Transcription of unrearranged antigen receptor genes in *scid* mice

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The *scid* mouse mutant is severely deficient in lymphocytes; cells of the B or T lymphocyte lineage cannot be detected by either serological or functional assays. However, as shown here, germ-line transcripts of B cell immunoglobulin (*Ig*) constant and variable region genes and of T cell receptor (*TCR*) genes are detectable in lymphopoietic tissues of *scid* mice, as well as B and T lineage-specific $\lambda 5$ and $T3\delta$ transcripts. We conclude that B and T lineage-committed cells do arise in *scid* mice and that their *Ig* and *TCR* genes are accessible to enzymes involved in their recombination. This suggests that *scid* impairs lymphopoiesis at the stage at which antigen receptor genes normally undergo rearrangement. Key words: antigen receptor genes/germline transcripts/*scid* mutation

Introduction

Distinct and separate gene segments [variable (*V*), diversity (*D*) and joining (*J*)] are recombined in combinatorial fashion during early lymphocyte differentiation to form the coding sequences for the variable regions of lymphocyte antigen receptors i.e. immunoglobulin (*Ig*) and T cell receptors (*TCR*) (for reviews, see Tonegawa, 1983; Kronenberg *et al.*, 1986). Recombination of *V*, *D* and *J* (or *V* and *J*) elements at both *Ig* and *TCR* loci appears to be catalysed by a common, site-specific recombinase system that recognizes the highly conserved heptamer–nonamer sequences flanking all such gene elements (Tonegawa, 1983; Kronenberg *et al.*, 1983; Yancopoulos *et al.*, 1986). Recombination of antigen receptor genes proceeds in an ordered fashion. In general, *D*-to-*J* recombination occurs first, followed by *V*-to-*DJ* recombination. In developing B cells, *VDJ*-assembly at the *Ig* heavy chain (*Igh*) locus precedes rearrangement of the *Ig* light chain (*Igl*) loci (for a review, see Alt *et al.*, 1986). Likewise, rearrangement of *TCR γ* and *TCR β* genes precedes rearrangement of the *TCR α* locus in developing T cells (Born *et al.*, 1985; Haars *et al.*, 1986).

We recently reported evidence that the murine mutation, *scid*, adversely affects the mechanism of antigen receptor gene recombination (Schuler *et al.*, 1986). Mice homozygous for this mutation (*scid* mice) are severely deficient in lymphocytes. However, transformed cell lines which have a phenotype characteristic of immature lymphocytes can be obtained from *scid* mice. Specifically, early B cells

with *Igh* rearrangements can be recovered by transformation of *scid* bone marrow cells with Abelson murine leukemia virus (A-MuLV) and early T cells with *TCR β* rearrangement can be recovered as spontaneous thymic lymphomas in ~15% of *scid* mice. The striking finding made in these transformed lymphocytes was that the majority of rearranged *Igh* and *TCR β* alleles incurred grossly abnormal *J*-associated deletions as an apparent result of attempted *D*-to-*J* joining. These results led us to hypothesize that *scid* causes highly error-prone recombination of antigen receptor genes. Accordingly, most developing *scid* lymphocytes would be unable to produce *Ig* or *TCR* proteins due to aberrant gene rearrangements at both alleles of a critical antigen receptor locus (e.g. *Igh* or *TCR β*). Our inability to detect these cells directly in *scid* lymphoid tissues, except by transformation, might indicate that they turn over very rapidly.

According to the above hypothesis, the effect of *scid* would become manifest when B or T lineage-committed cells attempt to rearrange their *Ig* or *TCR* genes. However, cells of either lymphoid lineage have not been directly detected in *scid* mice by B or T lineage-specific monoclonal antibodies (Bosma *et al.*, 1983; Habu *et al.*, 1987) or by functional assays for B or T cell progenitor cells (Dorshkind *et al.*, 1984). Therefore, to assess whether B and T lineage-committed cells actually develop in *scid* mice, we looked for transcripts of germ-line *Ig* and *TCR* genes in *scid* lymphopoietic tissues. Before such genes undergo rearrangement, they become transcriptionally active (Van Ness *et al.*, 1981; Yancopoulos and Alt, 1985; Furley *et al.*, 1986). This reflects the early commitment of lymphocytes to the B or T cell lineage and, presumably, the accessibility of *Ig* and *TCR* genes to the recombinase system (Van Ness *et al.*, 1981; Yancopoulos and Alt, 1985). As additional markers for lineage-committed lymphocytes, we also tested for transcripts of the $\lambda 5$ and $T3\delta$ genes whose expression is restricted to early B cells (Sakaguchi *et al.*, 1986) and to T cells (van den Elsen *et al.*, 1984) respectively. Our results show that germ-line transcripts of *Igh*, *Igl(κ)*, *TCR γ* and *TCR β* genes, along with transcripts of the $\lambda 5$ and $T3\delta$ loci are present in the lymphopoietic organs of *scid* mice. We conclude that B and T lineage-committed cells do arise in *scid* mice and that they reach the stage at which antigen receptor genes normally become accessible to the recombinase system and undergo rearrangement.

