RAG-1-Deficient Mice Have No Mature B and T Lymphocytes

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Summary

The V(D)J recombination activation gene RAG-1 was isolated on the basis of its ability to activate V(D)J recombination on an artificial substrate in fibroblasts. This property and the expression pattern in tissues and cell lines indicate that RAG-1 either activates or catalyzes the V(D)J recombination reaction of immunoglobulin and T cell receptor genes. We here describe the introduction of a mutation in RAG-1 into the germline of mice via gene targeting in embryonic stem cells. RAG-1-deficient mice have small lymphoid organs that do not contain mature B and T lymphocytes. The arrest of B and T cell differentiation occurs at an early stage and correlates with the inability to perform V(D)J recombination. The immune system of the RAG-1 mutant mice can be described as that of nonleaky scid mice. Although RAG-1 expression has been reported in the central nervous system of the mouse, no obvious neuroanatomical or behavioral abnormalities have been found in the RAG-1-deficient mice.

Introduction

The generation of antibody and T cell receptor (TCR) diversity is mediated to a large extent by the somatic DNA rearrangement known as V(D)J recombination, which joins variable (V), diversity (D), and joining (J) gene segments (Tonogawa, 1983). The genes for the antigen receptors are assembled through this process exclusively in lymphocytes. V(D)J recombination occurs at seven different loci: the immunoglobulin (g) heavy chain, κ, and λ light chain loci in B lymphocytes (Tonogawa, 1983), and the TCR α, β, γ, and δ chain loci in T lymphocytes (Davis and Bjorkman, 1989). The prevailing model is that a common recombinase is active in precursors of both B and T cells and that the sequential recombinations are executed by developmentally controlled targeting of the recombinase to the different loci (Alt et al., 1986).

The cis-acting sequences required for V(D)J recombination have been described in detail (Tonegawa, 1983; Hesse et al., 1989). A gene called the recombination activation gene (RAG-1) was isolated by virtue of its ability to activate V(D)J recombination in NIH 3T3 fibroblasts on an artificial recombination substrate carrying selectable markers (Schatz and Baltimore, 1988; Schatz et al., 1989). A second, structurally unrelated gene called RAG-2 was later identified in the immediate vicinity of RAG-1 (Oettinger et al., 1990). A model has been proposed in which RAG-1 and RAG-2 together are sufficient to induce V(D)J recombination on an artificial substrate in fibroblasts (Oettinger et al., 1990). The expression of both genes is concordant and restricted to cell lines displaying V(D)J recombination activity and to developing lymphoid tissues (Schatz et al., 1988; Oettinger et al., 1990; Boehm et al., 1991; Turke et al., 1991). The only reported discordancies in their spatial patterns of expression are transcription of only RAG-1 in the central nervous system of the mouse (Chun et al., 1991) and of only RAG-2 in the bursa of Fabricius of the chicken (Carlson et al., 1991).

We have introduced a deletion in the RAG-1 gene of pluripotent embryonic stem (ES) cells (Evans and Kaufmann, 1981) by the targeted gene disruption technique (Capocchi, 1989) and produced mice homozygous for the RAG-1 mutation (hereafter called mutant, homozygous mutant, or RAG-1-deficient mice). These mutant mice do not have any mature B and T lymphocytes. Flow cytometric analysis of lymphoid organs reveals a blockade of lymphocyte differentiation at an immature stage, similar to the situation described in the severe combined immunodeficiency (scid) mouse (Bosma et al., 1983; Bosma and Carroll, 1991). Southern blot analysis of DNA from thymus and bone marrow-derived Ablison-transformed cell lines indicates that both TCR and immunoglobulin gene loci remain in the germline configuration. In an accompanying paper (Shinkai et al., 1992 [this issue of Cell]), a similar phenotype is described in RAG-2-deficient mice. Taken together, these data suggest that RAG-1 and RAG-2 are both necessary in vivo either to activate or to catalyze the V(D)J recombination reaction.

In view of the reported expression of RAG-1 in the central nervous system (Chun et al., 1991), we have examined the brain of the RAG-1 mutant mice by histological means, but have not detected any overt structural abnormalities.

