in irradiated and control SCID animals. Finally, irradiation did not restore IgM rearrangement (*20*) or the transition of large $CD43^+$ pro-B cells to small $CD43^-$ pre-B cells in SCID mice (Fig. 1B, bottom), confirming that low-dose irradiation fails to rescue B cell development in SCID mice. The failure to induce B cell development is not a result of differential γ-radiation exposure of T and B lymphoid progenitors. A newborn mouse is about 1 cm thick, but the attenuation length of high-energy (662 keV) photons from a ^{137}Cs source is at least 10 cm when murine tissues are traversed. Because the distance traversed is small compared to the attenuation length, energy deposition is uniform throughout the animal (*21*).

To investigate the mechanism of rescued DP thymocyte development, we examined control and IRNB-SCID thymocytes for TCRβ rearrangement and expression, because both events are thought to participate in the transition from DN to DP (*10*, *11*). Southern (DNA) analysis of thymus DNA showed that diverse TCRβ rearrangements were induced concomitantly with the appearance of DP thymocytes in 1- and

Table 1. DNA-damaging agents induce T cell development in newborn SCID mice. C.B-17 SCID mice were bred and maintained under defined flora conditions. Newborn SCID mice (1 to 3 days old) received 100 cGy of irradiation from a ^{137}Cs source or an intraperitoneal injection of bleomycin (0.5×10^{-3} to 1×10^{-3} units per gram of body weight). Thymocytes were obtained at the indicated time points after treatment and stained as described (Fig. 1). For comparison, 3- to 8-week-old C.B-17 mice had 159 ± 60 thymocytes (n = 16) and 32 ± 14 LN cells (n = 9). Thymocytes from young adult C.B-17 mice contained an average of 84% $CD4^+CD8^+$, 10% $CD4^+CD8^-$, and 3% $CD4^-CD8^+$ cells. Numbers shown are means ± standard deviation of results obtained from 3 to 15 mice. When only two mice were tested, both values are shown.

Treatment	Mice (n)	Total cells (10^6)	Phenotype of thymocytes (percent of total cells)			
			$CD4^+CD8^-$	$CD4^+CD8^+$	$CD4^-CD8^+$	TCR^{hi}*
			1 week after treatment			
None	5	1 ± 0.4	0	3 ± 2	0	0
Irradiation	24	7 ± 4	0.3 ± 0.2	66 ± 19	11 ± 7†	0
Bleomycin	3/6*	6 ± 6	0.3 ± 0.2	48 ± 17	4 ± 1	0
			2 weeks after treatment			
None	2	0.5; 1	0.7; 1	0.2; 0.3	0.1; 0.3	0
Irradiation	11	21 ± 7	2 ± 1	72 ± 10	3 ± 2	0
Bleomycin	2/3‡	1; 1	0.8; 0.8	18; 10	0.8; 0.2	0
			3 to 4 weeks after treatment			
None	4	3 ± 1	0.2 ± 0.2	0.1 ± 0.2	0.2 ± 0.2	0.5 ± 0.1
Irradiation	14	18 ± 9	2 ± 1	63 ± 20	7 ± 7	2 ± 2

*Determined by staining with H57-597 (antibody to TCRβ) or 145-2C11 (antibody to CD3ε). †These are immature TCR^-CD8^+ transitional thymocytes that are $CD4^-$ or $CD4^{lo}$ (*14*). ‡Treatment with bleomycin induced development of DP thymocytes only in the indicated number of mice out of the total. In our SCID colony, <5% of the thymocytes were DP among control SCID mice less than 2 months old. Therefore, we scored only mice with ≥10% DP thymocytes as having responded to the treatment. All irradiated mice showed inductionof DP maturation by this criterion.

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2-week-old IRNB-SCID mice (Fig. 2A), whereas rearrangement of this locus was not detected in control SCID mice (*5*, *20*). In thymus DNA from wild-type C.B-17 mice, highly diverse rearrangements involving multiple V, D, and J loci gave rise to a characteristic smearing of the C_β germline bands (Fig. 2A). Thymus DNA from 1- to 4-week-old IRNB-SCID mice also showed this smearing, which indicates the polyclonal nature of the rearrangements. However, by 6 to 8 weeks after irradiation, predominant, oligoclonal TCRβ rearrangements were visible in all samples (Fig. 2A). By 11 to 15 weeks after irradiation, 60% (n = 16) of the IRNB-SCID mice tested developed DP thymic lymphoma, and by 17 to 20 weeks, tumor incidence was 100% (n = 26). Thus, some DP cells acquired mutations that enhanced their survival or proliferation relative to that of normal, short-lived DP thymocytes. Irradiation of adult SCID mice with a single dose of 150 or 175 cGy has been reported to induce DP thymic lympho-

