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T-cell receptor gene rearrangements in functional T-cell clones from severe combined immune deficient (scid) mice: Reversion of the scid phenotype in individual lymphocyte progenitors

(lymphocyte differentiation/immunodeficiency/variable–diversity–joining recombination)

JOHN H.-J. PETRINI*, ANN M. CARROLL, AND MELVIN J. BOSMA

Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111

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ABSTRACT **The severe combined immune deficient (scid) mouse mutant is characterized by a general absence of functional B and T lymphocytes. This deficiency appears to result from a defect in the variable–diversity–joining (VDJ) recombinase system, which is responsible for the assembly of V, D, and J gene segments that code for immunoglobulin and T-cell receptor (TCR) V regions. Most rearranged immunoglobulin or TCR genes in transformed scid lymphocytes contain abnormal J-associated deletions and are nonfunctional. A few functional lymphocyte clones do arise, however, in some young adult scid mice and in virtually all old scid mice; this phenomenon is referred to as leakiness. Alloreactive, CD3$^+$ T-cell clones were isolated from leaky scid mice and the status of their TCR β and γ loci was examined in an effort to assess the nature of the recombinase activity that gives rise to functional scid lymphocytes. The recombination junctions of six γ and two β alleles were sequenced, representing four alloreactive T-cell clones. All of the junctions were indistinguishable from those seen in normal cells. These results cannot be attributed to selection by antigen because other rearranged TCR genes account for the TCR molecules expressed by these T-cell clones. We conclude that reversion of the scid phenotype can occur in rare lymphocyte progenitors and may account for most functional lymphocyte clones in leaky scid mice.**

Mice that are homozygous for the *scid* mutation [severe combined immune deficient (scid) mice] are generally devoid of functional B and T lymphocytes. This deficit results from an impaired variable–diversity–joining (VDJ) recombinase system (1). Immature transformed scid lymphocytes contain a defective VDJ recombinase activity that cannot recombine antigen receptor gene segments (V, D, and J) at appreciable frequency, though this activity can specifically recognize and readily join the recombination signal sequences that flank these segments (2–4). Attempted D to J (1, 5–8) or V to J (3, 9) joining frequently results in deletion of one or both participating genomic segments. Despite the severity of this defect, oligoclonal B and T cells can be detected in about 15% of young adult scid mice and in virtually all old scid mice (≥1 year old) (10–12). We refer to these mice as leaky scid mice.

Two hypotheses for the appearance of oligoclonal B and T scid lymphocytes have been considered (10–13): The first postulates that the defective scid recombinase activity is capable of mediating normal rearrangement of V(D)J gene segments at very low frequency; the second postulates that the scid recombinase activity may be partially or completely normalized in rare lymphocyte progenitors as a result of some genetic or epigenetic change; we will refer to this as reversion of the scid phenotype. Although these two hypotheses are not mutually exclusive, they make different predictions about the status of the nonexpressed antigen receptor alleles in functional scid lymphocytes. Specifically, if the defective recombinase activity were capable of mediating successful V(D)J rearrangements at a very low frequency and were responsible for the appearance of functional lymphocytes, only those alleles whose expression is functionally selected by antigen should be normally rearranged. The nonexpressed loci would be expected to show the effect of the defective recombinase activity and contain aberrant deletions of V, D, or J gene segments. Alternatively, if functional scid lymphocytes were to result from a qualitative alteration in the scid recombinase activity, the structures of nonexpressed loci should reflect this and resemble normally rearranged loci.

