Recombinase-activating gene (RAG) 2-mediated V(D)J recombination is not essential for tumorigenesis in Atm-deficient mice


*Experimental Immunology Branch, †Genetics Department, and ‡Biostatistics and Data Management Section, Office of the Director of the Division of Clinical Sciences, National Cancer Institute; †Genetic Disease Research Branch, National Human Genome Research Institute; **Veterinary Resources Program, Office of Research Services; ¶National Institute on Aging, National Institutes of Health, Bethesda, MD 20892; and ¶¶Howard Hughes Medical Institute, National Institutes of Health Research Scholars Program, One Cloister Court, Bethesda, MD 20892

Edited by Michael Potter, National Institutes of Health, Bethesda, MD, and approved March 20, 2000 (received for review December 28, 1999)

The majority of Atm-deficient mice die of malignant thymic lymphoma by 4–5 mo of age. Cytogenetic abnormalities in these tumors are consistently identified within the Tcrαδ locus, suggesting that tumorigenesis is secondary to aberrant responses to double-stranded DNA breaks that occur during V(D)J recombination. Since V(D)J recombination is a recombinase-activating gene (RAG)-dependent process, we generated Rag2−/−Atm−/− mice to assess the requirement for RAG-dependent recombination in thymic lymphomagenesis. In contrast to expectation, the data presented here indicate that development of malignant thymic lymphoma in Atm−/− mice is not prevented by loss of RAG-2 and is therefore not dependent on V(D)J recombination. Malignant thymic lymphomas in Rag2−/−Atm−/− mice occurred at a lower frequency and with a longer latency as compared with Atm−/− mice. Importantly, cytogenetic analysis of these tumors indicated that multiple chromosomal abnormalities occurred in each tumor, but that none of these involved the Tcrαδ locus. Nonmalignant peripheral T cells from TCR-transgenic Rag2−/−Atm−/− mice also revealed a substantial increase in translocation frequency, suggesting that these translocations are early events in the process of tumorigenesis. These data are consistent with the hypothesis that the major mechanism of tumorigenesis in Atm−/− mice is via chromosomal translocations and other abnormalities that are secondary to aberrant responses to double-stranded DNA breaks. Furthermore, these data suggest that V(D)J recombination is a critical, but not essential, event during which Atm-deficient thymocytes are susceptible to developing chromosome aberrations that predispose to malignant transformation.

Ataxia telangiectasia (AT) is an autosomal recessive disease characterized by ataxia, ocular telangiectasias, humoral and cellular immunodeficiencies, increased incidence of cancers, and increased sensitivity to ionizing radiation (reviewed in ref. 1). The genetic defect causing this disease is mutation of the gene encoding the AT protein (2). AT appears to play an important role in cellular responses to the presence of double-stranded DNA (dsDNA) breaks, probably due to its function in cell cycle checkpoint control (3). Several components of the AT phenotype, such as sensitivity to ionizing radiation, infertility, immunodeficiency, and lymphoreticular malignancies with characteristics translocations, plausibly result from aberrant responses to dsDNA breaks. More specifically, it has been suggested that immunodeficiency and the increased incidence of lymphoid cancers seen in this disease are secondary to aberrant recognition and repair of dsDNA breaks that normally occur during V(D)J recombination and isotype switching in lymphoid cells (3, 4).

Support for this hypothesis comes from studies of the murine model of AT. It has been observed that the majority of Atm−/− mice die from malignant thymic lymphoma by 4–5 mo of age (5–7). The initial characterization of Atm−/− mice demonstrated that these lymphomas displayed translocations detected by spectral karyotyping (SKY) (5), and recent analysis demonstrated that 100% of thymic lymphomas from Atm−/− mice had translocations in chromosome 14. Notably, these translocations were shown by fluorescent in situ hybridization (FISH) to occur consistently within both alleles of the T cell receptor (Tcr) αδ locus (M. Liayanage, C.B., Z.W., A. Coleman, D. G. Pankratz, S. Anderson, A.W.-B., and T.R., unpublished work). The consistent involvement of the Tcrαδ locus suggests a mechanism of tumorigenesis in which dsDNA breaks occurring within this locus during V(D)J recombination are not efficiently handled in Atm-deficient cells during T cell development. The dsDNA breaks and increased chromosomal instability typically seen in AT (reviewed in ref. 1) predispose to development of malignant thymic lymphoma.

