The Absolute Number of Trans-Rearrangements between the TCRG and TCRB Loci Is Predictive of Lymphoma Risk: A Severe Combined Immune Deficiency (SCID) Murine Model

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ABSTRACT

Pilot studies in human populations have demonstrated a correlation between the level of antigen receptor trans-rearrangements and risk (at the population level) of lymphoid malignancy. Irradiation of newborn severe combined immune deficiency mice results in an increased risk of subsequent development of thymic lymphoma (100% of mice so irradiated are dead of thymic lymphoma by 20 weeks of age). We, therefore, assayed the occurrence of trans-rearrangements in this well-controlled mouse mutant system and found a 50–100-fold increase in the absolute number of TCRG-TCRB trans-rearrangements compared to unirradiated littermates (and a comparable fold increase over age-matched BALB/c mice) at 2 weeks following irradiation. We also found a marked disproportion in generating trans-rearrangements versus intralocus rearrangements in the severe combined immune deficiency system compared to BALB/c, independent of irradiation. The trans-rearrangements noted were polyclonal in nature. These data, again, suggest that the absolute level of antigen receptor trans-rearrangements may serve as a biomarker of lymphoma risk.

INTRODUCTION

Cancer is a disease caused by genetic instability. One particular kind of "physiological" genetic instability that has been implicated in contributing to lymphoid malignancy involves the DNA breakage and rejoicing events that have evolved primarily for the purpose of formation of functional immunoglobulin and T-cell receptor genes (1). These rearrangements, mediated by a particular complex of enzymes, V(D)J recombinase, identify variable (V), diversity (D), and joining (J) segments from disparate parts of an antigen receptor locus and bring them into contiguity. These reactions, although usually described as occurring within a given immunoglobulin or T-cell receptor locus, can also occur between two different loci (trans-rearrangement; Refs. 2–5). We have developed a PCR-based assay for detecting interlocus trans-rearrangements between (rather than within) T-cell receptor loci (6, 7). We have shown previously that such trans-rearrangements occur in humans at levels high enough to be demonstrated in most individuals but at levels low enough to allow comparisons between individuals or populations. Such comparisons have suggested that the absolute level of trans-rearrangements in peripheral blood is correlated (on a population level) with the risk of that population to develop lymphoid malignancy. This correlation is found whether the population being studied is at increased risk because of an inherited predisposition (e.g., AT2) or an acquired exposure (e.g., exposure of farmers to pesticides and exposure of patients with Hodgkin's disease to chemotherapy; Refs. 7–9). These initial pilot studies in human populations await extension and verification by large, prospective, long-term population-based molecular epidemiological studies in which occupational and environmental exposures and ultimate health outcome can be more rigorously quantified.

If an assay of the level of these trans-rearrangements can serve as a biomarker of lymphoma risk, it is crucial to be able to verify, in a more defined experimental system, the relationship between the generation of trans-rearrangements and cancer. To test this, we have developed a murine model for the occurrence of these events. The murine system allows for greater control over the genetics and destabilizing factors that seem to contribute to variability in the propensity of an organism to trans-rearrange.

SCID mice carry a mutation (10) that causes impaired V(D)J recombination and a general defect in double-strand DNA break repair (11–13). This results in a lymphocytic developmental arrest, B- and T-cell immunodeficiency, and radiosensitivity. The gene targeted by the SCID mutation has been cloned (14, 15). It is the catalytic subunit of a DNA-dependent protein kinase.

In this article, we report our analysis of trans-rearrangements in the SCID mouse model. The components of the SCID phenotype that drew us to study trans-rearrangements in this system include the findings that, after sublethal doses of newborn irradiation, V(D)J recombination within the TCRB locus is restored transiently in SCID mice, but at a price, because all mice so irradiated are dead of thymic lymphoma by 20 weeks of age (16). We therefore asked whether the level of trans-rearrangements in SCID mice following newborn irradiation would act as a biomarker of cancer risk and thus validate, in an experimental system, our earlier pilot studies in human populations.

