Transient Restoration of Gene Rearrangement at Multiple 
T Cell Receptor Loci in γ-Irradiated scid Mice

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Summary

The developmental arrest of thymocytes from scid mice, deficient in variable, (diversity), and joining, or V(D)J recombination, can be overcome by sublethal γ-irradiation. Since previous studies focused on restoration of rearrangement of the T cell receptor (TCR) β locus, productive rearrangement of which is selected for, we sought to examine to what extent locus specificity and cellular selection contributed to the observed effects. We report here that irradiation of newborn scid mice induces normal V-D-J rearrangements of the TCR γ locus, which like TCR β, is also actively rearranged in CD4-CD8- (double negative) thymocytes. In contrast, no complete V-Jγ rearrangements were detected. Instead, we detected substantial levels of hairpin-terminated coding ends at the 5' end of the Jγ locus, demonstrating that TCR γ rearrangements manifest the effects of the scid mutation. Irradiation, therefore, transiently compensates for the effects of the scid mutation in a locus-nonspecific manner in thymocytes, resulting in a burst of normal TCR β and γ rearrangements. Irradiation also allows the development of cells that can initiate but fail to complete V(D)J recombination events at the TCR α locus, which is normally inaccessible in scid thymocytes.

T lymphocytes are characterized by the expression of antigen-specific TCR molecules consisting of the clonotypic α and β or γ and δ chains. The V, (D), and J gene segments that encode the variable portion of these polypeptides are joined together during T cell development by a somatic gene rearrangement process termed V(D)J recombination (for a review see reference 1). A number of stages of intrathymic T cell development have been defined by the expression of various surface molecules, with several of the developmental transitions tightly coupled with the ordered assembly of TCR genes (for a review see reference 2). Thymocytes early in the pathway lack expression of CD4 and CD8 (hence referred to as double negative cells) and rearrange their β, γ, and δ TCR loci. Cells with a productive β rearrangement proliferate extensively and develop into CD4+/CD8- (double positive) thymocytes, initiate α gene rearrangement, and commit to the α/β T cell lineage, whereas double negative cells with a productive γ and δ rearrangement become γ/δ T cells (3, 4).

Mice genetically deficient in the V(D)J recombination process, either due to the naturally occurring scid mutation (5) or to the targeted disruption of one of the two recombination-activating genes, RAG1 or RAG2 (6, 7), are unable to generate functional TCR complexes, and their thymocytes are arrested at the double negative stage of development (8, 9). In RAG-/- lymphocytes, V(D)J recombination fails to initiate (10, 11), whereas in scid thymocytes, the recombination machinery generates double strand breaks adjacent to TCR gene segments but fails to join the coding segments properly. The unjoined coding ends of the TCR δ locus from scid thymocytes have been demonstrated to have a covalently sealed, hairpin structure (12). The rare coding junctions that do form are often characterized by large deletions of coding DNA (13) or the appearance of long palindromic sequences (termed P nucleotides [14]) at the coding junctions (15-17). The defect in scid mice has recently been demonstrated to be a deficiency in the catalytic subunit of the DNA-dependent protein kinase (DNA PK, 18, 19). The catalytic subunit is active only when bound to DNA, and this is mediated by the

1 Abbreviations used in this paper: DNAPK, DNA-dependent protein kinase; IRNB, irradiated newborn; RAG1, recombination-activating gene 1.
Ku nuclear complex, a heterodimer of 70- and 80-kD peptides (20).

It was recently shown that sublethal γ-irradiation of scid (21) or RAG−/− (22, 23) mice can overcome the developmental arrest and promote the appearance of large numbers of double positive thymocytes. The mechanism by which γ-irradiation produces this effect in RAG−/− mice is independent of a functional TCR β protein, since these mice are inherently unable to perform gene rearrangement (6, 7).