In a recent study designed to test this hypothesis, Liao and Van Dyke (8) used the fact that V(D)J recombination is a recombinase-activating gene (RAG)-dependent process and generated Rag1−/−Atm−/− mice to assess tumor incidence. If tumorigenesis is secondary to faulty V(D)J recombination, no tumors would be expected to develop in these double knockout mice. Consistent with this hypothesis, development of malignant thymic lymphoma did not occur in nine Rag1−/−Atm−/− mice, whereas five of five Rag1−/−Atm−/− control mice died of malignant thymic lymphomas by 7 mo of age.

We had independently initiated a similar series of experiments in which we generated Rag2−/−Atm−/− mice for assessment of tumor susceptibility. Surprisingly, in contrast to expectation and the results reported by Liao and Van Dyke (8), we observed development of malignant thymic lymphomas in Rag2−/−Atm−/− mice, although at a lower frequency and with a longer latency than in Atm−/− mice. Strikingly, cytogenetic evaluation of these Rag2−/−Atm−/− thymic lymphomas revealed an absence of the Tcrαδ locus translocations that were seen in lymphomas from Atm−/− mice with intact RAG function. These findings indicate that rearrangement of the Tcrαδ locus is a critical, but not...
essential, event during which Atm-deficient thymocytes are susceptible to developing translocations that predispose to malignancy. Furthermore, the data indicate that the role of ATM is not limited to recognition and repair of dsDNA breaks initiated by V(DJ) recombination, but rather that ATM functions more broadly in predisposing to translocation events and tumorogenesis.

Materials and Methods

Flow Cytometric Analysis. The following conjugated antibodies were used for three-color flow cytometric analysis: anti-CD8 FITC, anti-Thy 1.2 FITC, anti-CD4 phycoerythrin (Becton Dickinson Immunocytochemistry Systems), anti-CD3 FITC, anti-B220 phycoerythrin, anti-H57 biotin, and anti-V88 biotin (PharMingen). Anti-Leu-4 FITC, anti-Leu-4 phycorythrin, and anti-Leu-4 biotin (Becton Dickinson Immunocytochemistry Systems) were used as negative staining controls. Biotin-conjugated antibodies were revealed with streptavidin-CyChrome (PharMingen). Samples were analyzed on a FACScan (Becton Dickinson Immunocytochemistry Systems) using CellQuest software.

Breeding and Typing Mice. The creation of Atm-deficient mice (allele designation Atmins5790neo) has been described previously (5). Progeny of heterozygote matings were genotyped by PCR (9). Rag2-deficient mice (10) were identified phenotypically by flow cytometric analysis of peripheral blood lymphocytes as described above. Rag2+/− cells were CD4−CD8−B220−; Rag2− cells were CD4+, CD8+, or B220+. To generate mice deficient for both Rag2 and Atm, double heterozygotes were crossed and progeny were typed as described above. To create HyTcrTg+Rag2−/−Atm+/− mice, HyTcrTg+ mice (11) were first crossed with Rag2−/−Atm+/− mice. These mice were typed for the presence of transgene by flow cytometric analysis as described above. Peripheral blood lymphocytes from HyTcrTg+Rag2−/−Atm−/− mice, HyTcrTg+ mice (11) were first crossed with Rag2−/−Atm+/− mice. These mice were typed for the presence of transgene by flow cytometric analysis as described above. Peripheral blood lymphocytes from HyTcrTg+Rag2−/−Atm−/− mice were B220−, but had a population of Thy 1.2+V88− cells, whereas peripheral blood lymphocytes from HyTcrTg+Rag2−/−Atm−/− mice were Thy 1.2−B220+. HyTcrTg+Rag2−/−Atm−/−mice were then crossed with HyTcrTg+Rag2−/−Atm−/− mice.