MATERIALS AND METHODS

Preparation of Animals, Including Irradiation and Sacrifice. BALB/c and BALB/c SCID or CB-17 SCID mice were bred either in the Hospital for Sick Children animal facilities (Toronto, Ontario, Canada) or in the National Cancer Institute Frederick Cancer Research Facility (Frederick, MD). Irradiation of mice was performed as described previously (16).

DNA Extraction. Thymuses from the irradiated and unirradiated animals were isolated: a single-cell suspension of thymocytes was made, and DNA was extracted from these thymocytes by the "salting out" technique (17) or as described elsewhere (16). The DNA was resuspended overnight at 37°C and then sheared by 10 passages through a 26-gauge needle.

Nested PCR. To assay for recombinations within and between TCRG and TCRB loci, nested PCR was performed as a modification of methods described previously (16). Briefly, in the first amplification, limiting dilutions of DNA samples (from 1000 ng of genomic DNA, approximately 1.5 × 105 cell equivalents, down to 0.01 ng, approximately 1.5 cell equivalents) were suspended in a 50-μl solution containing 200 μM deoxynucleotides, 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM MgCl2, 100 μg/ml gelatin, the "a" set of primers (Table 1 and Fig. 1) at 100 ng each, and 1.25 units of Taq polymerase. The reaction was carried out at 95°C for 4 min for initial denaturation followed by 30 cycles of amplification consisting of 95°C for 15 s, 55°C for 15 s, and 72°C for 30 s plus a 6-s increase per extension cycle. After 30 cycles, 10 min at 72°C for elongation were allowed, and then 10% of the first amplification reaction

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The abbreviations used are: AT, ataxia-telangiectasia; SCID, severe combined immune deficiency; TCR, T cell receptor.

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2 The abbreviations used are: AT, ataxia-telangiectasia; SCID, severe combined immune deficiency; TCR, T cell receptor.
was nested using the same conditions with a “b” set of primers at 300 ng each. Nested PCR-generated products were resolved by 1.5% agarose gel electrophoresis. Southern blot transfer to Nytran (Schleicher & Schuell) membranes was performed followed by hybridization to α-32P end-labeled oligonucleotide probes internal to the amplification primer sequences of these loci.

For the intralocus rearrangements of the TCRG locus, primers were derived from the TCRGVSJI coding region (TCRGVS1Ja and TCRGVS1Jb; Ref. 18) and from the consensus sequences from the TCRGIJ1 and TCRGJ2 segments (TCRGJ1Ja and TCRGJ1Jb; Table 1; Ref. 19). A positive product was defined as one that hybridized to probes (“c” primers) from both sides of the rearrangement (e.g., TCRGV3S1c and TCRGV3Jc had to cohybridize to the same fragment). The germ-line diversity of the TCRG locus is quite limited, consisting of seven V segments in total (20). The primers chosen for the TCRGV amplification are homologous to one of these V segments. Thus, our TCRGVJ analysis is likely to measure a fraction of all of the TCRGVJ rearrangements in the population. There are 20 potential TCRBV segments (21), but our primers are homologous to only one of these, so that our TCRBVJ analysis is likely to have identified a smaller fraction of all of the TCRBVJ rearrangements that were present in the thymus. For the intralocus rearrangements of the TCRB locus, the specific pairs of primers and probes were from the TCRBV5S11 coding region (TCRBV5S1a and TCRBV5S1b for amplification and TCRBV5S1c as a probe; Ref. 18) and from the germ-line 3’ portion of the TCRBJ2S1-S7 cluster (TCRBJ2a and TCRBJ2b for amplification and TCRBJ2c as a probe; Ref. 22; Table 1).

For the trans-rearrangements, the same sets of primers for the TCRGV3S11 region of the TCRG locus and for the TCRBJ2J regions of the TCRB locus were used; again, the PCR products were of predicted sizes based on the specific J segments used. They were hybridized with the appropriate TCRGV3S1c and TCRBJ2c probes internal to the nesting set (Fig. 1). Every sample was subjected to at least two PCR-based dilutional analyses to verify the reproducibility of the assays. Samples were also checked for their comparable ability to amplify a genomic DNA dilution for a single copy gene.