Results

Evidence for B-lymphocyte lineage-committed cells in *scid* mice: expression of B-lymphocyte-specific genes
During early B cell differentiation in fetal liver of normal developing embryos there is a time-dependent change in the expression of different size transcripts of the *Igh* locus (Siden *et al.*, 1981), reflecting the ordered progression of *VDJ*-recombination. This is illustrated in Figure 1. In 16-day-old C.B-17 embryos, two transcripts of 2.6 kb and 2.1 kb

Fig. 1. Northern blot analysis of *Igh-Cμ* transcription in C.B-17-normal (N) and C.B-17scid (S) mice. Poly(A)⁺ RNA from fetal liver of 16-, 17- and 18 day-old embryos (12.5 μg/lane) and from adult bone marrow (3 μg/lane) was hybridized with pCμ3741. The filters were exposed for 20 h (a and d) and 60 h (b and e). To control for equal loading of RNA samples, the filters were rehybridized with the rpl7 ribosomal gene probe p49 (c and f). The size of the transcripts (in kb) is indicated on the left side of each panel in this and other figures and was estimated by comparison to a control lane containing known sizes of poly(A)⁺ RNA molecules (see Materials and methods).

were detected with the pCμ3741 probe specific for the constant region gene of the IgM heavy chain (*Igh-Cμ*) (Figure 1a and b). The same size transcripts were also detected with a probe specific for a region in the Jh-Cμ intron which is normally deleted during the processing of transcripts from completely assembled *Igh* genes (data not shown). These 2.6/2.1-kb Cμ⁺ transcripts are therefore designated here as Iμ. They represent sterile transcripts from unrearranged and partially (*Dh-to-Jh*) rearranged *Igh* genes (Kemp *et al.*, 1980a,b; Alt *et al.*, 1982; Nelson *et al.*, 1983; Lennon and Perry, 1985). Novel 2.4-kb and 2.1-kb Cμ⁺ transcripts appeared in 17-day-old C.B-17 embryos and increased in abundance at day 18 (Figure 1a and b). These transcripts have been shown previously to correspond to fully assembled *Igh* genes since they also hybridize to *Igh* variable region gene probes; the 2.4-kb RNA encodes the membrane form of IgM heavy chains (μ_m-mRNA), and the 2.1-kb RNA encodes the secreted form (μ_s-mRNA) (Alt *et al.*, 1980; Rogers *et al.*, 1980). [In our hands, the various Cμ⁺ transcripts were generally estimated to be ~0.3 kb shorter than reported earlier by others, but matched the sizes calculated from the known sequences assuming a 150-nt poly(A) segment. It is important to point out that, in contrast to earlier studies, we used poly(A)⁺ RNA markers. The discrepancy between calculated sizes and those estimated from gels calibrated with non-poly(A)⁺ RNA markers has been discussed by Nelson *et al.* (1983).] The dramatic increase in the abundance of the μ_m/μ_s-mRNA between days 16 and 18 of gestation probably reflects an increasing number of pre-B and B cells with fully assembled *Igh* genes. However, other factors may also play a role, such as increased stability of

Fig. 2. Northern blot analysis of *Igh-V558* transcription in BALB/c normal (N) and BALB/c-scid (S) fetal liver. Poly(A)⁺ RNA from 16- and 17-day-old embryos was hybridized with RNA probes derived from pV.A1Pst, specific for members of the *Igh-V558* gene family (a) and from spCμ, specific for *Igh-Cμ* (c). A longer exposure of the filter in (c) is shown in (d). To control for equal loading of RNA samples, the filter in (a) was rehybridized with p49 (b).

the μ_m/μ_s-mRNA compared to the sterile Iμ transcripts (Kelley and Perry, 1986).