To test the above hypotheses, we molecularly cloned and characterized eight recombinant T-cell receptor (TCR) loci from four alloreactive CD3$^+$ T-cell clones isolated from leaky scid mice. As reported earlier (13), Southern blot analysis of the recombinant TCR loci in these clones did not show evidence of aberrant deletions that characterize the majority of antigen receptor gene rearrangements in immature B and T cells of scid mice. This suggested that the VDJ recombinase activity expressed in the progenitors of these clones may have been qualitatively distinct from the defective recombinase activity that characterizes the scid phenotype (2–4). Compelling evidence for this possibility is presented in this report, where we show normal recombination junctions in all eight of the recombinant TCR loci analyzed. This normality cannot be explained by antigenic selection, because other rearranged TCR genes account for the expressed TCR molecules on these T-cell clones. We conclude that reversion of the scid phenotype can occur in lymphocyte progenitors to permit differentiation of functional B- and T-cell clones in an otherwise immunodeficient scid mouse.

MATERIALS AND METHODS

Cell Culture. Isolation and culture of T-cell clones has been described in detail (13).

DNA Preparation. Genomic DNA was prepared as described by Schuler *et al.* (1).

Polymerase Chain Reaction (PCR) Amplification. PCR was carried out using the following oligonucleotide primers. β locus primers: 3′ Jβ2 (GGGGAATTCGATTTCCCTCCCGGAGA); Jβ2b (CCCGAGCTCCCAACTTACCGAGAACAG); Jβ2c (CGCGGATCCAAACTACTCCAGGGA); UDβ1 (GGGGGATCCTTAGGTCAAAGGGCAAAGC); UDβ1.2 (CCCGGATCCCAGGAAAGCAGTCTCTAA); UDβ1.2b (CCCAAGCTTGCTGATAGGCGTGA); UDβ2 (GGGGAATTCTGTGGAGTCTCCTGGTAGGG). γ locus

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Abbreviations: V, variable; D, diversity; J, joining; TCR, T-cell receptor; scid, severe combined immune deficient; PCR, polymerase chain reaction.

*Present address: Dana–Farber Cancer Institute, Boston, MA 02115.

primers: Vγ1.2 (GGGAAGCTTTGCTTGCAGTTGG); Jγ2 (GGGGAATTCATCACTGGAATAAAGCAG); DVγ1.2 (GGGCTGCAGAGAGACAGATGA). Reactions were carried out in 100-μl volumes in the presence of 200 μM (each) dATP, dGTP, dCTP, and dTTP, 1 μM (each) oligonucleotide primer, 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 3.0 mM $MgCl_2$, and 0.01% (wt/vol) gelatin using 300 ng of genomic DNA as template. Genomic DNA was boiled for 5 min before addition to reaction mixes. Cycling was carried out in a Gene Machine programmable thermal controller (USA Scientific, Ocala, FL) using the following protocols. β locus amplification: 30 cycles of 1 min at 95°C, 1 min at 55°C, 2 min at 72°C, followed by 10 min at 72°C. γ locus amplification: 30 cycles of 1 min at 95°C, 1 min at 55°C, 1 min at 72°C, followed by 10 min at 72°C. PCR products were analyzed on 3% NuSieve gels (FMC) and Southern blotted as described below. Fragments were isolated and purified using GeneClean (Bio 101, La Jolla, CA). In some cases, a second round of PCR was carried out as above using the purified PCR product as the template with primer pairs consisting of one primer internal to the fragment and one of the original primers. DNA sequence of the PCR primer designated UDβ2 was derived from pDβ2, a 7-kilobase (kb) *Eco*RI fragment representing the Dβ2 and Jβ2 loci, which was the generous gift of S. Hedrick. PCR products were subjected to restriction enzyme digestion and cloned into pUC18 or pUC19 (14). DNA sequencing by the dideoxy chain-termination method (15) was carried out on double-stranded DNA using the method of Toneguzzo *et al.* (16). Autoradiograms were exposed for 18 hr at room temperature using Kodak X-Omat film.