Quantitation of Intralocus Rearrangements and Trans-Rearrangements and Statistical Analysis. For explanations of quantitation of intralocus rearrangements and trans-rearrangements, see legends to Tables 2 and 3. All analyses were performed using the Wilcoxon rank sum test, using exact permutational Ps. All Ps are two-sided.

sequencing of Trans-Rearrangements. One-third of the PCR products were run on a 1.5% agarose gel and purified with a QIAquick Extraction Kit (Qiagen, Chatsworth, CA), and then 2 ng of the purified PCR product were sequenced directly by PCR [fmol DNA Sequencing System (Promega, Madison, WI)] using a [γ-32P] end-labeled TCRGV3S1b primer.

RESULTS

There is an Absolute Increase in the Level of Trans-Rearrangements following Irradiation of Newborn SCID Mice. The levels of TCRGV-TCRBJ trans-rearrangements were determined in DNA extracted from thymocytes from 1–3-week-old littermate SCID mice, some of which had received 100 cGy of ionizing radiation within the first 48 h of life. Intralocus TCRGVJ and TCRBJVJ rearrangements were determined on the same samples. Unirradiated BALB/c mice of corresponding age were included in this study for comparison. The results of this analysis, displayed as the median of all of the mice of a given group tested, are presented in Table 2. The median number of TCRGV-TCRBJ trans-rearrangements in 13 irradiated SCID mice was 100 per 106 thymocytes. There was, on average, a 50–100-fold increase in the absolute number of trans-rearrangements in the irradiated SCID mice compared to their unirradiated littermates (P < 0.006).

SCID mice have been produced that lack a component of the V(DJ) recombinase complex (23). In such Rag2−/−/SCID mice, irradiation during the newborn period results in thymic hyperplasia but no evidence of TCRB gene rearrangement. Such mice do not suffer from the markedly elevated incidence of lymphoma that is seen in their Rag2−/− counterparts. We analyzed thymocytes from three Rag2−/−/SCID mice irradiated during the newborn period. No trans-rearrangements were detected (Table 2).

In SCID Mice, the Ratio of Trans-Rearrangements:Intralocus Rearrangements Is Skewed Regardless of Irradiation Status. Our hypothesis is that trans-rearrangements do not stand alone but rather are a reflection of some aspect of the activity or accessibility of the involved antigen receptor loci. We therefore explored our findings with reference to the activity of the relevant intralocus rearrangements, reported previously to be increased following newborn irradiation. To quantitate this, we expressed the level of trans-rearrangements as a function of the frequency of the relevant intralocus rearrangements (Table 3). In so doing, we found that there is a marked predisposition to trans-rearrange in SCID mice compared to BALB/c mice. Furthermore, this ratio of trans-rearrangements to intralocus rearrangements was abnormal in SCID mice regardless of whether or not they had been irradiated (P < 0.002).

In thymocytes from BALB/c mice of comparable age to the SCID mice studied, there is approximately one TCRGV-TCRBJ trans-rearrangement for every 50,000 TCRGVJ intralocus rearrangements noted (Table 3). This is quite comparable to the ratio of trans-rearrangements:intralocus rearrangements noted by us and others in human peripheral blood mononuclear cells (5, 7–9, 24, 25). In contrast, the number of TCRGV-TCRBJ trans-rearrangements compared to TCRGVJ intralocus rearrangements is approximately 100–500-fold higher in SCID mice than in BALB/c mice whether the SCID mice have been irradiated or not. There is a comparable 100-fold increase in the ratio of TCRGV-TCRBJ trans-rearrangements compared to TCRBVJ intralocus rearrangements.