Tumor Incidence Studies and Histopathological Analysis. Mice were examined daily for evidence of morbidity or mortality. Mice were killed when death was judged to be imminent; thoracic and abdominal organs were removed en bloc and preserved in 10% neutral buffered Formalin (Sigma) for necropsy. Organs from mice that were found dead were similarly preserved. Fixed tissues were embedded in paraffin blocks, sectioned, and stained with hematoxylin and eosin by American Histology Labs (12), and sections were examined by light microscopy.

Statistical Analysis. Survival time of mice was calculated from date of birth until date of death or date last known alive. All animals in which death was felt to be imminent were killed, and the underlying morbidity was recorded as the cause of mortality. Kaplan–Meier survival curves were generated separately for death due to malignant thymic lymphoma and death due to malignancy other than malignant thymic lymphoma (13). Animals were considered to have failed in the analysis of a given end point if a diagnosis of that end point was made; animals that died due to a cause other than that end point had their follow-up time censored in that curve at the date of death. Three mice were excluded from analysis because their survival durations were unknown. The statistical significance of differences between pairs of Kaplan–Meier curves, each representing a different group of animals, was determined by the Mantel–Haenszel method (14). Individual, unadjusted P values are reported and all were determined to indicate statistical significance at the 0.05 level in view of the multiple comparisons being made according to the method of Hochberg (15). All P values are two-tailed.

Thymic Lymphoma Cell Culture. Thymic lymphomas were harvested and single-cell suspensions were cultured in RPMI 1640 (Bio-Whittaker) supplemented with 15% FCS (Biofluids, Rockville, MD), sodium pyruvate (1%), nonessential amino acids (1%), l-glutamine (0.5%), 2-mercaptoethanol (5 × 10−5 M), penicillin (0.5%), streptomycin (0.5%), and either 20 IU/ml human recombinant IL-2 (Atm−/− lymphomas) or 100 IU/ml human recombinant IL-2 and 6 ng/ml rIL-7 (Rag2−/−Atm−/− lymphomas).

SKY and FISH. Metaphase chromosomes were prepared from tumor cell lines at early passage numbers (passages 0–2). Cells were incubated in 0.1 μg/ml Colcemid (GIBCO/BRL) for 30–60 min and then lysed in 0.075 M KCl. Chromosomes were fixed in 3:1 methanol/acetic acid and dropped onto glass slides. Thymocyte chromosomes were prepared by culturing single-cell thymocyte suspensions for 2 h in 0.1 μg/ml Colcemid and then lysing and fixing as described above. Splenocyte chromosomes were prepared from splenocytes cultured for 48 h in RPMI 1640 + 20% FCS with 6 μg/ml concanavalin A to preferentially stimulate T cells. After an additional 30-min incubation in 0.1 μg/ml Colcemid, cells were harvested and metaphase chromosomes were prepared as described. SKY of the tumor cell lines was performed as described previously (16, 17). Raw spectral images were visualized by assigning red, green, or blue colors to specific spectral ranges. Chromosomes were then unambiguously identified using a spectral classification algorithm that results in the assignment of a separate classification color to all pixels with identical spectra by use of SKy View software (Applied Spectral Imaging). aberrations were defined using the nomenclature rules from the Committee on Standardized Genetic Nomenclature for Mice (see the Mouse Genome Informatics web site at http://www.informatics.jax.org). Six to 10 metaphases were analyzed for each tumor, and 10–12 metaphases were analyzed for each splenocyte or thymocyte preparation. Bacterial artificial chromosome (BAC) clones for FISH analysis were labeled by nick-translation with biotin-dUTP (Cα) and Spectrum Orange-dUTP (Vα and Cδ), whereas the whole chromosome 14 paint was labeled with digoxin-dUTP and detected with mouse anti-digoxin followed by sheep anti-mouse IgG5.5. FISH results were imaged and analyzed using QFISH software (Leica, Cambridge, U.K.).

Results

Incidence of Malignant Thymic Lymphomas in Mice Deficient in Both ATM and RAG-2. To determine whether tumorogenesis in Atm−/− mice is dependent on V(DJ) recombination, we intercrossed Rag2−/−Atm−/− mice to generate Rag2−/−Atm−/− mice and littermate controls as described in Materials and Methods. Kaplan–Meier analysis of tumor-free survival was determined. If tumorogenesis is secondary to aberrant recognition of dsDNA breaks that occur during V(DJ) recombination of the Tcr α/δ loci, it would be expected that preventing V(DJ) recombination by deleting the Rag2 gene would prevent the development of malignant thymic lymphoma in Rag2−/−Atm−/− mice.