In contrast, in scid mice, irradiation induced the development of double positive thymocytes, many of which contained normal TCR β rearrangements and TCR β protein (21). The appearance of large numbers of double positive thymocytes containing normal TCR β rearrangements in irradiated newborn (IRNB scid mice raised two important issues. First, it was unknown whether the effects of irradiation were confined to the β locus or also extended to the TCR γ and δ loci (which are also actively rearranged in double negative thymocytes). Second, it was not known to what extent the double positive scid thymocytes induced by irradiation initiated rearrangements of the TCR α locus, and whether the rearrangements displayed the characteristics of wild-type or scid recombination. We have therefore analyzed TCR δ and α gene rearrangements in IRNB scid mice. We found that normal TCR δ rearrangements occur and that TCR α recombination is initiated after irradiation, but results in broken coding ends terminating in a hairpin structure. Therefore, irradiation compensates for the scid defect in V(D)J recombination in a transient and locus-nonspecific fashion, resulting in the development of double positive thymocytes that then initiate defective TCR α rearrangements.

Materials and Methods

Mice. C.B-17 scid/scid and wild-type BALB/c mice were bred and housed at the Hospital for Sick Children. Wild-type C57Bl/6 and AKR/J mice used as controls in some experiments were purchased from the Jackson Laboratory (Bar Harbor, ME) and housed at Yale Medical School. Newborn scid mice were treated with a single low dose of irradiation (100 cGy) as described previously (21).

Flow Cytometry. Flow cytometric analyses of thymocyte suspensions for CD4, CD8, and CD25 surface markers and for intracellular TCR β chain protein were carried out as previously described (21) on a FACSScan® flow cytometer with Lysis II software (Beckton Dickinson & Co., Mountain View, CA).

Nucleic Acid Preparation and Hybridization. High molecular weight DNA was prepared from total homogenized kidney or liver and single cell suspensions of total thymocytes with proteinase K digestion/phenol-chloroform extraction (24). 5–8 μg genomic DNA was restriction digested with the indicated enzymes (Boehringer Mannheim Corp., Indianapolis, IN or New England Biolabs, Beverly, MA) according to the manufacturers' instructions, electrophoresed through 0.7–0.8% agarose gels, and transferred to Gene Screen Plus nylon membrane (NEN DuPont, Boston, MA) on a Posiblot apparatus (Stratagene, La Jolla, CA). Two-dimensional agarose gel electrophoresis was carried out as described previously (12). Briefly, PstI-digested DNA in a wide lane was electrophoresed through a neutral agarose gel in the first dimension; the lane was excised and cut in half lengthwise. One half was saved as first dimension control, whereas the other half was equilibrated with alkaline running buffer (50 mM NaOH, 1 mM EDTA) and inserted into the alkaline agarose gel perpendicular to the direction of the first dimension. After second dimension electrophoresis, the gel was transferred along with the first dimension control as described above. Molecular weight markers were run both in the first and the second dimensions to ensure accurate size determination. Hybridization was carried out in the presence of 50% formamide (24) with random hexamer-primed [α-32P]dCTP (Amersham Corp., Arlington Heights, IL) labeled probes 4, 8, 9, 10, 11, 13, 14, 15, RAG-1 (4), and Cal or Call (25) as indicated. Autoradiography and quantitative analysis were performed using a PhosphorImager with ImageQuant 3.0 software (Molecular Dynamics, Sunnyvale, CA). The relative hybridization signal for a given probe was calculated as described (4).

DNA Sequencing. TCR V4(D)J1β and V5(D)J1β sequences were PCR amplified, restriction enzyme digested at artificially introduced enzyme sites, and directionally subcloned into pBluescript II vector (Stratagene) as described previously (4). DNA prepared from individually picked clones was double strand sequenced with the Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA), separated on denaturing polyacrylamide gel (National Diagnostics, Atlanta, GA), and analyzed on a Stretch automated sequencer (model 373; Applied Biosystems).