Results

Generation of the Mutation in RAG-1 in ES Cells and in Mice

The targeting vector pPMKC-31 (Figure 1) was constructed from a RAG-1 genomic clone isolated from a genomic DNA library made from ES cells of a 129/Sv mouse, in order to maximize homology between the targeting plas-
mid and the target sequences. Homologous integration would create a deletion of 1356 bp in the 5% end of the coding sequence of RAG-1. The resulting mutation is most likely a null mutation, as the deletion encompasses about half of the coding sequence of RAG-1 and includes both the putative nuclear localization signal and the zinc finger-like motif (Schatz et al., 1988). Moreover, as the neo-selectable marker is introduced in the opposite transcriptional orientation, a polypeptide correctly initiated at the translation start site would probably be prematurely terminated at the level of the neo gene.

The targeting strategy employed was to isolate a number of G418-resistant clones and analyze them individually by Southern blot, since previous experience in our laboratory had shown that the negative selection step utilizing the herpes simplex virus-thymidine kinase gene (Mansour et al., 1988) does not give a significant enrichement and that targeting frequencies among the G418-resistant clones are generally high enough for direct screening (Mombaerts et al., 1991; and data not shown). Using AB1 ES cells (McMahon and Bradley, 1990), we obtained targeted clones in two independent experiments: one (clone A103) out of 130 G418-resistant clones in one experiment and one (clone G113) out of 117 clones in another experiment.

Clone A103 was injected into C57BL/6J blastocysts. From those implanted females that became pregnant (representing 194 implanted blastocysts), 29 male, 1 hermaphrodite, and 12 female chimeras were born, with an average coat color chimerism of about 75%. Of 27 chimeric males that were tested mated to CD1 or C57BL/6 females, 17 had live offspring, and 12 were germline chimeras. One out of eight female chimeras had germline offspring. Clone A103 was also injected into eight BALB/c blastocysts, giving rise to two male chimeras and one female chimera. This female proved to be a germline chimera. Clone G113 did not give rise to good chimeras.

(129/Sv × CD1) F1 heterozygotes were intercrossed to produce homozygotes. Offspring were genotyped by Southern blot analysis of tail DNA (data from one litter are shown in Figure 2). Of 20 newborn animals, 6 were homozygous, and 33 out of 112 offspring genotyped after weaning were homozygous.

**Macroscopic Analysis**

Mice homozygous for the RAG-1 mutation have proven healthy and indistinguishable from their normal littermates by visual inspection up to 21 weeks of age. RAG-1 mutant mice are fertile. Two 12-week-old mutant males could readily fertilize either a homozygous or heterozygous mutant female. In the former case 12 pups were born, and in the latter, 11. Both litters were raised successfully to weaning.

We determined the number of cells in the lymphoid organs of twelve mutant mice of varying ages (newborn and 3- to 9-week-old mice) in comparison with corresponding wild-type or heterozygous littermates. The thymus of the mutant mice contained 15 to 130 times fewer cells than the wild-type or heterozygous littermates. One 4-week-old mutant mouse had the highest number of thymocytes among all mutant mice examined, but that was still 3.4 \times 10^8 compared with 230 \times 10^8 thymocytes in a wild-type littermate. The numbers of nonerythroid cells in the spleen of mutant mice of 3 weeks or older were between five and nine times smaller than those in corresponding wild-type
or heterozygous littermates. Visual inspection revealed a stroma-like structure in the site where the inguinal lymph nodes are located, but only a few nonerythroid cells if any could be recovered from these structures. No other obvious anatomical alterations were observed. As far as a potential neurological deficit is concerned (Chun et al. 1991), we have observed no obvious clinical symptoms to date. The mutant mice are active, have a firm grip with their forelimbs, can hear sounds, can walk on a pencil, can sense heat and feel pain, and can swim as well as their heterozygous or wild-type littermates.

**Absence of Mature B and T Lymphocytes**

Cells from thymus, spleen, and bone marrow were subjected to flow cytometric analysis. Data are shown for 4-, 5-, or 7-week-old mice (Figure 3 and Figure 4). The lymphocytes of the RAG-1-deficient mice are larger than those of wild-type or heterozygous mice, as indicated by the increased forward scatter (Figure 3A). No CD3-positive or TCR a/b-positive cells have been observed (Figure 3B). The thymocytes are CD8–CD4 double negative (Figure 3C), and almost all of them are interleukin-2 (IL-2) receptor-positive (Figure 3D). Finally, they are Thy-1 positive (Figure 3D), bright J11d–positive, bright Sca-1-positive,
and CD5-negative (data not shown). Thus, thymocyte development seems to be arrested at an immature stage.