Southern Blot Analysis. Southern blotting of genomic DNA was carried out as described (13). In some cases, Southern transfer of PCR products was effected using the Stratavac vacuum blotting system (Stratagene). In those experiments, hybridization conditions were as described in Hummel *et al.* (17). Hybridization probes used in this study were as follows: pVγ1.2, a subclone of p8/10-2γ1 (18) prepared by W. Schuler (Basel Institute for Immunology), and pJβ1 and pJβ2, described by Malissen *et al.* (19). The Jδ2 probe is a 3-kb *Sac* I fragment, which was generously provided by Y. Chien (Stanford University School of Medicine).

RESULTS

Configuration of TCR Loci in T-Cell Clones from Leaky scid Mice. Summarized in Table 1 are the configurations of TCR β and γ loci in the four alloresponsive, $CD3^+$ T-cell clones analyzed. Also included is a nonalloresponsive T-lineage clone, 1142, which is interleukin 2 dependent and $CD3^-$ (ref. 13; A.M.C., unpublished data). The indicated status of TCR β and γ rearrangements is based upon the following criteria. Rearrangement of the TCR γ loci gives rise to *Eco*RI restriction fragments of 14, 16, 17, and 22 kb upon Southern blotting with appropriate probes (18–21). Hence, aberrant rearrangements at these loci are identified by the creation of novel-size restriction fragments. Combinatorial diversity of rearrangements at the Dβ and Jβ loci is much greater than at the γ loci, hence it is frequently difficult to assess the quality of TCR β rearrangements by the size of the recombinant restriction fragment. Therefore, rearrangements that delete the *Pvu* II and *Cla* I restriction sites that lie just 3′ of the Jβ1 or Jβ2 loci, respectively (22, 23), are classified as aberrant since they fall outside of the involved Jβ cluster. Using the above criteria, all of the TCR β and γ gene rearrangements in clones 1280, 1287, and 7167 appear normal. In contrast, clone 1233 contains abnormal as well as normal rearrangements (13).

Table 1. Status of TCR loci in functional T-cell clones from leaky scid mice

	TCRβ		TCRγ			TCRδ	
Clone	Jβ1	Jβ2	JCγ1	JCγ2	JCγ4	Jδ1	Jδ2
1280	G, R	G, **R**	G, G	**R, R**	ND	–, –	–, –
1287	–, –	R, **R**	G, G	G, **R**	ND	–, –	–, –
7167	G, –	R, R	G, R	**R, R**	ND	–, –	–, –
1233	R, –	R, A	G, G	**R**, A	G, A	–, –	ND
1142*	A, –	**R**, A	A, A	R, R	ND	R, R	G, G

Isolation and characterization of the above T-cell clones was described previously (13). All clones are alloresponsive and $CD3^+$ unless indicated otherwise. The status of TCR β, TCR γ, and TCR δ alleles, as determined from Southern blot analysis (ref. 13; A.M.C., unpublished results), is indicated as follows: R denotes normal DJ, VJ, or VDJ rearrangement; inclusive of joins in and out of frame, **R** in boldface type denotes those alleles that have been sequenced and are shown in Fig. 1, A denotes aberrant rearrangement according to the criteria described in the text, G denotes germ-line configuration, dashes (–) denote deletion, and ND denotes that the status of the rearrangement has not been determined. β alleles inferred to be V to DJ rearrangements by the loss of sequences 5′ of the appropriate Dβ exon are underlined (A.M.C., unpublished results).
*Clone 1142 is nonalloresponsive and $CD3^-$.

DNA Sequences of Recombinant TCR Loci in T-Cell Clones from Leaky scid Mice. The TCR loci indicated in Table 1 with boldface type were molecularly cloned by PCR amplification, and DNA sequence analysis was carried out. Although the β gene rearrangement from the $CD3^-$ clone 1142 appeared to be normal by Southern blot analysis (see Table 1), it resulted in deletion of the Dβ1 and Jβ2.3 coding segments, with the breakpoints of the recombination falling 17 nucleotides upstream of Dβ1 and 12 nucleotides downstream of Jβ2.3 (resulting in a total deletion of 85 nucleotides). The DNA sequence of this rearrangement is shown in Fig. 1*a*. Deletions such as that seen in clone 1142 are typical of those reported in transformed cells from scid mice (1, 3, 6–9). Dβ to Jβ rearrangements from $CD3^+$ clones 1280 and 1287 were also cloned by PCR amplification. In contrast to the rearrangement from clone 1142, the TCR β rearrangements from clones 1280 and 1287 exhibit normal imprecision (including the insertion of non-germ-line-encoded nucleotides in 1287) at the junction of the Dβ and Jβ coding segments (see Fig. 1*a*).