Results

V-D-Jβ Rearrangements in Irradiated scid Thymocytes. To determine the effects of γ-irradiation on the TCR δ locus in scid thymocytes, genomic thymus DNA from irradiated and nonirradiated scid mice, and from control BALB/c mice, was subjected to Southern blot analysis. EcoRI digestion and hybridization with probe 4 (which lies between Jβ1 and Jβ2; Fig. 1A) allowed detection of any rearrangement to Jβ1. The BALB/c thymus DNA produced a characteristic pattern of bands (Fig. 1A, lane 2), many of which can be assigned to specific V-to-DJβ or D-to-Jβ rearrangements (4, 26, 27). Scid thymus DNA produced a quite different pattern of bands: the germline fragment was retained to a substantial extent, and only three distinct nongermline bands were observed (Fig. 1A, lane 3). The top-most nongermline band represents D2-J1 rearrangements, whereas the other two (black arrowheads) represent broken DNA molecules that terminate in a hairpin structure at the ends of the D82 and J81 coding regions (hereafter referred to as coding end molecules). These results agree with previous studies that demonstrated that thymocytes from nonirradiated scid mice initiate rearrangement of the TCR δ locus generating hairpin-terminated coding end molecules, with most of the events occurring at the D82 and J81 gene segments (12, 28).

We reasoned that if irradiation resulted in a period of normal δ rearrangements in the scid thymus, we should see the appearance of diverse nongermline bands similar to those seen in the wild-type thymus, and a decrease in the intensity of the germline-sized band. We might also expect to see the disappearance of the coding end molecules, due either to conversion to coding joint products or to degradation. Analysis of individual IRNB scid mice confirmed these predictions. We observed a significant reduction in
the intensity of the germline hybridization signal and the disappearance of the two bands corresponding to coding end molecules (Fig. 1 A, lanes 4 and 5, compare with lane 3). Furthermore, in the 1-wk-old sample (Fig. 1 A, lane 4) there are clearly detectable bands migrating as expected for V4DJ18 and V5DJ18 rearrangements that were also seen in independent DNA samples from 9-d-old IRNB scid mice (data not shown). The identity of these bands was corroborated by rehybridizing the same blot to a mixture of V84- and V85-specific probes (data not shown). The bands corresponding to complete V-D-J8 rearrangements became barely detectable at 2 wk after irradiation (Fig. 1 A, lane 5, and data not shown) and were not detectable at 3 wk after irradiation (data not shown). The appearance of V4- and V5-DJ18 rearrangements 7–9 d after irradiation indicates that a single dose of irradiation resulted in the generation of normal V-D-J8 recombination products in scid mice.

We also wanted to determine whether the disappearance of the TCR 8 coding end molecules was due to their conversion into the V-D-J8 joints or to their quantitative reduction in the large proliferative burst that follows irradiation (21). When scid mice are provided with a functional TCR B transgene (29), they develop thymocytes in comparable numbers and with comparable surface phenotypes to those seen in irradiated nontransgenic scid mice (data not shown). Southern blotting experiments identical to those
DNA sequences of 23 V4-D-J8 (top) and 25 V5-D-J8 (bottom) joints PCR-amplified from four independent thymus samples obtained from irradiated scid mice. T1 and T2.1 represent the samples of 1 wk and 2 wk after irradiation, respectively, shown in Fig. 1 A. T2.2 and T2.3 are two more independent thymus samples of 2 wk after irradiation. The entire junction and the ends of the variable and joining genes are shown in a 5′→3′ direction. The top lines indicate the germline (GL) sequences (28, 41), N indicates nongermline nucleotide addition. Potential P nucleotides are highlighted. Note that some AT dinucleotides shown as DSI sequences cannot be assigned unambiguously and are also highlighted as potential P nucleotides. (No) Number of occurrences of each sequence expressed as a ratio of the total number of sequences determined. The next column shows the percent unique sequences in each sample. The rightmost column indicates the reading frame of the sequences, "+" meaning the frame that could generate full-length TCR β protein.