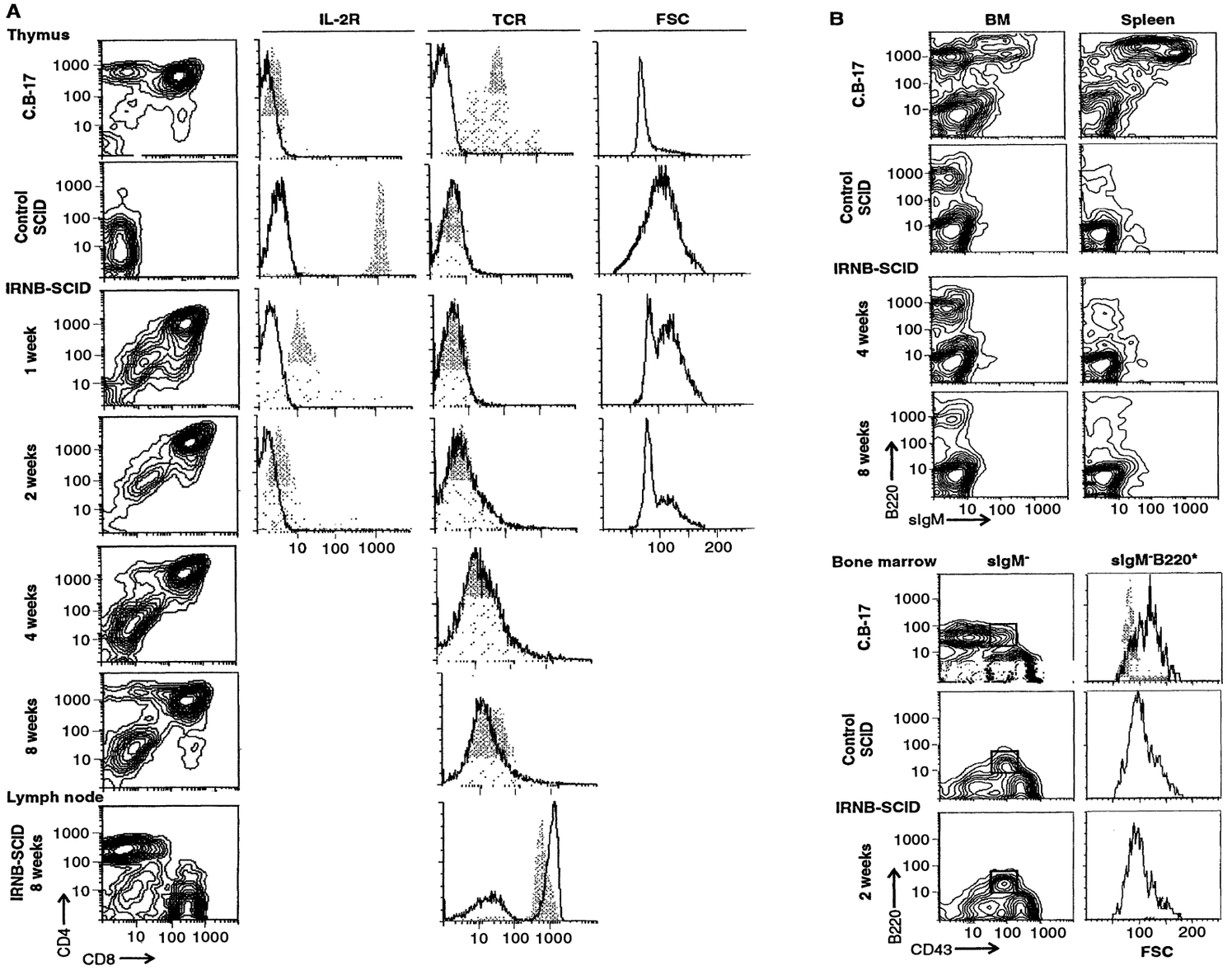

Fig. 1. Analysis of T and B lymphocyte development in IRNB-SCID mice. (**A**) T cell development as a function of time after irradiation. Thymocytes or LN cells were obtained at different times after irradiation (100 cGy) of newborn (1- to 3-day-old) SCID mice and analyzed for forward scatter (FSC) and expression of CD4, CD8, TCRβ, or IL-2Rα by multiparameter flow cytometry as described (*41*). Two-parameter contour plots (7% probability) show CD4 versus CD8 expression (far left). Single-parameter histograms show the expression of IL-2Rα (shaded histograms, left) or TCRβ (shaded histograms, middle), compared to staining with isotype-matched control antibodies (clear histograms). TCRβ expression by IRNB-SCID LN (shaded histogram) was compared to TCRβ expression by C-B.17 LN (clear histogram). The control SCID mouse was 1 week old. (**B**) Lack of B cell development in IRNB-SCID mice. Top: BM and spleen cells were obtained at the indicated times after irradiation (100 cGy) and analyzed by multiparameter flow cytometry for sIgM versus B220 expression. These results are representative of mice analyzed at 2 weeks (n = 3), 4 weeks (n = 7), and 8 weeks (n = 6) after irradiation. The frequencies of $B220^+sIgM^-$ pre-B cells in BM were as follows: control SCID: 15 ± 3%; IRNB-SCID: 18 ± 5%. Bottom: $sIgM^-$ BM cells from the indicated mice were analyzed for B220 versus CD43 expression to distinguish pro-B ($B220^+CD43^+$, square) from pre-B ($B220^+CD43^-$) cells. (Note that no $sIgM^+$ cells were detected in BM from IRNB-SCID mice.) In addition, the FSC profile of pro-B cells in each sample is shown. Note that pre-B cells in C.B-17 BM (shaded histogram) are predominantly small (FSC mean channel = 80). The frequency and size (FSC mean channel number) of the pro-B subset in each sample was as follows: C.B-17, 2.2% (114); control SCID, 8% (106); and IRNB-SCID, n = 3: 12 to 16% (103 to 108).

mas, but with lower incidence and longer latency (greater than 6 months) than we observed in newborn SCID mice (*22*). In contrast, young wild-type C57BL/6 and BALB/c mice rarely develop thymic lymphoma when irradiated with single doses as high as 700 cGy (*23*), which underscores the importance of the SCID DNA repair defect in susceptibility to radiation-induced thymic lymphomas.