The spleen does not contain any mature B cells, as judged by the lack of staining by anti-IgM (Figure 4A) or anti-IgD (data not shown) antibody. About one third of the splenocytes (on a narrow gate for the lymphoid population; see legend of Figure 4A) are B220 positive and may represent an early stage of B cell differentiation. A small fraction of the splenocytes is Mac-1 positive (data not shown) and could be macrophages, natural killer cells, or neutrophils. Likewise, the bone marrow contains no mature, IgM- (Figure 4B) or IgD-positive (data not shown) cells. About one fourth of the bone marrow cells (with a narrow gate on the lymphoid population; see Figure 4B legend) are B220-positive and could be early B cell precursors. The intensity of the B220 staining is lower than the majority of the B220-positive cells in the bone marrow of the normal mouse (Figure 4B). The composition of the lymphoid population in the lymphoid organs of the RAG-1-deficient mouse is similar to that of the scid mouse (Bosma and Carroll, 1991).

We also measured by ELISA serum IgM levels of seven mutant mice of 5–16 weeks and found that IgM is undetectable (data not shown). It has been shown that the serum IgM level is a sensitive indicator for leanness in the case of the scid mouse (Bosma and Carroll, 1991).

In summary, no mature B or T lymphocytes have been observed in the lymphoid organs of twelve RAG-1 mutant mice up to the age of 9 weeks, and no serum IgM up to the age of 16 weeks. It remains to be seen whether any leanness in the phenotype occurs at an older age.

Establishment of Abelson-Transformed Lines

The Abelson murine leukemia retrovirus was used to transform bone marrow cells from a 5-week-old female mutant mouse and from a wild-type female littermate. A similar number of cell lines grew up in both mice (transformation efficiency was \(1.19 \times 10^{-4}\) for homozygote and \(8.74 \times 10^{-5}\) for wild-type), as has been reported for the scid mouse (Vulpis et al., 1988). All of the Abelson retrovirus-transformed lines are B220 positive (data not shown) and contain RAG-2 and \(\lambda\)5 (Sakaguchi and Melchers, 1986) RNA (Figures 5A and 6C). It is therefore likely that these cell lines originated from immature B cells. Mutant RAG-1 transcripts are present in all of the cell lines established from the mutant mouse (Figure 5B).

Absence of V(D)J Recombination

To confirm that the absence of mature B and T lymphocytes is due to a defect in V(D)J recombination, Southern blot analysis was performed with DNA from the thymus and the Abelson-transformed lines. The \(3'J_{\beta}\) probe (Chien et al., 1987) allows the analysis of rearrangements involving \(D_{\beta}\) or \(J_{\beta}\) and all \(V_{\beta}-J_{\beta}\) rearrangements (in the latter case the TCR \(\beta\) locus is deleted). Thymus DNA, isolated from 4- or 7-week-old RAG-1-deficient mice, showed no rearrangements (Figure 6A), whereas thymus DNA of a 7-week-old scid mouse showed a low level of rearrangements (Figure 6A) that are presumably aberrant (Carroll and Bosma, 1991). When a \(5'J_{\mu}\) probe was used, no rearrangements were observed at the TCR \(\beta\) locus (Figure 6B), whereas the scid thymus shows a faint additional band that probably represents a D-J rearrangement (Malissen et al., 1984). A TCR \(\alpha\) probe was used as a control for loading and Southern transfer efficiency of the DNA samples (Figure 6C). Lanes 4 (sample of wild-type mouse) is clearly overloaded, but this does not affect the conclusions. We also analyzed DNA from the bone marrow-derived Abelson lines with an immunoglobulin \(J_{\mu}\) probe. All of the cell lines derived from the RAG-1-deficient mouse revealed a gerline configuration, but none of the lines derived from the wild-type mouse retained the IgH locus in a gerline configuration (Figure 7).

In conclusion, the inability to perform V(D)J recombination is the most likely explanation for the absence of mature B and T lymphocytes.