In contrast to C.B-17 normal embryos, C.B-17scid embryos did not express μ_m- or μ_s-mRNA (Figure 1a and b). The only Cμ⁺ transcripts detectable were the sterile Iμ transcripts. The abundance of these transcripts remained relatively constant between days 16 and 18 and was 65% of that seen in 16-day-old C.B-17 normal embryos, as estimated by densitometric scanning of the autoradiographs. Similar results were obtained with poly(A)⁺ RNA derived from adult bone marrow of normal C.B-17 versus C.B-17scid mice. Whereas the μ_m- and μ_s-mRNA was found in RNA from normal bone marrow (Figure 1d) no such transcripts were observed in the case of scid bone marrow; only sterile Iμ transcripts and Cμ⁺ transcripts of 1.9 and 1.6 kb were found (Figure 1e). The latter transcripts are also sterile transcripts that have been described previously (Kemp *et al.*, 1980b; Alt *et al.*, 1982; Nelson *et al.*, 1983) and are designated here as Sμ. They were regularly found in poly(A)⁺ RNA from A-MuLV-transformed pre-B cell lines derived from both normal and scid bone marrow (data not shown).

It has been shown previously (Yancopoulos and Alt, 1985) that unrearranged *Igh* variable region genes of the J558 gene family (*Igh-V558*) are expressed in fetal liver of BALB/c mice and in A-MuLV-transformed cell lines of BALB/c fetal liver. Moreover, this expression is not seen in T cells or other hematopoietic cells. Therefore, we looked for *Igh-V558* transcripts in fetal liver of 16- and 17-day-old BALB/c-scid embryos with BALB/c normal embryos serving as controls. Figure 2a shows a Northern blot analysis of fetal liver poly(A)⁺ RNA probed with pV.A1Pst, which was derived by subcloning the *Igh-V558*-specific pV.A1 probe of Yancopoulos and Alt (1985) into an SP6 expression vector. Various size transcripts were seen as noted previously by Yancopoulos and Alt (1985). In agreement with these investigators, two prominent germ-line *Igh-V558* transcripts of 0.75 kb and 4.3 kb were visualized in both BALB/c normal and scid mice (Figure 2a). μ_m- and μ_s-mRNA

Fig. 3. Northern blot analysis of *Igl-Cα* transcription in BALB/c normal (N) and BALB/c-*scid* (S) fetal liver. This analysis made use of the same filter as in Figure 2a; after removal of the first probe the filter was rehybridized to a probe ($p\kappa^0$) specific for the *Igl-Cα* locus.

Fig. 4. Northern blot analysis of $\lambda 5$ transcription in C.B-17 normal (N) and C.B-17-*scid* (S) mice. Poly(A)⁺ RNA from fetal livers of 16-, 17- and 18-day-old embryos (4 μ g/lane) and from adult bone marrow (3 μ g/lane) was hybridized to $p\lambda 5$ (a and c). To control for equal loading of RNA samples each filter was rehybridized with $p49$ (b and d). The bone marrow RNA containing filter in c and d is the same as in Figure 1d hybridized after the removal of the first probe.

(2.4 kb and 2.1 kb) that also hybridized to $pV.A1Pst$, and therefore expressed an *Igh-V558* gene, appeared at high levels in 17-day-old normal embryos (compare Figure 2a and c). However no 2.4-kb $C\mu^+$ transcripts were detected in BALB/c-*scid* embryos (Figure 2d) and thus the 2.4-kb transcripts seen with $pV.A1Pst$ in *scid* embryos (and perhaps also a portion of those seen in normal embryos) must correspond to additional germ-line *Igh-V558* transcripts. The level of these germ-line transcripts was estimated by densitometric scanning to be 60% of normal for day 17.