Southern analysis of three IRNB-SCID thymic lymphomas that arose 12 to 20 weeks after irradiation supported the idea that tumors arose from oligoclonal expansions of preneoplastic cells because only one or two TCRβ rearrangements were seen in each tumor (Fig. 2B). In addition, cell lines established from these primary tumors all had the same TCRβ rearrangements as the primary tumor (Fig. 2B). Tumors of B lymphoid or nonlymphoid origin were not observed, despite impaired dsDNA break repair in all tissues of SCID mice. Therefore, the T lineage specificity of neoplastic transformation induced by low-dose irradiation may reflect an interaction between defective dsDNA break repair and TCR rearrangement.

We examined whether TCRβ rearrangements observed 1 to 4 weeks after irradiation were expressed using a reverse transcription–polymerase chain reaction (RT-PCR) assay to detect transcripts derived from rearranged loci. TCRβ mRNA was expressed in all IRNB-SCID thymuses, but only rarely in age-matched controls (1 out of 26 animals) (Fig. 2C). These results, which are representative of 20 to 30 mice analyzed per group, suggest that radiation-induced TCRβ rearrangement invariably leads to expression of appropriately sized $VDJC_\beta$ transcripts. In contrast, VJC_α transcripts were not detected in 1- to 4-week-old control or IRNB-SCID mice (Fig. 2C). Thus, either TCRα rearrangements did not occur or they were nonproductive. Similar to results reported for TCRα-deficient mice (*11*, *24*), surface TCRβ was not detectable on most DP thymocytes found in 1- to 4-week-old IRNB-SCID mice (Fig. 1A), but we cannot exclude the possibility that a TCRβ-containing pre-T receptor complex (*25*) is present in small amounts. Even after 4 to 8 weeks, when surface TCRβ was found on mature thymocytes and LN cells (Fig. 1A), TCRα mRNA was rarely detected (Fig. 2C). Thus, in contrast to our finding that TCRβ rearrangement and expression was rapid and universal, TCRα expression was detected at low frequency and only at longer time points after irradiation. This apparent TCR locus specificity may indicate that V(D)J recombinase activity is only transiently restored and does not persist until the time when the TCRα becomes recombinationally active. This interpretation is based on the facts that rearrangement of TCRα is developmentally delayed relative to that of TCRβ (*9*) and that the maturational arrest conferred by the SCID mutation occurs before the TCRα locus becomes recombinationally accessible, as implied by the absence of TCRα germline transcripts (*26*).