The Absolute Number of Trans-Rearrangements Involving the TCRD Locus Is Not Increased following Irradiation of Newborn SCID Mice. We determined the level of other combinations of trans-rearrangements in the SCID system and in BALB/c mice using primer sets analogous to those we have described for our study of TCRGV-TCRBJ rearrangement (see listing of these primers in the legend to Table 3). Trans-rearrangements involving the TCRD locus occur at a low level in the SCID and BALB/c backgrounds (Table 4). Despite evidence of TCRD activation (26, 27), the absolute number of trans-rearrangements involving TCRD does not increase significantly with the stimulus to thymic proliferation supplied by 100 cGy of ionizing radiation.

SCID Mice Generate TCRGV-TCRBJ and TCRGV-TCRDJ Trans-Rearrangements That Are Indistinguishable from Those Generated in the BALB/c Background. Because of the defects in V(DJ) recombination that have been described as characteristic of the SCID mouse mutant (11, 12), we were interested in examining, at the level of genomic DNA sequence, the particular trans-rearrangements for which we were assaying. Because of the primers used in our study (see "Materials and Methods") and the criteria used for calling a PCR
Chromosome 6

The number of intralocus rearrangements and trans-rearrangements is expressed as the number occurring per 1.5 × 10^6 cells (approximately 1 μg of DNA). Each data point is expressed as the reciprocal of the highest dilution of DNA yielding an amplifiable product. For example, if a sample yields an amplifiable fragment at a 1:1000 dilution (1 ng of DNA) that hybridizes to probes from both the TCRG and TCRB loci, then the frequency of trans-rearrangements in the sample (per 1.5 × 10^6 cells) is 1000. For each group of mice (BALB/c, irradiated BALB/c, SCID, and irradiated SCID), we calculated the median frequency based on the individual frequencies for each member of the group (n, number of animals). Beside each median value is listed the interquartile range.

Comparing the absolute number of intralocus rearrangements (TCRBVJ or TCRGVJ) between SCID and SCID 100R, P < 0.001; comparing the absolute number of trans-rearrangements (TCRGV-TCRBJ) between SCID and SCID 100R, P < 0.006; comparing the absolute number of trans-rearrangements (TCRGV-TCRBJ) between SCID 100R and BALB/c, P < 0.02.

*p = 0.13.

**ND, not done.

<table>
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<th>TCRBVJ</th>
<th>TCRG</th>
<th>TCRG-TCRBJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCID (n = 7)</td>
<td>10 (1–11.375)</td>
<td>550 (475.5–575)</td>
</tr>
<tr>
<td>SCID 100R (n = 12)</td>
<td>550 (100–1375)</td>
<td>10,000 (2000–10,000)</td>
</tr>
<tr>
<td>BALB/c (n = 7)</td>
<td>1000 (1000–1250)</td>
<td>100,000 (7750–100,000)</td>
</tr>
<tr>
<td>BALB/c 100R (n = 5)</td>
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<td>ND</td>
</tr>
<tr>
<td>Rag2−/−/SCID 100R (N = 3)</td>
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<td>0</td>
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</table>

The absolute number of intralocus rearrangements and trans-rearrangements is expressed as the number occurring per 1.5 × 10^6 cells (approximately 1 μg of DNA). Each data point is expressed as the reciprocal of the highest dilution of DNA yielding an amplifiable product. For example, if a sample yields an amplifiable fragment at a 1:1000 dilution (1 ng of DNA) that hybridizes to probes from both the TCRG and TCRB loci, then the frequency of trans-rearrangements in the sample (per 1.5 × 10^6 cells) is 1000. For each group of mice (BALB/c, irradiated BALB/c, SCID, and irradiated SCID), we calculated the median frequency based on the individual frequencies for each member of the group (n, number of animals). Beside each median value is listed the interquartile range.

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*p = 0.13.