Constant region transcripts of the unrearranged Ig κ light chain locus (*Igl-Cκ*) also serve as a specific marker of B cells (Nelson *et al.*, 1985; Cook and Balaton, 1987). These germ-line transcripts initiate in a region ~8 kb upstream from the *Igl-Cκ* gene (Van Ness *et al.*, 1981). A 400-nt RNA probe ($p\kappa^0$) containing this transcriptional initiation site was derived from a DNA clone previously described by Van Ness *et al.* (1981) and was used to probe the poly(A)⁺ RNA from fetal liver of BALB/c normal and BALB/c *scid* embryos. As shown in Figure 3, 1.1-kb transcripts were revealed in both normal and *scid* embryos. These 1.1-kb transcripts are processed derivatives of an 8-kb nuclear transcript (Kelley *et al.*, 1988) and must derive from unrearranged *Igl-Cκ* genes because $p\kappa^0$ represents a DNA sequence that is normally lost upon $V\kappa$ -to- $J\kappa$ recombination. The 1.1-kb κ^0 transcript was less abundant in the case

Fig. 5. Northern blot analysis of T lymphocyte-specific gene expression. Poly(A)⁺ RNA from C.B-17 normal (N) and C.B-17-*scid* (S) thymus of 6- to 8-week-old mice was blotted onto separate filters and hybridized to $pC\gamma 2$, $pC\beta 2$, $pC\alpha$, and $pT3\delta$. The amount of RNA applied to each lane of the respective filters (normal versus *scid*) was: 2.0 μ g versus 0.2 μ g (a) and 0.5 μ g versus 3.0 μ g (b–d).

of *scid* where it was estimated by densitometric scanning to be 21% of normal for day 17.

Sakaguchi *et al.* (1986) recently described a new gene, $\lambda 5$, which appears to be expressed only in early B cells. $\lambda 5$ shows sequence homology to Ig λ light chain genes but is not rearranged during lymphocyte development (Sakaguchi and Melchers, 1986) and is thus not considered an antigen receptor gene. The B cell specificity of $\lambda 5$ expression was confirmed in the present study by Northern blot analysis of poly(A)⁺ RNA extracted from a panel of A-MuLV-transformed cell lines and early T cell lymphomas of both C.B-17-*scid* and C.B-17 normal mice (data not shown). The Northern blots in Figure 4a and b show that $\lambda 5$ is expressed in fetal liver and adult bone marrow. In fetal liver of both C.B-17 normal and *scid* embryos there is a dramatic increase in the abundance of $\lambda 5$ transcripts between days 16 and 17 of gestation, followed by a slight decrease at day 18. As estimated by densitometric scanning, $\lambda 5$ transcription in liver of 17- and 18-day-old *scid* embryos reached a level of 50% compared to that found in respective normal tissue. No difference was seen for day 16 of gestation between *scid* and normal embryos.

Evidence for T-lymphocyte lineage-committed cells in *scid* mice: expression of T-lymphocyte specific genes

Two sizes of *TCRα*, *TCRβ* and *TCRγ* transcripts are generally found in thymocyte RNA of normal mice, reflecting different stages of developing T-lymphocytes. These are 'full-length' transcripts produced from fully assembled *TCR* genes and shorter transcripts which derive from germ-line or partially rearranged genes (Chien *et al.*, 1984; Saito *et al.*, 1984; Siu *et al.*, 1984; Snodgrass *et al.*, 1985). The sizes of these transcripts are 1.5/1.3 kb for *TCRγ*, 1.3/1.0 kb for *TCRβ* and 1.7/1.4 kb for *TCRα* genes. As demonstrated by Northern blot analyses shown in Figure 5a–c, C.B-17-*scid* thymocytes expressed only the 1.3-kb *TCRγ* and 1.0-kb *TCRβ* transcripts; *TCRα* transcripts were undetectable. In contrast, thymocytes of C.B-17 normal mice expressed both the full-length and shorter *TCRγ*, *TCRβ* and *TCRα* transcripts. Interestingly, the 1.3-kb *TCRγ* transcript was more abundant in *scid* than in normal thymocytes, whereas the converse was true for the 1.0-kb *TCRβ* transcript. [Notice that 10 times more RNA from normal thymocytes compared to *scid* was loaded on the gel to demonstrate *TCRγ* transcription (Figure 5a), whereas a 6-fold amount of *scid* versus normal RNA was loaded to demonstrate *TCRβ*, *TCRα* and *T3δ* transcription (Figure 5b–d).] The absence of full-length *TCRγ* and *TCRβ*

transcripts in *scid* thymocytes is in accord with our previous inability to detect *TCR γ* and *TCR β* rearrangements in these cells (Schuler *et al.*, 1986). Thus, we feel that the 1.3-kb *TCR γ* and 1.0-kb *TCR β* transcripts correspond to germ-line transcripts. It should be noted here that the 1.0-kb *TCR β* transcript is generally believed to be a product of partially rearranged (i.e. D β -to J β) *TCR β* genes (Siu *et al.*, 1984; Clark *et al.*, 1984). However, these studies could not formally rule out transcription from an unrearranged *TCR β* gene, and at least one T cell line has been reported, a human leukemic cell, that transcribes the *TCR β* gene from germ-line configuration and gives rise to the shorter *TCR β* transcript (Furley *et al.*, 1986).