We determined the frequency of radiation-induced rescue of productive, in-frame TCRβ rearrangements by flow cytometric analysis of intracellular TCRβ protein in IRNB-SCID thymocytes. After permeabilization with saponin, cells were analyzed by two-color immunofluorescence for expression of CD4 and TCRβ. In all mice analyzed (n = 12) 1 week after irradiation, 50 to 90% (mean: 71%) of the $CD4^+$ thymocytes coexpressed cytoplasmic TCRβ (Fig. 3). As expected, no $CD4^+$ or $TCR\beta^+$ cells

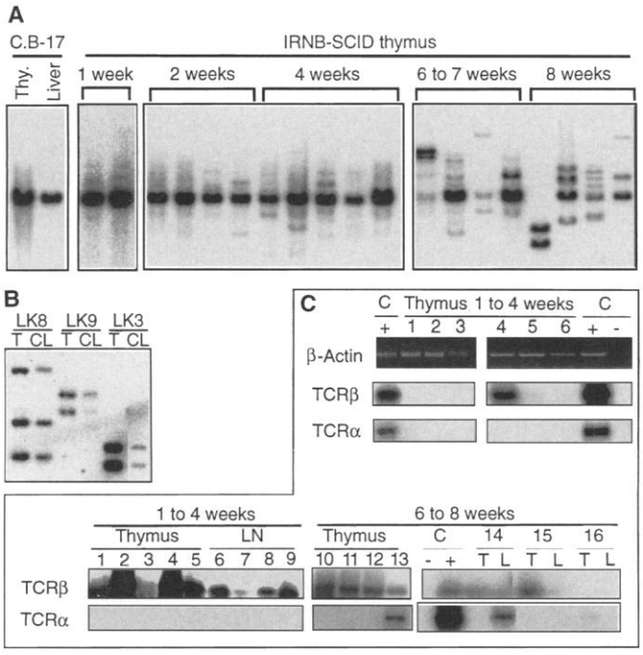

Fig. 2. Rearrangement and transcription of the TCRβ locus occur rapidly after irradiation of newborn SCID mice. (**A**) Southern analysis of the TCRβ locus in IRNB-SCID mice. Genomic DNA was prepared from normal C.B-17 thymus and liver and from the individual thymuses of IRNB-SCID mice of the indicated ages. DNA samples were digested with Pvu II and separated on 0.7% agarose gels by electrophoresis. Southern blot filters were prepared on GeneScreen Plus membrane (NEN Dupont) and hybridized to a [^{32}P]deoxycytidine 5′-triphosphate–labeled TCR C_β cDNA probe. Phosphorimages were obtained after a 2-day exposure with the use of a Molecular Dynamics imaging system. (**B**) Southern analysis of the TCRβ locus in radiation-induced SCID thymic lymphomas. IRNB-SCID mice (designated LK8, LK9, and LK3) that showed signs of respiratory distress at 12 to 20 weeks after irradiation were found to contain thymic lymphomas that filled the whole chest cavity. Cell lines were established from each primary tumor by established techniques. Southern analyses were performed on DNA extracted from a tissue fragment of each primary tumor (T) and each cell line (CL) as described in (A). (**C**) Comparison of TCRβ and TCRα transcripts and surface TCRβ expression in control SCID (top) and IRNB-SCID mice (bottom) 1 to 4 and 6 to 8 weeks old. Expression of TCR mRNA was detected by RT-PCR assay that used degenerate, consensus V_β or V_α sense primers and homologous C_β or C_α antisense primers, respectively, to detect V(D)J transcripts from rearranged loci (*42*). Each cDNA sample contained equivalent amounts of β-actin cDNA. Positive and negative controls for each set of PCR reactions were cDNAs from reactions seeded with 1 to 2 μg of C.B-17 thymus RNA with and without reverse transcriptase, respectively. Lanes C, C.B-17 thymus cDNA synthesized with (+) or without (−) reverse transcriptase. Top: control 1- to 4-week-old SCID thymus. Bottom: thymus (T) and LN (L) from 1- to 4-week-old and 6- to 8-week-old IRNB-SCID mice.