Histological Analysis of the Brain

In light of the reported presence of RAG-1 transcripts in the brain of the mouse (Chien et al., 1987), we assessed the effect of the RAG-1 mutation on the structure of the mouse brain by histological means, although no obvious behavioral deficit was observed (see above). Brains of 7- and 8-week-old male homozygous mutant and wild-type littermates were examined in serial sagittal sections stained with cresyl violet.

No significant defect could be found in the structure of
Figure 6. Southern blot Analysis of TCR Rearrangements in Thymus
(A) DNA from 5 × 10^6 cells was digested with EcoRI. The Southern blot was hybridized to the S1 probe. The size of the germline fragment is 7.5 kb. Lane 1, AB1 E6 cells; lane 2, thymocytes from a 4-week-old wild-type mouse; lane 3, thymocytes from a homozygous mutant mouse that is a littermate of the mouse in lane 2; lane 4, thymocytes from a 7-week-old wild-type mouse; lane 5, thymocytes from a homozygous mutant littermate of the mouse in lane 4; lane 6, thymocytes from a 7-week-old SCID mouse. There is no evidence for rearrangements in the thymus of the mutant mice. The aged thymus has two faint bands (indicated with arrows) that probably represent D-D or D-J rearrangements.
(B) The blot was stripped and hybridized to the 5′ D1 probe. The germline band is at 0.1 kb. No rearrangements are observed in lanes 3 and 5. In lanes 2 and 4, no signal is visible at the level of the germline band as the DNA sequences complementary to this probe are deleted (V-D-J rearrangement) or have an altered size (D-J rearrangement). A faint band (indicated with an arrow) is visible in the aged thymus and probably corresponds to a D-J rearrangement.
(C) The blot was stripped again and hybridized to a TCR C′ probe. The intensity of the bands serves to control for variations in amount of DNA loaded and Southern transfer efficiency. As the DNA sample from the wild-type mouse in lane 4 is underloaded, the data in lane 4 of Figures 6A and 6B are not relevant, but this does not affect the conclusions.

alterations (Nowakowsky, 1984; Joyner et al., 1991), their normal appearance in the mutant suggest that the absence of RAG-1 function does not interfere in a major way with developmental events such as neurogenesis, migration and differentiation.

Discussion

RAG-1 and RAG-2 Are Both Required for V(DJ) Recombination

In this report, we describe the generation and the initial analysis of mice with a mutation of the RAG-1 gene, which has been implicated in the regulation of the catalysis of V(DJ) recombination (Schatz et al., 1989). The data show that this gene is necessary in vivo for V(DJ) recombination to occur. The presence of RAG-2 transcripts in the Abelson-transformed lines derived from the RAG-1 mutant mouse indicates that the mutation introduced in the RAG-1 gene does not inhibit the expression of the closely linked RAG-2 gene. Formal proof, however, that the lack of V(DJ) recombination is due solely to the targeted disruption of RAG-1 awaits complementation of the V(DJ) recombination defect either by transfection of the Abelson-transformed lines with a RAG-1 expression plasmid or crossing the RAG-1-deficient mice to RAG-1 transgenic mice. RAG-1 transcripts of a slightly longer size that are observed in the RAG-1-deficient lines are unlikely to give rise to any functional protein (see Results).

Our data, taken together with those of the RAG-2-deficient mice described in the accompanying paper (Shinkai et al., 1992), support the hypothesis that both the RAG-1 and RAG-2 gene products are required in vivo for V(DJ) recombination, as in transfected fibroblasts (Oettinger et al., 1990). The low level of rearrangements observed with an artificial substrate upon transfection of either the RAG-1 or RAG-2 gene into 3T3 fibroblasts (Schatz...
Immature B and T Cells

Flow cytometric analysis of cells from lymphoid organs reveals the absence of mature B and T lymphocytes, which is most likely the result of the deficit in V(D)J recombination. B and T cell precursors need to produce functional antigen receptors on their surface in order to survive (Rolink and Melchers, 1991; von Boehmer, 1988). In the case of the T cell lineage, it is well established that the precursors that fail to produce a TCR do not undergo the processes of positive selection (von Boehmer, 1988). Thus, like in the scid mouse (Bosma and Carroll, 1991), the thymus of the RAG-1 mutant mouse remains small and contains immature, large, CD8–CD4 double negative thymocytes expressing the IL-2 receptor. Likewise, the bone marrow and the spleen of the RAG-1 mutant mouse contain a population of dull B220-positive cells, a fraction of which may represent pro-B cells. Some of these cells may serve as targets for transformation by the Abelson retrovirus.