In contrast to this prediction, however, Rag2−/−Atm−/− mice did in fact develop malignant thymic lymphomas (Fig. 1). Of 24 Rag2−/−Atm−/− mice followed, 6 (25%) were diagnosed with malignant thymic lymphoma at necropsy as compared with 0 of 13 Rag2−/−Atm−/− mice (P = 0.0018 by Mantel–Haenszel test comparing overall probabilities as a function of time). Age at death ranged from 7–17.5 mo, with the mean age at death being 12 mo. Rag2−/−Atm−/− littermate controls had a higher frequency of tumorogenesis and a shorter latency to tumor development (Fig. 1). Seven of 12 Rag2−/−Atm−/− mice (58%) developed malignant thymic lymphoma as compared with 0 of 13 Rag2−/−Atm−/− mice (P = 0.0012 by Mantel–Haenszel test) or as compared with 6 of 24 Rag2−/−Atm−/− mice (P = 0.036 by
The curves for these two groups are at 100% event-free survival. To represent the sacrifice or death of an animal that was not subsequently diagnosed with malignant thymic lymphoma. Tick marks were used to indicate the death of an animal diagnosed at necropsy with malignant thymic lymphoma. For purposes of statistical analysis (Mantel–Haenszel test). Age at death ranged from 4–7.75 mo, with a mean age at death of 6.4 mo. Since T cell development is arrested at the CD4+CD8− stage in Rag−/− mice (10), it was possible that the occurrence of thymic lymphomas in Rag−/− mice is related to the developmental arrest in Rag2-deficient thymocytes. To test this possibility, we generated HyTcrTgRag−/− mice, in which the arrest in T cell development due to Rag2 deficiency is rescued by Tcr transgene expression (18), and monitored these mice for development of thymic lymphoma. Preliminary study of HyTcrTgRag−/− mice revealed that malignant thymic lymphomas do develop in these mice and thus demonstrates that lymphomagenesis in Atm-deficient mice is independent of T cell developmental arrest.

In addition to malignant thymic lymphomas, Atm-deficient mice also showed an increased incidence of nonthymic tumors as compared with control mice. Seven of 24 Rag−/−Atm−/− mice (29%) and 2 of 12 Rag−/−Atm−/− mice (17%) developed various forms of nonthymic malignancy as compared with 0 of 13 Rag−/−Atm−/− mice (P = 0.0134 and P = 0.0072, respectively, by Mantel–Haenszel test). These malignancies included two cases of disseminated lymphoma (Rag−/−Atm−/− mice), a variety of sarcomas (Rag−/−Atm−/− and Atm−/− mice), and ovarian granulosa cell tumors (Rag−/−Atm−/− and Atm−/− mice). No mice in control groups (Rag−/−Atm+ and Rag+Atm+ mice) developed any malignant tumors. As might be expected, the early morbidity and mortality of Rag−/−Atm−/− mice were often attributable to infectious causes such as pneumonia, myocarditis, candidal esophagitis, subcutaneous abscess, or colitis secondary to Helicobacter infection. The majority of Rag−/−Atm+ (8/13) mice were alive when this study was terminated.

**Flow Cytometric Analysis of Rag−/−Atm−/− Tumors.** Flow cytometric analysis of Rag−/−Atm−/− and HyTcrTgRag−/− malignant thymic lymphomas was performed to evaluate surface phenotypes. Although nearly all Atm−/− thymic lymphomas have been reported to have a CD4+CD8− phenotype (5–8), analysis of Rag−/−Atm−/− tumors revealed variable profiles. All tumors were negative for surface TCR (Cβ and/or CD3) as expected in Rag−/− mice; however, surface expression of CD4 and CD8 differed among tumors (data not shown). One tumor had a CD4+CD8− phenotype (1107); one was primarily CD4−CD8+, with a subpopulation of CD8+ cells (2865); one was predominantly CD4+ with a subpopulation that was CD4+CD8+ (8039); and two tumors were CD4−CD8+ (2998, 8097). The HyTcrTgRag−/−Atm−/− thymic lymphomas analyzed also expressed varying levels of CD4 and/or CD8 and were TCR Vβ8+, consistent with expression of the HyTcr transgene (data not shown). All Rag−/− and HyTcrTgRag−/−Atm−/− thymic lymphomas were Thy 1.2+, verifying that they were of T cell origin.