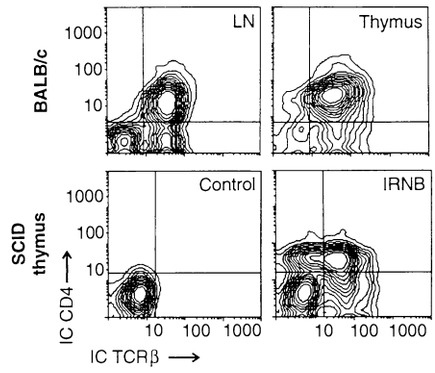

Fig. 3. Analysis of intracellular TCRβ chain expression in IRNB-SCID mice. Thymocytes were obtained from IRNB-SCID mice 1 week after irradiation (100 cGy) and analyzed for intracellular expression of CD4 and TCRβ by multiparameter flow cytometry. Intracellular staining was done as described (*43*). Fluorescence was analyzed as described for Fig. 1. Intracellular (IC) CD4 versus TCRβ expression is displayed on two-parameter contour plots (7% probability). Staining of wild-type BALB/c LN and thymocytes and age-matched control SCID thymocytes are shown for comparison. These results are representative of several experiments analyzing a total of 12 IRNB-SCID mice 1 week after irradiation. Twenty to 65% (mean: 41%) of total IRNB-SCID thymocytes expressed IC TCRβ.

were detected in control SCID animals, whereas most BALB/c thymocytes coexpressed CD4 and cytoplasmic TCRβ. Other studies indicate that DP thymocytes develop in SCID mice in response to several different experimental manipulations, including adoptive transfer of wild-type BM cells or T cells or treatment with antibody to asialo GM-1 (*27*), but whether these treatments act by restoring V(D)J recombinase function and expression of TCRβ protein has not been determined. In contrast, we show that a single exposure of newborn SCID mice to low-dose irradiation selectively induces maturation of large numbers of DP thymocytes that express TCRβ protein.

We tested two possible explanations for the rapid enhancement of thymus cellularity and coincident rescue of intracellular TCRβ protein expression in 50 to 90% of IRNB-SCID DP thymocytes (Table 1 and Fig. 3). DNA damage could promote oligoclonal expansion of rare DP cells that made successful TCRβ coding joints, resulting in limited TCRβ diversity. Alternatively, DNA damage could restore normal VDJ_β recombination in many precursors, resulting in a diverse TCRβ repertoire. To distinguish between these alternatives, we analyzed the structure and diversity of TCRβ transcripts expressed in IRNB-SCID thymocytes by nucleotide sequence analysis of cloned RT-PCR products (Fig. 4). TCRβ transcripts containing V_β8.1, V_β8.2, and V_β8.3 were selected for analysis, because members of this family are expressed at a high frequency in wild-type T cells (*28*). In addition, V_β14-containing transcripts were chosen to evaluate inversional recombination, because this gene lies in opposite transcriptional orientation to D_β and J_β elements (*29*) and can rearrange only by inversion. Eighty percent (29 of 36) of the TCRβ clones derived from two 1-week-old IRNB-SCID mice had distinct sequences (Fig. 4), which indicates a high degree of diversity. These clones showed combinatorially and somatically diversified coding joints and contained four distinct germline J_β elements derived from both the J_β1 and J_β2 gene clusters (*29*). Ninety percent (26 of 29) of the sequences were in-frame, consistent with a previous study of TCRβ rearrangements in thymocytes from TCRα-deficient mice (*30*), which, like IRNB-SCID thymocytes, are not subject to TCRαβ-based selection events. These data suggest selection for productive TCRβ rearrangement during development of IRNB-SCID DP thymocytes.

The high frequency of productive coding joints we observed in IRNB-SCID thymocytes contrasts with the inability of SCID pre-T and pre-B cell lines to generate normal coding joints in recombination signal sequence–dependent, artificial recombination substrates (*31*, *32*). The short deletions of V_β and J_β terminal nucleotides in IRNB-SCID thymocytes (Fig. 4) were indistinguishable from normal mouse TCRβ joints and contrast with the extensive coding sequence deletions observed in spontaneous SCID thymic lymphomas and Abelson murine leukemia virus–transformed SCID BM cells (*5*, *7*, *33*). In addition, alignment of the IRNB-SCID TCRβ sequences with germline V, D, and J sequences showed that each clone contained unique, N-region base additions, without palindromes (P nucleotides) derived from the terminal nucleotides of germline V or J segments (*34*). Both features distinguish IRNB-SCID TCRβ coding joints from the rare productive joints seen in "leaky" SCID antigen receptors, which usually contain excessive P nucleotide insertions and short or absent N regions (*35*). In summary, the TCRβ coding joints rescued by low-dose irradiation are diverse and lack the structural anomalies that characterize rare SCID antigen receptors, which suggests that normal V(D)J recombination has been restored.