No Leakedness Observed

The combination of the flow cytometric data, Southern blot analysis, and serum IgM ELISA measurements has revealed no leakedness in the phenotype to date. It remains to be seen whether any leakedness will be observed when older mice or a larger number of mice are examined. For instance, since serum IgM is a sensitive indicator of B cell development, we intend to follow it by serial bleeding as mice age. We can also follow immunoglobulin and TCR rearrangements by the sensitive polymerase chain reaction technique.

The observations made to date are in contrast to those in the scid mouse. Using the same 3J probe (Chien et al., 1987) as the one used in our study, D–J rearrangements at the TCRδ locus have been reported for scid mice (Carroll and Bosma, 1991), as can be seen in Figure 6A. As these mice grow older, some B and T cells express a functional antigen receptor and form an oligoclonal repertoire (Bosma et al., 1988; Carroll and Bosma, 1988; Carroll et al., 1989; Bosma and Carroll, 1991). This leakedness is partly the result of reversion of the mutation in individual lymphocyte progenitors (Petriti et al., 1990). As a result, serum levels of IgM increase in scid mice as they age (Bosma and Carroll, 1991).

If the RAG-1-deficient mice indeed turn out to be nonleaky, as the data obtained to date suggest, these mutant mice may prove to be a useful alternative to the scid mouse for a variety of studies, such as the efforts to produce a human immune system in the mouse—the “SCID-hu mouse” (McCune et al., 1988; Mosier et al., 1988)—as an animal model for AIDS, and reconstitution or infection experiments (McCune, 1991). Indeed, in addition to the leakedness, the scid mutation has at least two other drawbacks compared with the RAG-1 mutation: the gene has not been cloned, and the genetic defect is known to be pleiotropic affecting other processes, such as double-strand break-related DNA repair, which results in increased sensitivity to ionizing radiation (Fulop and Phillips, 1990; Biedermann et al., 1991; Hendrickson et al., 1991).

No Overt Structural Alterations in the Brain

The brain is the only nonlymphoid organ in which RAG-1 RNA has been claimed to exist (Chun et al., 1991). Furthermore, unlike lymphoid organs in which RAG-1 and RAG-2 are invariably coexpressed (Oettinger et al., 1990), the brain has been claimed not to express RAG-2 (Chun et al., 1991). On the basis of these observations, it has been speculated that a V(D)J or V(D)J-like recombination may
take place in the brain (Chun et al., 1991; Alt et al., 1991). Our RAG-1 mutant mice offer a direct test of the hypothesis that the RAG-1 gene product plays a physiological role in the brain including promoting somatic DNA rearrangement. However, our initial analysis of such mutant mice revealed no obvious behavioral or neuroanatomical abnormality. In particular, we observed no cytoarchitectural alterations in the cerebellum or the hippocampus, the sites at which the highest levels of RAG-1 transcripts in the central nervous system were reported (Chun et al., 1991). Both regions are sensitive to perturbations in pattern formation, making the absence of a structural defect all the more intriguing.

Several explanations can be offered. First, the observed RAG-1 transcripts may be superfluous and have no physiological function. For instance, they may not be translated. Second, there may be an alternative mechanism for whatever function RAG-1 performs in the brain, i.e., there could be genetic redundancy. Third, the absence of the RAG-1 protein may affect development and functioning of the mouse brain, but the repercussions may be subtle and have escaped our analytical methods.

With respect to the last possibility, it is interesting to note that in the immune system V(D)J recombination is essential for maturation and expansion of lymphocytes and that the mutation in RAG-1 leads to gross diminution of lymphoid organs. By contrast, there clearly is no dependence of this scale on the RAG-1 product in any of the major subregions of the brain. It is, however, feasible that functional deficits exist in the absence of obvious structural alterations. We will continue to look for such deficits, especially in the areas of olfaction (Buck and Axel, 1991) and learning and memory.