TCR (α,β) proteins are associated with three other membrane proteins, T3 γ , T3 δ and T3 ϵ , which together form a multi-subunit receptor complex (for a review see Weiss *et al.*, 1986). Expression of the gene encoding the T3 δ chain is restricted to the T-lymphocyte lineage (van den Elsen *et al.*, 1984) and as shown in Figure 5d, T3 δ is clearly expressed in C.B-17*scid* thymocytes although at a lower level than in C.B-17 normal thymocytes. T3 δ is expressed at very early stages in T-lymphocyte development, probably prior to the expression of full-length *TCR β* transcripts since *scid* thymocytes lack such transcripts. Moreover, the human leukemic cell line mentioned above, having both *TCR β* alleles still in germ-line configuration, also expresses the T3 δ gene (Furley *et al.*, 1986).

Discussion

The preceding results show that transcripts of unrearranged *Igh* and *TCR* genes as well as $\lambda 5$ and T3 δ transcripts are present in lymphopoietic organs of *scid* mice. The relevance of these findings (i) to our earlier hypothesis (Schuler *et al.*, 1986) that recombination of antigen receptor genes is highly error prone in developing *scid* lymphocytes, and (ii) to the 'accessibility model' of regulated *Ig* gene rearrangement (see Alt *et al.*, 1986), is discussed below.

One of the predictions of our earlier hypothesis, which we will refer to as the deregulated recombinase hypothesis, is that B and T lineage-committed cells should develop in *scid* mice. However, previous attempts to demonstrate these cells by either serological or functional assays have been negative (Bosma *et al.*, 1983; Dorshkind *et al.*, 1984; Habu *et al.*, 1987). Further, we have been unable to detect *Ig* or *TCR* rearrangements in *scid* lymphoid tissues (Schuler *et al.*, 1986). These negative results emphasize one of the difficulties of the deregulated recombinase hypothesis; namely that it is based on studies of transformed *scid* lymphocytes where neither the stage of the lymphoid progenitor that gives rise to the resulting transformed cells nor the effect of the transforming agents is known.

Direct evidence of B lineage-committed *scid* cells is now provided in the present study by Northern blot analysis of poly(A)⁺ RNA of fetal liver and adult bone marrow. Transcripts of $\lambda 5$ and of *Igh-C μ* , *Igh-V558* and *Igh-C κ* germ-line genes were readily detected in these tissues. As already shown by others (Kemp *et al.*, 1980a; Alt *et al.*, 1982; Van Ness *et al.*, 1985; Yancopoulos and Alt, 1985; Sakaguchi *et al.*, 1986; Cook and Balaton, 1987) all of the above transcripts, except the sterile C μ ⁺ transcripts, are expressed exclusively in cells of the B lymphocyte lineage. The apparent absence of μ_m - and μ_s -mRNA transcripts is

consistent with our previous inability to detect *Igh* rearrangements by direct analysis of *scid* bone marrow or fetal liver (Schuler *et al.*, 1986).

Likewise, direct evidence of T lineage-committed *scid* cells was obtained from Northern blot analysis of poly(A)⁺ RNA of thymus. Transcripts of the T3 δ locus and the unrearranged *TCR γ* and *TCR β* loci were present in the thymus of 6- to 8-week-old *scid* mice. Whereas *TCR γ* expression is seen in both early T and early B cells (Cook and Balaton, 1987), the expression of T3 δ and *TCR β* is restricted to T cells (van den Elsen *et al.*, 1984; Yanagi *et al.*, 1984; Cook and Balaton, 1987). The immature nature of the thymic T cells in young adult *scid* mice is evident from the results shown in Figure 5. Relative to normal thymus, *scid* thymus contained very high levels of the 1.3-kb *TCR γ* transcript, low levels of the 1.0-kb *TCR β* transcript and no detectable *TCR α* transcripts. This pattern of *TCR* expression resembles that seen in fetal thymus of 15-day-old normal embryos (Raulet *et al.*, 1985; Snodgrass *et al.*, 1985; Haars *et al.*, 1986). 'Full-length' transcripts derived from fully assembled *TCR γ* or *TCR β* genes were not found in *scid* thymocytes. This is again consistent with our previous inability to demonstrate *TCR γ* and *TCR β* rearrangements in *scid* thymocytes (Schuler *et al.*, 1986).