Successful rearrangement of antigen receptor genes is a common and critical aspect of both T and B lymphocyte maturation. Our demonstration that DNA-damaging agents rescue neither IgM rearrangement (*20*) nor B cell development (Fig. 1B) provides evidence that the V(D)J recombinase machinery is not identical, or is not regulated identically, in T and B cell progenitors. Additional support for this idea comes from the finding that transgenic overexpression of the *bcl-2* proto-oncogene in SCID mice enhances the survival of SCID B cell progenitors and restores IgM rearrangement and B cell development to a limited extent, but does not affect T cell development (*36*). However, the frequency, structure, and diversity of the IgM rearrangements were not directly evaluated in that study, so the mechanism by which *bcl-2* overexpression promoted limited B cell

Clone	V_β sequence	D_βN region sequence	J_β sequence		In frame
V_β8.1	GTATATTTCTGTGCCAGCAGTGAT				
1	GTATATTTCTGTGC	GCTG	1.3	AATACGCTCTATTTTGGAGAAGGAAGCCGGCTCATTGTTGTAG	+
2	GTATATTTCTGTGCCAGCAGT	GCCGA	2.3	GAAACGCTGTATTTTGGCTCAGGAACCAGACTGACTGTTCTC	−
3	GTATATTTCTGTGCCAGCAGT	GGACAGGCAAACTCCGAC	2.3	TATTTTGGCTCAGGAACCAGACTGACTGTTCTC	+
4	GTATATTTCTGTGCCAGCAGTG	CCGA	2.3	AGTGCAGAAACGCTGTATTTTGGCTCAGGAACCAGACTGACTGTTCTC	−
5	GTATATTTCTGTGCCAGCA	GCGGTCAAGGCGCT	2.3	GAAACGCTGTATTTTGGCTCAGGAACCAGACTGACTGTTCTC	+
6	GTATATTTCTGTGCCAGC	ATCGTA	2.4	AACACCTTGTACTTTGGTGCGGGCACCCGACTATCGGTGCTA	+
7	GTATATTTCTGTGCCAGCAGTG	GACAGGCAAACTCCGACTACACTTCGGCT	2.5	GACACCCAGTACTTTGGGCCAGGCACTCGGCTCCTCGTGTTA	+
V_β8.2	GTGTACTTCTGTGCCAGCGGTGAT				
8	GTGTACTTCTGTGCCAGCGGTGA	CAGT	1.3	TCTGGAAATACGCTCTATTTTGGAGAAGGAAGCCGGCTCATTGTTGTAG	+
9	GTGTACTTCTGTGCCAGCGG	GGACAGT	1.3	TCTGGAAATACGCTCTATTTTGGAGAAGGAAGCCGGCTCATTGTTGTAG	+
10	GTGTACTTCTGTGCCAGCGG	GTCAG	1.3	CTGGAAATACGCTCTATTTTGGAGAAGGAAGCCGGCTCATTGTTGTAG	+
11	GTGTACTTCTGTGCCAGCG	GACCTG	2.3	AAACGCTGTATTTTGGCTCAGGAACCAGACTGACTGTTCTC	+
12	GTGTACTTCTGTGCCAGCGGTG	ACGTA	2.3	CTGTATTTTGGCTCAGGAACCAGACTGACTGTTCTC	+
13	GTGTACTTCTGTG	TGACCTGGAGGCTGTG	2.3	AGTGCAGAAACGCTGTATTTTGGCTCAGGAACCAGACTGACTGTTCTC	−
14	GTGTACTTCTGTGCCAGCGGTGA	GTCT	2.3	ACGCTGTATTTTGGCTCAGGAACCAGACTGACTGTTCTC	+
15	GTGTACTTCTGTGCCAGCGGTGAT	GGGGCA	2.4	ACCTTGTACTTTGGTGCGGGCACCCGACTATCGGTGCTA	+
16	GTGTACTTCTGTGCCAGCGGT	GTTGCAACGTTC	2.4	AACACCTTGTACTTTGGTGCGGGCACCCGACTATCGGTGCTA	+
17	GTGTACTTCTGTGCCAGCGGT	GCTA	2.4	CTTGTACTTTGGTGCGGGCACCCGACTATCGGTGCTA	+
18	GTGTACTTCTGTGCCAGCGGTGAT	GCT	2.4	CAAAACACCTTGTACTTTGGTGCGGGCACCCGACTATCGGTGCTA	+
19	GTGTACTTCTGTGCCAGCGGTG	GGGGGGGG	2.4	CAAAACACCTTGTACTTTGGTGCGGGCACCCGACTATCGGTGCTA	+
V_β8.3	TTGTACTTCTGTGCCAGCAGTGAT				
20	TTGTACTTCTGTGCCAGCAGTGA	GCCG	1.3	AATACGCTCTATTTTGGAGAAGGAAGCCGGCTCATTGTTGTAG	+
21	TTGTACTTCTGTGCCAGCAGT	GATCGG	1.3	ACGCTCTATTTTGGAGAAGGAAGCCGGCTCATTGTTGTAG	+
22	TTGTACTTCTGTGCCAGCAGT	TCGAT	1.3	AAATACGCTCTATTTTGGAGAAGGAAGCCGGCTCATTGTTGTAG	+
23	TTGTACTTCTGTGCCAGCAGTGAT	GCA	1.3	GGAAATACGCTCTATTTTGGAGAAGGAAGCCGGCTCATTGTTGTAG	+
24	TTGTACTTCTGTGCCAGCAGT	GCTAGG	1.3	CTCTATTTTGGAGAAGGAAGCCGGCTCATTGTTGTAG	+
25	TTGTACTTCTGTGCCAGCAG	GGTTCAGCA	2.4	CACCTTGTACTTTGGTGCGGGCACCCGACTATCGGTGCTA	+
V_β14	TTCTACCTCTGTGCCTGGAGTCT				
26	TTCTACCTCTGTGCCTGGAGT	GGTCCGA	1.3	GAAATACGCTCTATTTTGGAGAAGGAAGCCGGCTCATTGTTGTAG	+
27	TTCTACCTCTGTGCC	GGTGCAGGGG	2.4	ACACCTTGTACTTTGGTGCGGGCACCCGACTATCGGTGCTA	+
28	TTCTACCTCTGTGCC	CGCGTGGGTCC	2.5	CACCCAGTACTTTGGGCCAGGCACTCGGCTCCTCGTGTTA	+
29	TTCTACCTCTGTGCC	GGCGAGGGC	2.5	GACACCCAGTACTTTGGGCCAGGCACTCGGCTCCTCGTGTTA	+