**ND, not done.

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<td>550 (475.5–575)</td>
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<tr>
<td>SCID 100R (n = 12)</td>
<td>550 (100–1375)</td>
<td>10,000 (2000–10,000)</td>
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<tr>
<td>BALB/c (n = 7)</td>
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<td>100,000 (7750–100,000)</td>
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<tr>
<td>BALB/c 100R (n = 5)</td>
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<td>Rag2−/−/SCID 100R (N = 3)</td>
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<td>0</td>
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</table>

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Comparing the absolute number of intralocus rearrangements (TCRBVJ or TCRGVJ) between SCID and SCID 100R, P < 0.001; comparing the absolute number of trans-rearrangements (TCRGV-TCRBJ) between SCID and SCID 100R, P < 0.006; comparing the absolute number of trans-rearrangements (TCRGV-TCRBJ) between SCID 100R and BALB/c, P < 0.02.

*p = 0.13.

**ND, not done.

a Ratios of trans-rearrangements to relevant intralocus rearrangements were obtained by dividing the median frequencies of the trans-rearrangements by the median frequencies of the intralocus rearrangements (if the results are expressed, instead, by performing the calculation in a different sequence of steps, i.e., by computing the trans-rearrangement: intralocus ratio for each individual mouse and then defining a median ratio for the different groups, the results are essentially the same). Comparing the ratio of trans-rearrangements (TCRGV-TCRBJ) to intralocus rearrangements (TCRBVJ or TCRGVJ) between BALB/c and SCID, whether irradiated or not, P < 0.002.
TCRG-TCRB trans-rearrangements in SCID mice

DISCUSSION

The absolute number of TCRGV-TCRBJ trans-rearrangements shows a 50-100-fold increase in newborn SCID mice exposed to 100 cGy of irradiation within 2 weeks of this destabilizing exposure compared to unirradiated SCID or age-matched BALB/c control mice. Such irradiated mice have been shown previously to be remarkably predisposed to cancer, with 100% succumbing to thymic lymphoma by 5 months of age (16). The lymphoma risk of unirradiated SCID mice is significantly lower than the 100% lymphoma mortality by 20 weeks of age seen in irradiated newborn SCID mice (29-32). Low-level irradiation of BALB/c mice does not markedly increase lymphoma risk (33); nor does it cause a marked increase in the absolute number of trans-rearrangements. Thus, in the SCID murine model, as in the previous pilot studies in human populations, an assay of the absolute number of a particular trans-rearrangement is predictive of risk of lymphoid malignancy. As in the studies cited previously, the increase in trans-rearrangements is polyclonal in nature. Thus, we are not observing a single premalignant clonal proliferation but, rather, an absolute increased frequency of rearrangement between antigen receptor loci. There is no evidence that cells carrying trans-rearrangements are at a selective advantage in SCID because of the trans-rearrangement. There might be some evolutionary idiotypic selection of a given intralocus rearrangement over a trans-rearrangement, but the frequency in-frame versus out-of-frame trans-rearrangements (of 13 TCRGV-TCRB trans-rearrangement products sequenced at the genomic level following newborn SCID irradiation, 8 maintained an open reading frame) suggests that they are either treated identically to intralocus rearrangements or are at a slight disadvantage. In-frame genomic TCRB intralocus rearrangements in the thymus have been reported to be as high as 75% (34, 35). The finding of a fraction of in-frame trans-rearrangements in the SCID model is similar to our previous sequence analyses of trans-rearrangements in the peripheral blood of patients with AT or non-AT controls (7) and in studies by others of thymus from "normal" humans (24). With regard to functional selection of mature T cells, we have looked at CD4⁺CD8⁺ double-positive thymocytes compared to CD4⁺CD8⁻ and CD4⁻CD8⁺ thymocytes from BALB/c mice and do not find any significant difference in the frequency of TCRGV-TCRBJ trans-rear-

Table 4 Absolute number of trans-rearrangements involving the TCRD locus per $1.5 \times 10^6$ thymocytes