The expression of CD4 and/or CD8 on the cell surface of multiple Rag−/−Atm−/− thymic lymphomas was unanticipated as RAG-2 is necessary for V(D)J recombination and is therefore normally required for T cell development past the CD4−CD8− stage (10). These results are similar to the previous finding that CD3−CD4+CD8+ thymic lymphomas develop in Rag−/−p53−/− and Rag−/−p53−/− mice (19). To further clarify the circumstances under which CD4 and/or CD8 expression might be occurring, we analyzed thymocytes from non-tumor-bearing Rag−/−Atm−/− mice by flow cytometry. Thymocytes of three Rag−/−Atm−/− mice had a CD3−CD4−CD8− phenotype as expected in mice with a Rag2-deficient background, whereas thymocytes of three other Rag−/−Atm−/− mice had a

![SKY analysis of a metaphase cell from Rag2−/−Atm−/− thymic lymphoma 2998 displaying chromosome aberrations.](image-url)
CD3−CD4+CD8null phenotype (data not shown). This unexpected phenotype of dull CD8 expression could signify a bypass in Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> mice of the blockade in thymocyte development imposed by the absence of RAG-2. Interestingly, nonmalignant thymocytes from Rag2<sup>−/−</sup>/p53<sup>−/−</sup> and Rag2<sup>−/−</sup>/p53<sup>−/−</sup> mice have also been shown to have an unexpected CD4<sup>+</sup>CD8<sup>+</sup> phenotype (19).

**Cyto genetic Analysis of Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> Tumors.** To investigate chromosomal alterations that might contribute to underlying mechanisms of tumorigenesis, we performed cytogenetic analysis on early passage cells cultured from multiple Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> and HyTcrTg<sup>−/−</sup>Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> thymic lymphomas. SKY analysis was used to identify chromosomal structural aberrations, whereas FISH analysis using gene-specific probes for the variable and constant regions of the Tcr αβ locus was carried out to determine the presence or absence of translocations within this locus. SKY analysis of Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> and HyTcrTg<sup>−/−</sup>Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> tumors revealed multiple translocations (Fig. 2), with chromosome 14 being involved in several of these tumors (Table 1). However, unlike Atm<sup>−/−</sup> tumors, Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> and HyTcrTg<sup>−/−</sup>Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> tumors exhibited colocalization of the variable and constant region Tcr αβ locus FISH probes, indicating an absence of translocations within this locus (Fig. 3 and Table 1). Interestingly, although no consistent translocation can be identified in Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> and/or HyTcrTg<sup>−/−</sup>Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> tumors studied, every tumor did exhibit a translocation involving either chromosome 14 or chromosome 12, both of which are homologous to human chromosome 14. Moreover, the breakpoints approximate the same band regions (14D and 12D–F) as has been observed in Atm<sup>−/−</sup> tumors (Fig. 2, Table 1). The predilections for translocations within these chromosomes may be related to the conformation of chromatin structure. If the chromatin is in a more open conformation in these regions, as is necessary for efficient V(D)J recombination (20), these loci might be more susceptible to chromosomal translocations.

If the chromosomal abnormalities observed in these Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> and HyTcrTg<sup>−/−</sup>Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> thymic lymphoma