Experimental Procedures

Construction of Targeting Vector

RAG-1 was cloned by screening a genomic EMBL-3 phage library prepared from D3 ES cells (Gossler et al., 1986) with a probe generated by the polymerase chain reaction technique (gift from A. Abele). One positive phage out of 1.2 million plaques screened was purified and mapped by restriction enzyme analysis. A 9 kb ClaI and a 6.5 kb BamHI-ClaI fragment were subcloned into pBluescript SK- (Stratagene) and mapped further. The targeting vector pPMKO-31 was constructed in a quinuclomerate ligation reaction. Using a 5 kb ClaI-ApaI fragment upstream of the RAG-1 coding sequence (position 462), a 1.8 kb fragment containing the pgl-neo gene (Adra et al., 1987) excised with Apal and BamHI, a 1.277 to BgllII-MluI fragment containing sequences between positions 1837 and 3113, and the plasmid pGEM7 (Promega) cut with ClaI and MluI. This targeting vector is designed to delete 1356 nucleotides of the RAG-1 coding sequence between positions 482 and 1337 and replace it with the pgl-neo gene, transcribed in the opposite orientation. The source of the pgl-neo gene is the plasmid pBlUI described in Tyluswicz et al. (1991).

Targeting Experiment

ES cells were grown on mitomycin C or γ-irradiated primary embryonic fibroblasts, and during the selection on G418-resistant fibroblasts isolated from transgenic embryos carrying a neo resistance gene (Gossler et al., 1986). The medium composition was as described in Robertson, 1987. About 5 x 10⁵ ABI (McMahon and Bradley, 1990) ES cells were electroporated with 75 μg of MluI linearized pPMKO-31 using a Bio-Rad Gene Pulser set at 800 V and 3 μF. Selection was initiated 20 hr later at a concentration of 125 to 150 μg/ml active concentration of G418 ( Gibco). Colonies were picked from day 6 to day 9 of selection into 96 or 24 well dishes (Costar) and expanded. Half of a 24 well dish was frozen down and the other half used to isolate DNA for Southern blot analysis. The colonies were screened individually by cutting genomic DNA with BamHI and XhoI and probing the Southern blots with the external probe M-N, a 268 bp fragment containing RAG-1 coding sequences between positions 3113 and 3380 (Schatz et al., 1989).

Generation of Chimeric Mice

Chimeras were generated as described in Papaioannou (1981) and Bradley (1987). Litters of heterozygote intercrosses were housed in autoclaved microisolator cages with autoclaved food and water beginning at birth.

Screening of Mice

Mice were screened for homozygosity by Southern blot analysis on genomic tail DNA isolated according to Laird et al. (1991). The DNA samples were cut with BamHI and NcoI (see Figure 2) or with PstI, and the Southern blots were probed with the 3113-3380 bp fragment containing RAG-1 coding sequences between positions 1837 and 3380 (Schatz et al., 1989). Southern blots were exposed using a Fujix BioImage Analyzer BAS2000.

Flow Cytometric Analysis

10⁴ to 10⁵ cells were precipitated in 96-well round bottom dishes (Costar) for 20 min in 12.5 μl containing 5 μl of staining solution (composed of phosphate buffered saline [PBS], 0.1% sodium azide, 1% fetal calf serum) and 2.6 μl each of normal hamster, normal mouse (Jackson ImmunoResearch), and normal rat serum (Caltag). The preincubation with serum was omitted in the stainings with goat anti-mouse IgM. The samples were then stained by adding another 12.5 μl of staining solution containing 0.25 μl of antibodies either biotinylated or conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE). The antibodies used were: 2C11 for CD3 (Pharminagen), H57-597 for TCR αβ (Pharminagen), 53-6.7 for CD8 (Becton Dickinson), GK1.5 for CD4 (Pharminagen), S3-2.1 for Thy-1.2 (Pharminagen), PC 61.5 for IL-2 receptor/CD25 (gift from Kunio Sano), goat anti-mouse immunoglobulin μ (Caltag), RA-3-682 for B220 (Boehringer Mannheim), M1/70 for Mac-1 (Boehringer Mannheim), 53-7.3 for CD5 (gift from Pablo Pereira), J11d (gift from Pablo Pereira), and anti-Sca-1 (gift from Peggy Goodell). Cells were stained for 30 min. washed twice with staining solution, nucubated in 25 μl of staining solution containing streptavidin-PE (Southern Biotechnology) at a 1:250 dilution, washed once in staining solution, once in PBS containing propidium iodide, and finally resuspended in 200 μl of PBS. Cells were kept on ice during the staining procedure and spun in a refrigerated centrifuge. Flow cytometric analysis was carried out with a Becton Dickinson FAC-Scan using FACSscan software. Dead cells were gated out by means of propidium iodide staining. Five thousand to seventeen thousand events were acquired using a large gate and the lymphoid population was analyzed with a narrower gate based upon forward scatter and side scatter.