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Sequence analysis revealed predominantly normal V-D-J8 joints (Table 1), with only 8 of the 43 (18.6%) unique, nonprocessed V, D, or J ends having P nucleotide sequences of 3 bp or longer (potentially representative of scid-type aberrant rearrangements). Analysis of previously reported (4) V4/V5-D-J18 rearrangements from normal thymocytes and peripheral T cells found 13 of 162 (8.0%) unique, nonprocessed ends carrying P nucleotides of similar length (data not shown). In contrast, 5 of 13 (38.4%) independent coding ends of D2-J18 rearrangements amplified from nonirradiated scid thymus (the only rearrangements visible by Southern blot; Fig. 1 A, lane 3) exhibited 3 bp or longer P nucleotide sequences (data not shown). We also note that whereas 90% of the previously reported independent TCR β rearrangements were in-frame (21), only 9/20 (45%) of the δ rearrangements were in-frame, which is not statistically significantly different from the 33% expected for random rearrangements (P >0.19). The fact that there is no significant skewing toward in-frame δ rearrangements, yet the majority of the TCR δ sequences are normal (as judged by the absence of extensive P nucleotide addition) demonstrates that normal V(D)J recombination events observed after irradiation do not depend upon cellular selection of the recombination products. This suggests that the previous

Table 1. V4-D-J8 and V5-D-J8 Joints from Irradiated scid Thymocytes

| Source | V84 | N | D81 | N | D82 | N | J81 | No | unique | FRAME |
|--------|-----|---|-----|---|-----|---|-----|----|--------|-------|
| GL     | G84A | G | GT  | GC | GC  | GC | CT  | CT | GAT   | CTGAT |
| T2.2   | G84A | A | GA  | GC | GC  | GC | CT  | CT | GAT   | CTGAT |
| T2.2   | G84A | A | GA  | GC | GC  | GC | CT  | CT | GAT   | CTGAT |
| T2.2   | G84A | A | GA  | GC | GC  | GC | CT  | CT | GAT   | CTGAT |

| Source | V85 | N | D81 | N | D82 | N | J81 | No | unique | FRAME |
|--------|-----|---|-----|---|-----|---|-----|----|--------|-------|
| GL     | G85A | G | GT  | GC | GC  | GC | CT  | CT | GAT   | CTGAT |
| T1     | G85A | A | GA  | GC | GC  | GC | CT  | CT | GAT   | CTGAT |
| T1     | G85A | A | GA  | GC | GC  | GC | CT  | CT | GAT   | CTGAT |
| T1     | G85A | A | GA  | GC | GC  | GC | CT  | CT | GAT   | CTGAT |

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Figure 2. Initiation of TCR α gene rearrangement in irradiated scid thymocytes. (A) Graphical summary of SphI + EcoRI-digested Southern blots of wild-type C57Bl/6 (Bl/6) and nonirradiated or irradiated scid thymocytes. Four independent Bl/6, seven independent irradiated 1-wk-old and one nonirradiated 2-wk-old thymus samples were analyzed with probes 10, 11, 13, 14, 15, and Ca as explained in detail previously (4). The abscissa shows the probes, the ordinate indicates the average relative hybridization signal of the corresponding probe calculated as explained previously (4). The positive standard deviation from the average is shown on the top of the columns. The location of the probes in the context of the murine J-Cα locus is shown below the graph along with the relevant restriction sites and genetic elements. Symbols are as in Fig. 1, B and C. (B) PstI Southern blot analysis of the 5' end of the Jα locus shows the appearance of bands migrating at the predicted size of JαS0 coding end breaks (arrowheads) and the lack of V-Jα rearrangements present in normal BALB/c (B/c) thymus (arrows, left; compare lanes 2 with lanes 3–6) in irradiated or TCR Jα transgenic scid mice. (Dashes, left) Position of molecular weight standards in kilobases. Lane 1 contains wild-type BALB/c kidney DNA (K) as germline control, and lane 7 contains the same DNA double digested with PstI + Asel to generate a marker band that is 10 bp smaller than the predicted full-length coding end break (see map in C). Note the similar appearance of JαS0 coding end breaks in irradiated scid thymus samples of 1 (T1), 2 (T2), and 3 (T3) wk after irradiation (lanes 3–5) and in nonirradiated TCR β transgenic scid thymus (lane 6). (C) The 5' end of the Jα locus is shown with the position of the JαS0 RSS highlighted. (Double arrows) Size of the predicted fragments corresponding to germline and to JαS0 coding end break. Other symbols are as in Fig. 1, B and C.
observation of polyclonal, structurally normal V-D-Jβ rearrangements in IRNB scid mice (21) did not arise merely because of the strong selection for a functional TCR β protein.