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**Table 1. Summary of structural aberrations in Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> and HyTcrTg<sup>−/−</sup>Rag2<sup>−/−</sup>/Atm<sup>−/−</sup> thymic lymphomas identified by SKY and FISH analysis**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Genotype</th>
<th>SKY-detected clonal aberrations&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TCR V/C C&lt;sup&gt;b&lt;/sup&gt; FISH&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2593</td>
<td>Atm&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>T(9;14)&lt;sup&gt;+&lt;/sup&gt;, T(12;9), T(7;4)</td>
<td>V/C rearranged</td>
</tr>
<tr>
<td>2865</td>
<td>Rag2&lt;sup&gt;−/−&lt;/sup&gt;/Atm&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>T(9;17), T(12;9), T(18;12)</td>
<td>V/C colocalization</td>
</tr>
<tr>
<td>1107</td>
<td>Rag2&lt;sup&gt;−/−&lt;/sup&gt;/Atm&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>T(2;14), T(7;4), T(14;2;1)</td>
<td>V/C colocalization</td>
</tr>
<tr>
<td>8097</td>
<td>Rag2&lt;sup&gt;−/−&lt;/sup&gt;/Atm&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>T(17;1), T(8;14), T(14;8)</td>
<td>V/C colocalization</td>
</tr>
<tr>
<td>2998</td>
<td>Rag2&lt;sup&gt;−/−&lt;/sup&gt;/Atm&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>T(12D;15A), Dic(12E;15E), Dp(14), T(17;1)</td>
<td>V/C colocalization</td>
</tr>
<tr>
<td>8039</td>
<td>Rag2&lt;sup&gt;−/−&lt;/sup&gt;/Atm&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>T(4;9), T(12;15), Del(5)</td>
<td>V/C colocalization</td>
</tr>
<tr>
<td>3013</td>
<td>HyTcrTg&lt;sup&gt;−/−&lt;/sup&gt;Rag2&lt;sup&gt;−/−&lt;/sup&gt;/Atm&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>T(14;1)</td>
<td>V/C colocalization</td>
</tr>
<tr>
<td>3021</td>
<td>HyTcrTg&lt;sup&gt;−/−&lt;/sup&gt;Rag2&lt;sup&gt;−/−&lt;/sup&gt;/Atm&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>T(14;1n14;16)</td>
<td>V/C colocalization</td>
</tr>
</tbody>
</table>

<sup>a</sup>Structural aberrations were considered clonal if present in two or more metaphases. Gains and losses were considered clonal if present in at least two or three metaphases, respectively. Aberrations involving chromosomes 12 or 14 are shown in bold. At least 10 metaphase cells were analyzed per tumor.

<sup>b</sup>Rearrangement within the Tcr α locus was detected by FISH with two BAC clones, one each from the variable and constant regions of the gene, as described in Materials and Methods.

<sup>c</sup>V, variable region; C, constant region; FISH, fluorescence in situ hybridization.
mas are early events that occur in precursors of thymic lymphomas and predispose to malignant transformation, one would expect to see similar chromosomal abnormalities in mature T cells from nonmalignant mice of these genotypes. To investigate this possibility, SKY analysis of nonmalignant spleen and thymus T cells from HyTcrTg\(^{Rag2^{-/-} / Atm^{-/-}}\) mice was performed. A high frequency of chromosomal aberrations was observed in two of three mutant mice studied (Table 2). Although most chromosomal aberrations were not clonal, thymocytes from one mouse (8644) did harbor recurrent chromosomal aberrations. No aberrations were found in spleen or thymus T cells from a HyTcrTg\(^{Rag2^{-/-} / Atm^{-/-}}\) mouse (Table 2), and SKY analysis of T cells from wild-type mice did not reveal any chromosome aberrations in over 30 metaphases from three experiments (data not shown).