Taken together, the preceding results indicate that B and T lineage-committed cells do arise in *scid* mice and develop to a stage where antigen receptor genes are transcribed in germ-line configuration. As shown by others (Van Ness *et al.*, 1981; Yancopoulos and Alt, 1985; Furley *et al.*, 1986) *Ig* and *TCR* genes become transcriptionally active prior to their rearrangement. This transcriptional activity appears to be a prerequisite of gene rearrangement and is thought to reflect the accessibility of antigen receptor loci to the recombinase system (for a review, see Alt *et al.*, 1986). In this respect, developing lymphocytes in *scid* mice appear to reach the stage at which constant and variable region genes become accessible to the recombinase system. According to the deregulated recombinase hypothesis, recombination of antigen receptor genes does occur in these cells, but it is very imprecise and generally non-functional. Affected cells would be unable to produce functional *Ig* or *TCR* proteins and presumably would turn over rapidly or be eliminated by myeloid cells (e.g. macrophages) and/or natural killer (NK) cells. Consistent with the latter idea are recent reports of successful outgrowth of *scid* B cells in long-term bone marrow cultures under conditions in which myeloid and NK cells do not grow (Witte *et al.*, 1987; Hirayoshi *et al.*, 1987). Interestingly, the cultured *scid* B cells show *Igh* rearrangements but, in contrast to B cells derived from normal bone marrow, fail to produce μ chains.

The observation that transcripts of *Igh-V558*, *Igl-C κ* and *TCR β* germ-line genes and those of the $\lambda 5$ and T3 δ loci were more abundant in lymphoid tissue of normal mice than of *scid* mice is also consistent with the deregulated recombinase hypothesis. This difference would reflect the contribution of cells that have already undergone antigen receptor gene rearrangement and that persist in the lymphoid tissues of normal mice but are eliminated in those of *scid* mice.

Finally, with respect to the concept of ordered rearrangement of *Igh* and *Igl* genes and the accessibility model of regulated *Ig* gene rearrangement (reviewed in Alt *et al.*, 1986), it is of interest that *Igl-C κ* is transcribed in germ-line configuration in *scid* fetal liver. One of the postulates

of this model is that the product of a functionally rearranged *Igh* allele (μ chain) would signal the 'opening' and transcriptional activation of the *Igl*(κ) locus. This locus would now presumably be accessible to the recombinase system. However, we were unable to detect *Igh* gene rearrangements in freshly harvested lymphoid cells from *scid* mice (Schuler *et al.*, 1986). Furthermore, as shown here, μ_m^- or μ_s^- mRNA was not detectable in *scid* fetal liver. Thus, in *scid* mice, germ-line *Igl-C κ* transcripts appear to be made by cells that are committed to the B lymphocyte lineage but that have not rearranged their *Igh* genes. This raises the possibility that non-immunoglobulin factors may be sufficient to induce the transcriptional activation of *Igl-C κ* genes. This possibility is supported by the *in vitro* finding that bacterial lipopolysaccharide can induce transcription of the unrearranged *Igl-C κ* genes in pre-B cell lines in the absence of functionally rearranged *Igh* genes and independently of DNA and protein synthesis (Nelson *et al.*, 1985).

Materials and methods

Mice

The *scid* mutation occurred in the C.B-17/Icr (C.B-17) inbred strain, an *Igh* congenic strain of BALB/cAnIcr (BALB/c). C.B-17 mice homozygous for *scid* are designated C.B-17*scid* mice. The *scid* locus was introduced into BALB/c mice by selective breeding; these mice are designated as BALB/c-*scid* mice. All mice were bred and maintained under specified pathogen-free conditions in the barrier facility of the Institute for Cancer Research, Philadelphia, PA. C.B-17*scid* and BALB/c-*scid* mice were housed in microisolator cages. Embryos at different days of gestation were derived from timed matings; the day of vaginal plugs was counted as day 0.