In contrast to the diversity of TCR V-D-Jβ junctions identified from IRNB scid thymi (81% unique; 21), TCR θ rearrangements were oligoclonal (Table 1). Only 20 of 45 TCR θ sequences (44%) were unique, a somewhat surprising result given the relatively strong hybridization signal seen for V4- and V5-Dβ rearrangements after irradiation (2 and 5% of the total genomic hybridization intensity, respectively; Fig. 1, lane 4). By contrast, our previous analysis of wild type V-D-Jβ rearrangements (4) found that >95% were unique (data not shown). In IRNB scid thymocytes oligoclonal V-D-Jβ rearrangements, representing 2–5% of the genomic loci, indicate that large numbers of double positive thymocytes containing the same V-D-Jβ rearrangement may arise from a limited pool of double negative precursors.

Coding End Molecules of the TCR α Locus in Irradiated scid Thymocytes. TCR α gene rearrangement normally initiates in double positive thymocytes (31) after productive TCR β gene rearrangement (32). Since irradiation of newborn scid mice leads to the appearance of normal β rearrangements followed by the emergence of large numbers of double positive thymocytes (21), it was of interest to determine the degree to which TCR α gene rearrangement was initiated in these cells. We first employed a quantitative multiprobe Southern blot analysis we have recently developed (4, 25) to assess the extent of rearrangements throughout the TCR α locus. Southern blots hybridized simultaneously to probes 10, 11, 13, 14, 15, and Cal (for details, see reference 4) were quantitated on a phosphorimager; the retention of hybridization intensity in the germline size band, after normalization for loading differences, was determined for each probe (Fig. 2 A). As expected, there was no significant TCR α gene rearrangement in nonirradiated scid mice. In contrast, in IRNB scid mice, ~30% of the 5′ end of the θα locus had lost its germline configuration, whereas at the more distal 3′ θα regions, there was no significant rearrangement and/or deletion.

To investigate the molecular structure of the α rearrangements in IRNB scid mice, we focused on the most 5′ θα gene, θα50 (33). We have recently demonstrated that this gene segment participates in a large number of diverse V-θα rearrangements and also exhibits high levels of broken DNA molecules terminating in a recombination signal sequence (signal end molecules) in wild-type thymocytes (25). PstI Southern blots were hybridized to probe 9, which lies just downstream from θα50 and defines a signal germline hybridization fragment spanning exclusively the θα50 gene (for map, see Fig. 2 C). V-θα rearrangements were identified by the appearance of novel nongermline fragments present in wild-type thymus but not in kidney DNA (25, and compare Fig. 2 B, lanes 1 and 2). A fragment corresponding to potential θα50 coding end breaks was also readily identified because double digestion of the germline DNA with PstI + Asel (Fig. 2 B, lane 7) results in a restricted fragment only 10 bp shorter than a putative θα50 coding end molecule (for map, see Fig. 2 C). No band of this size was visible in total wild-type thymus and the closest visible band has been shown to represent complete V-Jα (mostly Vα8 and Vα11) rearrangements (25). In contrast, θα thymocytes, 1, 2, or 3 wk after neonatal irradiation, exhibited one clear band migrating exactly at the position expected for a θα50 coding end molecule (Fig. 2 B, lanes 3–5, compare to the marker band in lane 7). Thymocytes from nonirradiated TCR β transgenic scid mice also showed similar quantities of θα50 coding end molecules (Fig. 2 B, lane 6). None of these samples, however, contained visible bands corresponding to V-θα rearrangements, as found in wild-type thymus. In separate experiments, we found θα50 signal end molecules and reciprocal products in IRNB scid thymus samples (data not shown), consistent with previous results demonstrating the capacity of scid cells to form signal joints (34).