Southern and Northern blot Analysis

TCR α and β rearrangements were analyzed by cutting genomic DNA from thymus with EcoRI and hybridizing with probes described in Chien et al., (1987) (gift from Yoichi Shinoki). TCR γ rearrangements were analyzed by hybridizing with a 5'D probe, a 1.2 kb PstI fragment isolated from cosmide 2.3W (Malissen et al., 1994). The TCR C probe was a 0.5 kb BglII-BamHI fragment (Mombaerts et al., 1991). Immunoglobulin heavy chain rearrangements were analyzed by cutting with EcoRI and hybridizing with a 1.9 kb BamHI/EcoRI JH probe (Sakamoto et al., 1990).

The RAG-2 probe is a 246 bp fragment cloned into pUC12 with the following polymerase chain reaction primers: 5'-ATG TGC CGG ATG ACA AAG GAC TGG AGA GGT-3' (position 156 to 176 in Oettinger et al., 1990) and 5'-GCG TCT GAC GAC GTC TTT ATC-3' (position 401 to 381 in Oettinger et al., 1990). The 5S (Sakaguchi and Melchior, 1989) DNA probe was a gift from Eugene Oltz.

Establishment of Abelson-Transformed Cell Lines

The Abelson murine leukemia virus was used to transform bone marrow cells as described in Rosenberg and Baltimore, 1976. Transformation efficiency was determined by counting the number of colonies in soft agar per 2 x 10⁴ infected bone marrow cells.
Histology of the Brain

Mice were deeply anesthetized with avertin, perfused for 2 min with PBS, and then with 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4) for 15 min. Following perfusion, the brain was removed from the skull and stored in fresh paraformaldehyde overnight at 4°C. The brains were bisected at the midline and embedded in paraffin wax (polyethylene glycol 4000) according to the protocol of Muller (1977). Serial ten micron sections were collected, mounted on gelatinized slides, and stained with cresyl violet.

Acknowledgments

P. M. wishes to thank En Li, Yoshi Ishikawa, and Alan Clarke for introducing him to ES cell technology, and Pablo Perera, Juan Lafaille, and Shige Ishihara for advice in the art of the PACScan. The authors thank Rolf Kemler and Rudolf Jaensch for providing neo-resistant transgenic mice, Alan Bradley for ES cells and advice, Michael A. Rudnicki or providing ptk-neo, Asa Abeliou for the RAG-1 probe, Alcino Silva for the use of the D3 genomic library, Michele Maxwell, Lil Wang, John Anderson, and James Spencer for technical help, Chris Newcomer and his staff for facilities, Bruce Spiegelman for advice and encouragement, Yoichi Shikita for the IL-2 probe, Gene Odzo for the I2 probe, Peggy Goodell, Kunio Sano, Juan Lafaille, and Pablo Perera for antibodies, and Antonio Coutinho and Juan Lafaille for critical review of the manuscript. We thank Fred Alt and Yoichi Shikita for sharing unpublished information. P.M. is a Howard Hughes Medical Institute Predoctoral Fellow in Biological Sciences; J.J. is supported by a National Institutes of Health postdoctoral training grant; and R.S.J. is an Albert L. Ryan Predoctoral Fellow. This work was supported by grants from the Howard Hughes Medical Institute and the National Institutes of Health to S.T., by NS18381 to K.H., and by HD27985 to V.E.P.

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Received December 17, 1991; revised January 13, 1992.

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phenotype of RAG-1-deficient mice


Note Added in Proof

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