Hybridization probes

Plasmid pC μ 3741 is a cDNA clone encoding most of the *Igh-C μ* region (Marcu *et al.*, 1980). Plasmid pC α is a 370-bp *Xho*II–*Ava*II fragment, spanning the *TCR α* constant region gene, cloned into pUC 12 (pC α was a kind gift of A. Iwamoto). Plasmid pPEM-T3 δ contains a cDNA encoding the mouse T3 δ chain (van den Elsen *et al.*, 1985). The recombinant cDNA clone p49 encodes the ribosomal protein L7 (Meyuhas and Perry, 1980). ³²P-Labeled hybridization probes were derived from these plasmids by nick-translation (Rigby *et al.*, 1977). The probe spC μ was constructed by subcloning a 567-bp *Bam*HI–*Pst*I fragment (of a C μ -containing cDNA) into the transcription vector pSP64 (Promega, Madison, WI). The *Igh-V558* specific probe pV.A1 *Pst*I was derived by subcloning a 200-bp *Pst*I–*Pst*I fragment from the A1 cDNA clone (Yancopoulos and Alt, 1985) into the transcription vector pGEM-3 (Promega). (The A1 cDNA clone was a kind gift of G. Yancopoulos.) Probe p λ 5 was obtained by subcloning the 260-bp *Eco*RI–*Xho*II fragment from plasmid pZ183-1aEX (Sakaguchi *et al.*, 1986) into pGEM-3. (Plasmid pZ183-1aEX was kindly provided by F. Melchers.) The p κ^0 probe was constructed by subcloning the 400-bp *Eco*RI–*Taq*I κ^0 fragment of pKp6 (Van Ness *et al.*, 1981) into pSP64. Probe pC β 2 was derived by subcloning a 2.2-kb *Pst*I–*Hind*III fragment containing the *TCR-C β 2* constant region gene (Malissen *et al.*, 1984) into pSP64; pC β 2 also hybridizes to *TCR-C β 1*. Plasmid pC γ 2 hybridizes to the *TCR-C γ 1*, *TCR-C γ 2* and *TCR-C γ 3* constant region genes and was derived from the cDNA clone p8/10-2 γ 1.1 (Iwamoto *et al.*, 1986) by subcloning the 850-bp *Ava*I–*Eco*RI fragment, containing *TCR-C γ 2*, into the expression vector pSP65 (p8/10-2 γ 1.1 was a kind gift of A. Iwamoto). The restriction fragments subcloned into the transcription vectors were in reverse orientation with respect to the SP6 promoter. ³²P-Labeled RNA-hybridization probes were derived from these plasmids by transcription of the linearized plasmids employing SP6 RNA polymerase and using [³²P]GTP (Melton *et al.*, 1984).

RNA analyses

Bone marrow and thymus glands from 6- to 8-week-old mice and fetal livers were immediately frozen after dissection. Total cellular RNA was extracted from these tissues following the guanidinium/cesium chloride method given by Chirgwin *et al.* (1979). The poly(A)-containing RNA fraction was purified by oligo-deoxythymidylic acid–cellulose chromatography (Maniatis *et al.*, 1982). For Northern blot experiments, poly(A)⁺ RNA was electrophoresed through 1.2% agarose/formaldehyde gels (Maniatis *et al.*, 1982), transferred

to Nytran membranes (Schleicher and Schuell, Keene, NH) and cross-linked to the membrane by UV irradiation (Church and Gilbert, 1984). Hybridization with ³²P-labeled probes was carried out following a modification of the procedure described by Singh and Jones (1984). Briefly, hybridization was carried out for 21 h at 42°C with nick-translated hybridization probes, or at 65°C with RNA hybridization probes, in 4 × SET (1 × SET: 150 mM NaCl; 2 mM EDTA; 30 mM Tris, pH 7.4) supplemented to contain 50% formamide, 0.5 mg/ml heparin, 0.1% sodium pyrophosphate and 1% SDS. After hybridization, filters were washed at 68°C in 0.1 × SET/1% SDS and then autoradiographed. The sizes of the RNA components were estimated relative to a poly(A)⁺ RNA ladder (9.5, 7.5, 4.4, 2.4, 1.4 and 0.3 kb; BRL, Gaithersburg, MD) which was run in a parallel track and was also transferred to the Nytran membrane. After processing of the membrane the marker lane was cut off and the marker bands visualized by staining with methylene blue (Maniatis *et al.*, 1982).

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