DNA sequences from six of the seven Vγ1.2 to Jγ2 rearrangements contained in clones 1280, 1287, 7167, and 1233 are shown in Fig. 1*b*. In all cases, the V and J segments are joined with normal amounts of base loss and addition at the junction. Two of the characterized γ loci are in the correct reading frame and could encode a subunit of the expressed heterodimeric antigen receptor (Fig. 1*b*, 1280a and 1287). However, as these γ loci are derived from cells that have deleted their TCR δ loci, it is unlikely that they encode a subunit of the expressed T-cell receptor. In-frame joins of Vγ1.2 to Jγ2 have been observed previously in cells expressing α/β TCR molecules (24). We were unable to amplify sequences from either of the rearranged γ2 alleles in clone 1142, suggesting that these rearrangements are aberrant, as are the β locus rearrangements in this clone (ref. 13; this report). The Jγ2 primer is homologous to sequences 75 bp 3′ of the heptameric joining signal that flanks the Jγ2 coding exon. A deletion on the order of 100–200 bp would not be detectable by Southern blotting but would delete sequences recognized by the Jγ2 primer and would explain our inability to clone such rearrangements using PCR.

We observed the insertion of non-germ-line-encoded nucleotides in several of the loci analyzed. This activity is a feature of VDJ recombination in normal cells (25, 26) and it appears to be independent of the defective recombinase activity expressed in transformed scid cell lines, because

We are unable to detect any Jδ-hybridizing segments in clones 1280, 1287, and 7167 (data not shown). Presumably, both alleles of the TCR δ locus have been deleted by rearrangement of the TCR α locus. This strongly implies that these clones express α/β and not γ/δ TCR molecules.