<table>
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<tr>
<th>Mouse code/type</th>
<th>TCRGV-TCRDJ</th>
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<th>TCRDV-TCRDJ</th>
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<tr>
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<td>0 (n = 7)</td>
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<td>SCID</td>
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<td>0.3 (n = 6)</td>
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<tr>
<td>SCID 100R</td>
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<td>0.3 (n = 12)</td>
<td>0 (n = 7)</td>
<td>1.4 (n = 9)</td>
</tr>
<tr>
<td>SCID 100R</td>
<td>0 (n = 11)</td>
<td>0.3 (n = 12)</td>
<td>0 (n = 7)</td>
<td>1.4 (n = 9)</td>
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</tbody>
</table>

*a These trans-rearrangements were detected using the same primers for the TCRB and TCRD loci. For TCRDJ, the primer sequences were: for TCRDJia, OCT TAC TCA ACA AGCTTTATAGC TTT GACTAGTCAA AAC ACCTTG; for TCRDJib, TAC TTC CAA CCT TT TAG GT (48); for TCRDJic, TT CCA CAG TCA CTT GGG TTC (49); for TCRDV7.3a, CAG TFC ATC CCA CAG TCA CIT GGG 'FTC (50). Quantitation of these trans-rearrangements was performed as described in Table 2, n, number of animals.

Not detectable.

Table 5 Junctional sequences of TCRGV-TCRBJ and TCRGV-TCRDJ trans-rearrangements: P (palindromic), N (nontemplated), and D (diversity) sequences

<table>
<thead>
<tr>
<th>Mouse code/type</th>
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<td>TCC TAC GGC TAA AG</td>
<td>T AAC TAT GCT GA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Trans-rearrangements sequences between TCRG and TCRD

<table>
<thead>
<tr>
<th>Mouse code/type</th>
<th>Sequence</th>
<th>TCRGV-TCRDJ</th>
<th>TCRGV-TCRDJ</th>
<th>TCRGV-TCRDJ</th>
<th>TCRGV-TCRDJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germ line</td>
<td>TCRGV1S1</td>
<td>TCC TAC GGC TAA AG</td>
<td>CT ACC GAC AAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germ line</td>
<td>TCRDJ1</td>
<td>TCC TAC GGC TAA AG</td>
<td>CT ACC GAC AAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>77B/SCID 100R</td>
<td>2</td>
<td>TCC TAC GGC T</td>
<td>CT ACC GAC AAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>84/CSCID 100R</td>
<td>4A</td>
<td>TCC TAC GGC C</td>
<td>CT ACC GAC AAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>67A/SCID 100R</td>
<td>9B</td>
<td>TCC TAC GGC T</td>
<td>GAC AAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G10/SCID 100R</td>
<td>3A</td>
<td>TCC TAC GGC T</td>
<td>GAC AAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>84A/SCID 100R</td>
<td>1A</td>
<td>TCC TAC GGC T</td>
<td>C TAT GAA CAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>84A/SCID 100R</td>
<td>1B</td>
<td>TCC TAC GGC T</td>
<td>C TAT GAA CAG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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rangements in any of these populations (data not shown). Thus, the absolute number of trans-rearrangements are most consistent with a measurement of a kind of genetic instability, not a particular process of selection of trans-rearrangements or trans-rearrangement idiotypes.

We initially approached the SCID mouse model thinking that the level of trans-rearrangements might be vanishingly low because the level of intralocus rearrangements was known to be diminished markedly (1, 36). We were thinking that, as the level of TCRB-DJ rearrangements increased following newborn irradiation, there would be a related stimulus to trans-rearrangement which would provide us with (a) a means of studying the necessary and sufficient features for the occurrence of trans-rearrangements and (b) a system for assessing the destabilizing effects of a variety of biological and chemical agents. We were surprised to discover that trans-rearrangements were easily observable in SCID mice without irradiation, in spite of the relatively low level of intralocus rearrangements. This suggests to us that there is some fundamental difference between the SCID mutant and wild-type mice that makes trans-rearrangements more likely to occur in SCID. This increased tendency to trans-rearrange in SCID mice is not “reversed” by low-dose radiation in the newborn period, despite the evidence that such irradiation transiently releases the block to intralocus V(DJ) recombination of the TCR and TCRB loci and is accompanied by a wave of T-cell proliferation and differentiation within the thymus (16). DNA hairpin formation at coding ends of D and J sequences have been demonstrated in the SCID system (37–39). These hairpin ends are believed to be intermediates in the V(DJ) recombination process, which are quickly resolved in non-SCID animals. One possibility is that the persistence of these potentially reactive intermediates in the SCID mice is a factor in their predisposition to develop trans-rearrangements (and lymphoma). If these intermediates were subject to intranuclear “drift,” it might make more likely the possibility of interaction with more distant and noncontiguous loci once the release of the hairpin occurred (possibly as a direct or indirect result of irradiation; Ref. 26). This would increase the frequency of antigen receptor trans-rearrangements as well as translocations involving potential growth-dysregulating genes that could contribute directly to malignant transformation. In the absence of an essential component of the recombinein complex (Rag2−/−/SCID), the V(DJ) rearrangement process is not initiated, there are no trans-rearrangements formed, and there is no increased lymphoma risk in the SCID animals.