Fig. 4. Expression of normal, diverse VDJ_β rearrangements in IRNB-SCID thymocytes. Thymocyte RNA was isolated from two 7-day-old IRNB-SCID mice. TCRβ chains with V_β8.1, V_β8.2, V_β8.3, or V_β14 genes were amplified by RT-PCR as described for Fig. 2C, cloned into a plasmid vector, and sequenced by dideoxynucleotide chain termination (*44*). Clones 1 to 4, 9, 12, 13, 17, and 23 were derived from IRNB-SCID mouse 1; the rest were derived from the second animal. The nucleotide sequences are grouped according to utilization of germline V_β (underlined) and J_β gene segments. The average number of nucleotides deleted at the $V_\beta \rightarrow D_\beta$ joint is 3.7 ± 2.9, and the average number deleted at the $D_\beta \rightarrow J_\beta$ joint is 6.0 ± 3.9; the average number of D_βN region nucleotides is 7.6 ± 4.5. Although no P nucleotides corresponding to germline sequences were found, clone 16 has a 4-bp, nontemplated inverted repeat (GTTGCAAC) inserted between V_β8.2 and J_β2.4. The germline V and J segments in this clone are each deleted by 3 bp.

maturation was not linked to restoration of normal V(D)J recombinase activity.

Induction of cellular DNA damage can cause rapid apoptosis, or cell cycle arrest and transcription of genes required for DNA repair (*37*). One hundred centigrays of radiation is not sufficient to induce apoptosis of SCID thymocytes (*20*); therefore, restoration of many normal, diverse TCRβ coding joints most likely results from radiation-induced DNA repair of free coding ends. If repair is mediated by the *scid* gene product itself, it would suggest that the SCID mutation affects the regulation, rather than the function, of the *scid* gene product, which is consistent with the observed "leakiness" of the mutation and the low frequency of apparently normal V(D)J recombination events (*31*, *38*). Alternatively, radiation could stimulate other DNA repair activities that can functionally complement the *scid* gene at this critical phase of T cell maturation. These might include two genes, *XR-1* and *Xrs-6*, that participate in V(D)J recombination in fibroblasts transfected with the genes for RAG-1, RAG-2, and extrachromosomal recombination substrates (*8*). Our data indicate that simultaneous rescue of V(D)J recombination and proliferation in T cell precursors that have an intrinsic defect in DNA repair may enhance the generation of illegitimate recombination events (*39*), contributing to the invariant development of thymic lymphoma.