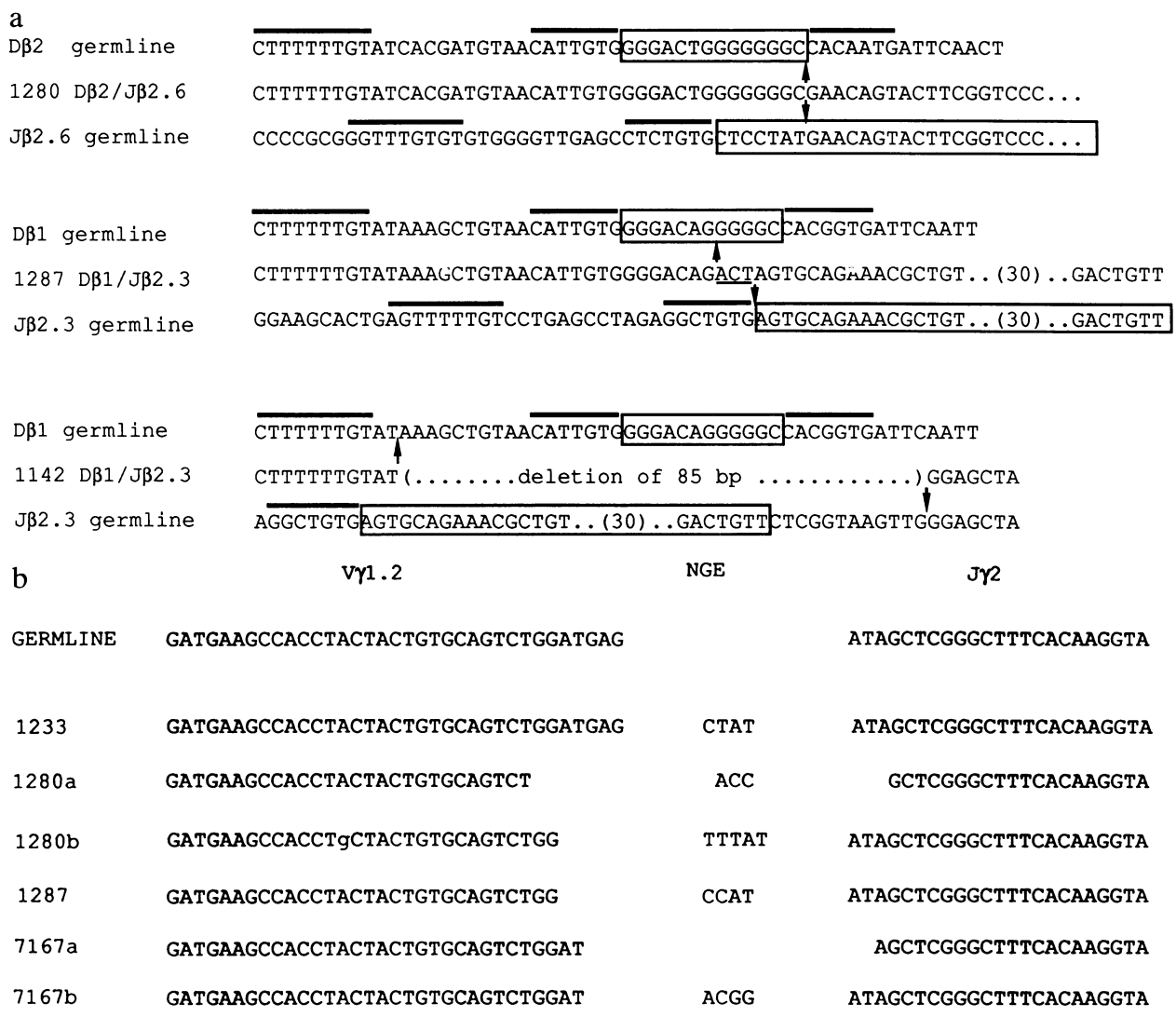

FIG. 1. DNA sequences of TCR gene rearrangements. (*a*) β locus rearrangements were molecularly cloned after two rounds of PCR amplification. Primers 1.2 kb upstream of Dβ1, or 500 base pairs (bp) upstream of Dβ2 (UDβ1.2 and UDβ2, respectively), were used in conjunction with primers homologous to sequences 75 bp 3′ of the last Jβ1 or Jβ2 coding exon. After 30 cycles of PCR, an aliquot of the reaction mixture was used in a Southern blot to determine the approximate size of the amplified fragment. Material in the appropriate size range was then isolated and subjected to secondary PCR using an alternative primer pair (as described in the text). Boxed nucleotides represent coding segments as delineated by Malissen *et al.* (19), and recombination signals are overlined. The sequence of the indicated locus is shown, with the appropriate germ-line Dβ and Jβ sequence given above and below (19, 22, 23). Point(s) of recombination are indicated by an arrowhead. (*b*) Rearrangements of Vγ1.2 to Jγ2 were molecularly cloned after 30 cycles of PCR amplification using primers 14 bp 3′ of the Jγ2 coding exon (Jγ2), and *ca.* 300 bp upstream of the 3′ border of Vγ1.2 (Vγ1.2 or DVγ1.2). Restriction sites were included in the PCR primers to facilitate subcloning. Sequences from the indicated clones are shown in comparison to the germ-line sequences (20, 21). Non-germ-line-encoded (NGE) nucleotides are indicated. The lowercase "g" in the sequence of 1280b appears to be a mutation, either somatically introduced or due to an artifact of PCR.

non-germ-line-encoded nucleotides have been observed in recombination junctions of scid immunoglobulin heavy chain genes (6) and in signal and hybrid joints of recombinant plasmid substrates recovered from recombinase-active scid cells (2).