The lack of detectable V-θα rearrangements and the appearance of a band at the size expected for θα50 coding end molecules in irradiated scid thymocytes suggested that θα rearrangements were being performed by a θα-type V(D)J recombination machinery. If this were the case, then the θα50 coding end molecules from IRNB scid thymus should terminate in covalently sealed hairpin ends, as has been demonstrated for the Dβ and Jβ genes in nonirradiated scid thymus (12). To test this hypothesis, two-dimensional Southern blots were performed as described previously (12). Total thymus DNA from wild-type and from 9–d-old irradiated scid mice was digested with PstI and electrophoresed in a neutral agarose gel in the first dimension. Approximately half of each sample was then electrophoresed in a neutral agarose gel in the first dimension and distributed along the diagonal of the gel. This result given the relatively strong hybridization signal of θα50 coding end molecules (Fig. 2 B, lane 4). By contrast, our previous analysis of wild type V-D-Jβ rearrangements (4) found that >95% were unique (data not shown). In IRNB scid thymocytes oligoclonal V-D-Jβ rearrangements, representing 2–5% of the genomic loci, indicate that large numbers of double positive thymocytes containing the same V-D-Jβ rearrangement may arise from a limited pool of double negative precursors.

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Discussion

The exact mechanism of V(D)J recombination remains to be elucidated, but it now appears that the early steps in the process (DNA recognition and cleavage at recombination signal sequences) are catalyzed by the RAG1 and RAG2 proteins (36, 37), whereas later steps (end process-
Figure 3. Two-dimensional Southern blot analysis of wild-type and irradiated scid thymus DNA. PstI-digested DNA from wild-type and from irradiated 9-d-old scid thymus was electrophoresed through a neutral agarose gel in the first dimension (1D), half of the samples were then separated in denaturing alkaline agarose gels in the second dimension (2D), transferred, and hybridized to probe 9. (insets) Schematic representation of the various signals obtained in the hybridization analysis. (Arrows, sides) Location of germline (GL), rearranged (V-Jα), and potential coding end breaks (Ja50) as they migrate in the first dimension. (Arrowheads) Position of the same bands after two-dimensional separation. Note the diagonal migration of V-Jα rearrangements in wild-type thymus as opposed to the irradiated scid sample, where the single non-germline band corresponding to the Ja50 coding end break migrates off the diagonal (see arrowhead and gray dot B, inset). No clear signal is visible on the diagonal in this sample (see small arrow and open circle A, inset), suggesting that the majority of fix50 coding end breaks terminate in a hairpin (for details see text). Two-dimensional separation of kidney DNA double digested with PstI + AseI confirmed that a restriction fragment of similar size as the Ja50 coding end break would migrate in the diagonal (data not shown). (Dashes) At left of the 1D lanes and beneath the 2D blots, molecular weight standards in kilobases, as follows: 1.6, 2.0, 3.0, 4.1, 5.1, and 6.1.

Linking and joining) require the direct or indirect involvement of the components of the DNAPK (18, 38). The scid mutation, which affects the catalytic subunit of this kinase, interferes with the formation of proper coding joints of the antigen receptor genes (34) and with DNA double strand break repair, resulting in increased sensitivity to agents causing DNA breaks such as γ-irradiation (39–41). Aberrant V(D)J recombination in scid lymphocytes is manifested in three ways: (a) extensive deletions of coding sequences of Ig (13) and TCR (17) genes; (b) persistence of high levels of hairpin-terminated coding end molecules of the TCR δ locus (12); and (c) long P nucleotide additions at the junctions of the rare D2-J18 (42, and our data) or V-Jy (17) rearrangements. It has been suggested that the principal defect in scid V(D)J recombination is the inability to resolve hairpin coding end intermediates into coding joints (43). Instead, coding end molecules appear to be resolved either through generation of abnormal joints with long P nucleotides or loss of DNA sequences due to nuclease digestion or illegitimate recombination (44).

A principal goal of this study was to determine whether the effects of γ-irradiation on V(D)J recombination in scid thymocytes were TCR locus specific. The disappearance of δ coding end molecules and the identification of complete V-D-Jδ rearrangements, which are not selected for in double positive, α/β-lineage thymocytes (4, 30), indicate that irradiation permits productive V(D)J recombination to occur at multiple antigen receptor loci accessible at the time of irradiation. However, this effect is temporally restricted because in the TCR α locus, which is not rearranged to any significant extent before generation of double positive thymocytes (31), no complete V-Jα joints were detectable with our assay, and only rare TCR α VJC transcripts have been observed (21). Instead, we have shown accumulation...
of hairpin-terminated Jo50 coding end molecules. The persistence of the Jo50 coding end molecules for several weeks, coincident with the loss of V-D-J8 alleles (see below), suggests that initiation of TCR α rearrangement continues at substantial levels throughout this time.