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41. For two-color analysis, thymocytes or LN cells were stained with: (i) biotinylated antibody to CD4 (anti-CD4) (YTS 191.1, Cedarlane Labs) and fluorescein isothiocyanate (FITC)–conjugated anti-CD8 (YTS 169-4, Cedarlane Labs) or (ii) biotinylated anti-TCRβ (H57-597) or anti-CD3ε (145-2C11, Cedarlane Labs) and FITC-conjugated anti–IL-2Rα (7D4, Pharmingen) as described [C. J. Guidos, J. S. Danska, C. G. Fathman, I. L. Weissman, *J. Exp. Med.* **172**, 835 (1990)]. For analysis of B cell development (Fig. 1B), spleen or BM cells were stained with biotinylated anti-B220 (RA3-6B2, Cedarlane) and FITC-conjugated anti-IgM (F9259, Sigma). Biotinylated antibodies were revealed with phycoerythrin (PE)-conjugated streptavidin (Caltag). For three-color analyses, cells were stained with PE-coupled anti-B220, biotinylated anti-IgM, cychrome 5–PE-avidin, and FITC anti-CD43. Dead cells and debris were excluded by gating on cells with high forward scatter and low propidium iodide fluorescence. Fluorescence was analyzed on a FACScan flow cytometer (Becton Dickinson) with Lysis II software.
42. Total RNA was isolated from the thymus and lymph nodes of individual mice, reverse-transcribed into complementary DNA (cDNA), and subjected to RT-PCR analysis as described (*40*). We confirmed cDNA quality by seeding 10% into RT-PCR reactions with actin primers. TCRβ cDNA was amplified with a degenerate, consensus V_β primer specific for sequences in the V_β1, 2, 5, 6, 8, 10, 12, 13, 15, and 16 genes (J. Danska, unpublished observations), together with a C_β antisense primer designed from a conserved sequence in the C_β1 and C_β2 genes. TCRα cDNA was amplified with a degenerate, consensus V_α primer with specificity for V_α 1 to 9 and 11 to 14, together with a C_α primer (*40*). PCR cycling conditions for V region consensus primers were as described (*40*). For actin PCR, cycling conditions were 94°C denaturation, 55°C annealing, and 72°C extension for 1 min each. PCR reactions contained each primer at 0.5 μM concentration, 5 U of Taq polymerase (Perkin-Elmer Cetus), and 0.2 mM deoxynucleotide triphosphates and were performed in a Perkin-Elmer 480 thermalcycler. PCR products were resolved on 2% agarose gels, stained with ethidium bromide, and photographed under ultraviolet light (actin) or transferred to nylon membranes and hybridized to ^{32}P-radiolabeled C_β or C_α probes. Photographs and autoradiograms were scanned with an Apple One scanner. The sequences of the primers were as follows: 5′ β-actin: TGG GTC AGA AGG ACT CCT ATG; 3′ β-actin: CAG GCA GCT CAT AGC TCT TCT; V_β consensus: TAA GCG GCC GCA TGS LYT GGT AYW XXC AG [S = G or T; L = A, G, or T; Y = C or T; W = A or C; X = A or G]; and C_β antisense: CCA CGT GGA GCT GAG CTG.
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44. RNA was extracted from 0.5×10^6 to 1×10^6 fresh IRNB-SCID thymocytes, reverse-transcribed, and PCR-amplified, with a C_β antisense primer paired with either a sense primer specific for the V_β8 family (V_β8.1, V_β8.2, and V_β8.3) (TAA GCG GCC GCG AGG CTG CAG) or a sense primer specific for V_β14 (CTG GAG CTC GAA TTC GCT CAG). As a negative control for each PCR reaction, mock cDNA (synthesized without reverse transcriptase) was amplified with the same PCR reagent mix; it was always found to be negative. PCR products of appropriate size were agarose gel–purified (Gene Clean, Bio-Rad), ligated into pCR-2 (Invitrogen), and electroporated into INV-OneShot bacteria (Invitrogen). Bacterial clones were screened on colony lifts and Southern blots for hybridization to V_β and C_β probes with the use of standard techniques. Double-stranded plasmid DNA from V_β- and C_β-positive clones was sequenced (U.S. Biochemicals) with the C_β and universal primers and resolved by electrophoresis on urea-polyacrylamide gels. DNA sequence analysis was performed with MacMolly software (Soft Gene GmbH, Köln, Germany).

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