DISCUSSION

Transfection of transformed scid cell lines with extrachromosomal recombination substrates (2) or recombinant retrovirus vectors (4) has shown that the scid VDJ recombinase activity can specifically recognize and join recombination signal sequences that flank V, D, and J coding elements but that it cannot efficiently join coding elements to form functional V(D)J genes. Studies with extrachromosomal recombination substrates have indicated that the frequency of coding joint formation is at least 1000-fold less than signal joint formation (2). Coding joints have been detected with recombinant retrovirus vectors (4, 27) and more recently in rearranged κ light chain genes of lipopolysaccharide-stimulated, recombinase-defective scid pre-B cells, transformed by Abelson murine leukemia virus; the frequency of wild-type κ rearrangements in the latter case is approximately 1 per 1000 cells (E. Hendrickson, M. Schlissel, and D. Weaver, personal communication). Normal coding joints also have been detected in some rearranged TCR loci of scid T-cell lymphomas (W. Schuler, M. Amsler, and M.J.B., unpublished results). These lymphomas typically contain multiple TCR gene rearrangements, which are grossly abnormal (1, 9).

At issue in the present study is whether the very low ability of the defective scid recombinase activity to form coding joints is sufficient to account for the pauciclonal B and T lymphocytes in leaky scid mice. Our results argue against this possibility, since three of the four alloreactive scid T-cell

clones (1280, 1287, and 7167) show reversion of the scid phenotype. Of the 12 rearranged TCR loci described in these clones, 3 correspond to the expressed β alleles, which are by definition normally assembled. The normal status of the remaining 9 loci (13) is confirmed in seven cases by the DNA sequences of Fig. 1. Given that the defective scid recombinase activity has a very low probability of forming coding joints, one would not expect to find coding joints at the unexpressed rearranged loci in addition to those at the expressed α and β loci. Therefore, we conclude that the recombinase activity expressed during the differentiation of clones 1280, 1287, and 7167 was qualitatively distinct from that of scid cells and resembled that of normal cells.

The one clone that might have arisen as a result of the low ability of the defective scid recombinase activity to form coding joints is clone 1233. This clone contains normally and abnormally rearranged TCR loci and therefore bears the imprint of a defective scid recombinase activity. However, the presence of a nonexpressed, but normally rearranged, γ allele (see Fig. 1*b*), in addition to an expressed β and α allele, raises the possibility that partial or complete reversion of the scid recombinase activity may have occurred during the ontogeny of the 1233 clone. Partial reversion might result in an increase in the frequency at which the defective recombinase mediates normal rearrangement but would not necessarily result in wild-type recombinase activity. Alternatively, complete reversion of the scid recombinase activity may have occurred during ongoing TCR gene arrangement and resulted in normal and abnormal rearrangements.

One way of directly testing for partial or complete reversion is to transfect selected recombinase-active scid pre-B clones (transformed by Abelson murine leukemia virus and positive for cytoplasmic μ chains) with extrachromosomal recombination substrates as described by Hesse *et al.* (28). To date, one of several such clones from leaky scid mice has been successfully transfected and shown to express a normal VDJ recombinase activity (D. Kotloff, N. Ruetsch, and M.J.B., unpublished results). This preliminary result adds support to our interpretation of T-cell clones 1280, 1287, and 7167 and indicates that reversion of the scid phenotype is possible in individual lymphocyte progenitors.

The expression of wild-type recombinase activity in a given lymphocyte progenitor could reflect reversion of one or the other *scid* allele; alternatively, it could reflect some genetic alteration other than that of the *scid* locus. Resolution of this issue will obviously require cloning the *scid* locus.

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