In addition to the TCRG–TCRB trans-rearrangement, we have studied, in analogous assays, other trans-rearrangement combinations among the TCR loci. In the unirradiated SCID animals, every type of trans-rearrangement is, in general, increased disproportionately compared to relevant intralocus VJ rearrangement. However, not all trans-rearrangements show the remarkable increase in absolute number following newborn irradiation. Trans-rearrangements in which the TCRD locus is a partner do not show an increased frequency following the 100 cGy stimulus. The TCRD locus has been reported to be the most active in the unirradiated SCID mouse, with a relatively large number of TCRDDJ rearrangements occurring. Following irradiation, Southern blot analyses by us (data not shown) or other investigators (26, 27) demonstrate TCRD locus rearrangement and also activation of the TCRα locus (as manifested by DNA hairpin formation at TCRαI coding ends (26, 27). These data as well as earlier reports (40, 41) suggest that the TCRD locus, embedded within TCRα, is excised from the chromosome at the time of TCRα activation. Thus, it is possible that an explanation for the decreased participation of TCRD in trans-rearrangement reflects either the timing of its rearrangement in the SCID system (activated prior to activation of other loci), its disposition in the cell (extrachromosomal at the time of maximal TCRB or TCRG chromosomal rearrangement), or both.

The two conditions (AT in humans and SCID in mice), in which we observed the most dramatic correlation between increase of trans-rearrangements and risk for lymphoid tumors, are inherited syndromes associated with mutations in genes (ATM and DNA-PKcs, respectively) that share a COOH-terminal phosphatidylinositol 3-kinase motif (42–44). The family of genes that share this motif include a number with protein kinase activity that can be stimulated by the presence of double-stranded DNA ends (44). The sharing of this motif by the AT and SCID gene products suggests a connection between DNA break repair and propensity to V(DJ) recombinase-mediated trans-rearrangement.

In summary, TCRGV–TCRB is a possible biomarker for population risk of lymphoid malignancy in the SCID mouse mutant system. As in the human populations that we have studied, the absolute number of such trans-rearrangements appears to be proportional to lymphoma risk. As a marker of lymphoma risk it is not fortuitous that trans-rearrangements show such a correlation, because the mechanism that causes their formation, V(DJ) recombination, is at least in part the same mechanism that causes the majority of malevolent translocations associated with lymphoid malignancy (45–47). This murine model might serve eventually as a screen for lymphomagenic agents (or conversely for agents that are protective against lymphomagenic insults). The model also appears relevant to considerations of the structural and physiological requirements of V(DJ) recombination. Moreover, this model may allow for a dissection of critical themes and features that promote chromosomal aberrations, a particularly dramatic form of genetic instability associated with malignant transformation.

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The Absolute Number of Trans-Rearrangements between the 
\( TCRG \) and \( TCRB \) Loci Is Predictive of Lymphoma Risk: A Severe Combined Immune Deficiency (SCID) Murine Model

Florigio Lista, Virginia Bertness, Cynthia J. Guidos, et al.


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