It appears that only a limited number of IRNB scid thymic precursors perform correct rearrangements at the TCR δ locus. Irradiation results in rearrangement of a substantial fraction of scid TCR δ alleles, with the TCR δ germline hybridization signal in the irradiated scid thymus approaching the levels seen in normal α/β T cells (Fig. 1 A, lanes 4 and 5). At 1 wk after irradiation, the reduction in germline δ alleles is accompanied by the appearance of readily detectable bands corresponding to V-D-J8 rearrangements. However, these bands do not accumulate to the extent seen in wild-type thymocytes, and they show a substantial decline in intensity by 2 wk after irradiation (Fig. 1 A, lane 5). In addition, V-D-J8 rearrangements cannot be detected 3 wks after irradiation, despite the fact that the hybridization intensity of the germline band remains low (data not shown). Importantly, the V4- and V5-D-J18 rearrangements appear to be oligoclonal, even at 1 wk after irradiation (Table 1), and only 42% of the examined V-D-J6 rearrangements were unique. This contrasts with the previously reported diversity (81%) of TCR β joints observed after irradiation, (21), and with our previous finding of >95% diversity of TCR δ joints in normal thymocytes (data not shown). Collectively, these data are consistent with the hypothesis that irradiation allows normal V-D-J8 and V-D-J6 rearrangements to occur independently in relatively few individual precursors. Since productive TCR β but not TCR δ rearrangements drive expansion of these precursors (32) it is likely that only a fraction of TCR β+ thymocytes will contain TCR δ gene rearrangements. The limited effect of irradiation on precursor cells might be due to a short period of time during which TCR β or δ alleles can be rearranged normally, and/or to a poor survival of irradiated cells.

A combination of several factors may explain the disappearance of bands corresponding to V-D-J8 rearrangements at longer time points after irradiation. First, because the effect of irradiation on V(D)J recombination is transient, no new V-D-J8 rearrangements are generated over time. Second, scid double positive thymocytes initiate a substantial amount of TCR α rearrangement (Fig. 2, A and B), and each such event places the δ locus on an extrachromosomal circle or on a signal end molecule (4, 45, and data not shown). These can be physically lost from the cells, particularly if cellular division occurs. Third, the population of thymocytes in an irradiated scid animal becomes oligoclonal over time, and all such mice eventually develop thymic lymphomas (21). Oligoclonality of TCR β rearrangements is clearly manifest on Southern blots by 6–8 wk after irradiation, and the reduction in cellular heterogeneity, presumably caused by proliferation of (pre-) malignant cells, was likely underway some time before this.

The mechanism by which γ-irradiation allows the production of normal V(D)J recombination products in scid thymocytes has not been determined. It might directly affect the V(D)J recombination machinery or result in increased production of normal rearrangement products through an independent mechanism that does not involve the scid gene product. In the former case, induction of either functional DNAPK or some alternative pathway would allow normal V(D)J recombination to occur. Alternatively, irradiation might significantly extend the G1 phase of cell cycle which in turn could increase the production of complete rearrangements by allowing more time for the resolution of coding end breaks. Whatever the effect of γ-irradiation on V(D)J recombination, our data demonstrate that it is temporally restricted, but not locus specific. It remains to be seen whether the scid-type initiation of TCR α rearrangements can similarly be corrected with subsequent exposures to γ irradiation, thereby allowing the generation of significant numbers and diversity of mature α/β T cells in scid mice.

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Note added in proof: While this work was under review, similar results on normal V-D-J6 rearrangements and generation of TCR α coding end molecules after γ irradiation of scid mice were reported (Bogue, M.A., C. Zhu, E. Aguilar-Cordova, L.A. Donehower, and D.B. Roth. 1996. p53 is required for both radiation-induced differentiation and rescue of V(D)J rearrangement in scid mouse thymocytes, Genes & Dev. 10: 553–565).
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