**Table 2. Chromosome aberrations in HyTcrTg\(^{Rag2^{-/-} / Atm^{-/-}}\) preclinical thymus and spleen**

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Genotype</th>
<th>Thymus</th>
<th>Spleen</th>
<th>No. of aberrant metaphases/total analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>8046</td>
<td>HyTcrTg</td>
<td>T(11;9)</td>
<td>T(10;10),T(11;3)</td>
<td>Thymus: 2/10 ~ 20%</td>
</tr>
<tr>
<td></td>
<td>Rag2(^{+/-}) / Atm(^{+/-})</td>
<td>T(12;14)</td>
<td>T(12;3)</td>
<td>6/12 = 50%</td>
</tr>
<tr>
<td></td>
<td>Rag2(^{-/-}) / Atm(^{-/-})</td>
<td>T(12;15)(^{\dagger})</td>
<td>Spleen: Rb[T(11;4.4)(^{\ast})] Rb[X.Del(X)]</td>
<td></td>
</tr>
<tr>
<td>8644</td>
<td>HyTcrTg</td>
<td>T(12;4)(^{\dagger})</td>
<td>Not analyzed</td>
<td>Thymus: 8/10 ~ 80%</td>
</tr>
<tr>
<td></td>
<td>Rag2(^{-/-}) / Atm(^{-/-})</td>
<td>T(12;15;4)(^{\dagger})</td>
<td>None Not analyzed</td>
<td></td>
</tr>
<tr>
<td>8645</td>
<td>HyTcrTg</td>
<td>None</td>
<td>Not analyzed</td>
<td>Thymus: 0/10 = 0%</td>
</tr>
</tbody>
</table>

*Structural aberrations were determined by SKY analysis. Most aberrations were not clonal (see below for exceptions).†This translocation was present in two metaphase cells.\(^{\ast}\)This translocation was present in peripheral T cells from wild-type mice (M. Liyanage, C.B., Z.W., A. Coleman, D. G. Pankratz, S. Anderson, A.W.-B., and T.R., unpublished work), suggesting that the translocations within the Tcr \(\alpha/\delta\) locus seen in Atm-mutant thymic lymphomas occur early in the process of tumorigenesis. To address the same question in the development of thymic lymphomas in Rag2\(^{-/-}\) / Atm\(^{-/-}\) mice, we analyzed thymocytes from HyTcrTg\(^{Rag2^{-/-} / Atm^{-/-}}\) mice. If the chromosomal structural abnormalities observed in thymic lymphomas from Rag2\(^{-/-} / Atm^{-/-}\) mice reflect predisposing events in malignant transformation of lymphocytes, we would expect an increased frequency of thymic or peripheral T cells with similar chromosomal aberrations in HyTcrTg\(^{Rag2^{-/-} / Atm^{-/-}}\) mice. Analysis of nonmalignant T cells from HyTcrTg\(^{Rag2^{-/-} / Atm^{-/-}}\) mice indeed revealed multiple chromosome aberrations in thymocytes and splenocytes. These data suggest that the chromosomal structural abnormalities seen in Rag2\(^{-/-}/Atm^{-/-}\) thymic lymphomas represent early events in the pathway of tumorigenesis and can predispose to selective expansion of lymphocyte clones, as has been observed in the human disease (4, 23).

Because both RAG-1 and RAG-2 are required for V(D)J recombination, with the elimination of either resulting in the absence of V(D)J recombination, one might expect tumor susceptibility in Rag1\(^{-/-} / Atm^{-/-}\) mice to be similar to that observed in Rag2\(^{-/-} / Atm^{-/-}\) mice. However, recent results reported by Liao and Van Dyke (8) indicated that malignant thymic lymphomas were not observed in Rag1\(^{-/-} / Atm^{-/-}\) mice, in marked contrast to the results reported here of tumor occurrence in Rag2\(^{-/-} / Atm^{-/-}\) mice. This unexpected discrepancy may be accounted for by the fact that the majority of Rag1\(^{-/-} / Atm^{-/-}\) mice in the study published by Liao and Van Dyke (8) died or were killed by 9 mo of age, whereas nearly all of the tumors that developed in Rag2\(^{-/-} / Atm^{-/-}\) mice in the study described here appeared at a later age. Alternatively, a difference in lymphoma susceptibility between Rag1\(^{-/-} / Atm^{-/-}\) and Rag2\(^{-/-} / Atm^{-/-}\) mice could signify previously undescribed differences in RAG-1 and RAG-2 function or could be
attributable to differences in genetic background and/or environmental differences in mouse colonies used in these studies. A comparison of $\text{Rag1}^{-/-}\text{Atm}^{-/-}$ and $\text{Rag2}^{-/-}\text{Atm}^{-/-}$ mice has been initiated to address these questions.

We extend sincere thanks for the excellent animal care provided by Genevieve Sanchez, Amy Werling, and the staff at Bioqual, Inc. We are also grateful to Andre Nussenzweig and Al Singer for careful reading of this manuscript and constructive comments.
