

REARRANGEMENT AND EXPRESSION OF T CELL ANTIGEN RECEPTOR AND γ GENES DURING THYMIC DEVELOPMENT

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T cell precursors arise from pluripotent hematopoietic stem cells that are located in the fetal liver or the adult bone marrow (1–6). These precursors migrate to the thymus, where they proliferate and differentiate, giving rise to functional T lymphocytes that can respond to antigen. Immunocompetent T cells generated in the thymus eventually emigrate to the peripheral lymphoid tissues such as the spleen and lymph nodes (7–9). The thymus consists of >95% T lymphocytes at different stages of maturation, and a variety of nonlymphoid stromal cells that are thought to play an important role in T cell development. In addition to their role in promoting T cell development, nonlymphoid cells in the thymus may influence the specificity of the T cell antigen receptors that are expressed by mature T cells (10–14), so that they recognize antigens predominantly or exclusively in the context of self MHC-encoded molecules. This process is often referred to as thymic selection or education (15–17).

The T cell antigen receptor has recently been defined as a heterodimeric, transmembrane glycoprotein composed of disulfide-bonded α and β polypeptide chains (18–20). cDNA clones corresponding to both of these chains have been isolated, and the nucleotide sequence of these clones shows that they have regions homologous to Ig V and J gene segments and to C region genes (21–25). The genomic organization and mechanism of rearrangement of the α and β genes also show striking homology with the Ig genes (reviewed in 26–28). In addition, the γ gene is also transcribed specifically in T cells, exhibits sequence similarity compared with Ig gene segments, and undergoes gene rearrangement (29–31). However, the product of the γ gene has not been shown to be part of the serologically-defined T cell receptor (32–33), and its function is unknown. Cell surface expression and function of the T cell receptor apparently requires coexpression of another cell-surface molecule, T3 (34), that is not directly

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involved in antigen recognition. In humans, the T3 molecule is composed of three invariant polypeptide subunits (35). The entire sequence of two of these subunits has been determined (36, 37) and neither resembles Ig.

The rearrangement and expression of T cell receptor genes is clearly an important step in T cell differentiation, and is a prerequisite for the selection of a repertoire of T cell antigen receptors that recognize antigens along with self MHC molecules. To understand these processes better, we have studied the timing and location of β and γ gene segment rearrangement and expression of the α , β , and γ genes, and the gene encoding the δ subunit of the T3 molecule (37) in fetal animals. We have also determined the extent of β gene rearrangement in several adult thymocyte populations. We find that the rearrangement and expression of the α , β , and γ gene families in fetal tissues occur in a well-defined order. Although some γ gene segment rearrangement occurs in fetal liver cells that have not migrated to the thymus, all detectable α and β gene expression and β gene rearrangement occur in the thymus. In adult thymocytes and T cells, β gene rearrangements generally occur on both chromosomal homologs, even in subpopulations in the adult thymus that do not contain functional cells. The implications of these findings for the intrathymic maturation of T cells and the selection of T cell antigen receptors is discussed.

Materials and Methods

Animals. BALB/cJ and C57BL/6J mice were obtained originally from The Jackson Laboratories, Bar Harbor, ME. C57BL/Ka mice have been bred at Stanford University since 1952 and were originally obtained from Dr. L. Strong at Yale University (New Haven, CT). C.AL-20 mice have been bred at Tufts University (Sommerville, MA) since 1978 from stock originating from M. Potter, National Institutes of Health (Bethesda, MD). C.AL-20 is an Ig H chain allotype-congenic strain that has the AKR/J allotype, Igh-1^d, on a BALB/cAnN background. Fetal thymus tissue and fetal livers were obtained from inbred matings of C57BL/6J or C.AL-20 mice. Females and males were caged overnight and checked for vaginal plugs the next morning. The day of vaginal plug was designated day 0. For the preparation of RNA from fetal thymus tissue and livers, the tissues were frozen immediately after dissection. For DNA preparation, a cell suspension was prepared by collecting fetal thymus tissue on a stainless steel mesh submerged in medium (RPMI 1640 with 5% FCS). The tissue was teased over the mesh with a glass pestle.

Hybridomas and Cell Populations. Hybridomas derived from fetal tissue or adult bone marrow were prepared and characterized as described previously (38). All hybridomas listed in Table 1 were derived from C.AL-20 mice, except the bone marrow-derived hybridomas, which are from AKR mice. Hybridomas were derived from fusions with the T lymphoma BW5147, except FBL12.3, which was fused to the P-3U1 myeloma. Adult bone marrow cell hybridomas were made from mice homozygous (A3) or heterozygous (B6) for an allele of the nude (*nu*) mutation. Thymocyte cell suspensions from 5-7-wk-old C57BL/6J mice were separated by panning with peanut agglutinin (PNA)¹ into PNA^{hi} and PNA^{lo} populations as described by (39). Thymocytes were also separated by FACS according to cell-surface phenotype using the antibodies MEL-14 (40) and 20.8.4S for H-2K^b (41). When sorting for the level of expression of MEL-14, FACS gates were chosen to sort for the 5% of thymocytes that have the highest MEL-14 expression (MEL-14^{hi}), and the 10% that express the lowest levels (42). Similarly, FACS fluorescence gates were chosen to sort thymocytes that lack detectable expression of H-2K^b; a coselection by forward light scatter restricted sorting to small thymocytes. This population contained cells that were <1% MEL-14^{hi}, and were >95% Lyt-2⁺, L3T4⁺.

¹ Abbreviation used in this paper: PNA, peanut agglutinin.

Hybridization Probes. T cell receptor β gene probes (Fig. 5) were purified restriction fragments derived from the β gene cosmid 2.3W7 (43) or subclones derived from this cosmid (43–45). The cDNA probe for the C region of the α gene has been described previously (46). A γ gene cDNA probe was provided by Dr. A. Winoto (California Institute of Technology). It was obtained by screening a cDNA library constructed from BALB/cJ adult thymus RNA with an oligonucleotide probe specific for the C region of the γ gene cDNA clone, pHDS4 (29). The 700 bp cDNA insert contains both V γ and C γ sequences (A. Winoto, unpublished results). Plasmid pPEM-T3d, containing a cDNA insert coding for the mouse T3 δ chain, was provided by Drs. P. van den Elsen and C. Terhorst (Dana-Farber Cancer Institute, Boston, MA) (37). A *c-myc* probe provided by Dr. R. Barth (California Institute of Technology) was used as a hybridization standard in some of the densitometric measurements. The probe is a 1.75 kb Sac I/Hind III fragment containing part of the second intron and third exon of the germline *c-myc* gene.

Southern Blotting and Densitometry. High molecular weight DNA was prepared from mouse livers, thymus tissue, and hybridomas by the method of Blin and Stafford (47). Genomic DNA was digested with either a 10-fold excess of restriction enzyme for 5 h or a 5-fold excess of restriction enzyme for 12–14 h at 37°C. Digested DNA was electrophoresed on 0.8% agarose gels, transferred to nitrocellulose or Nytran (Schleicher and Schuell, Inc., Keene, NH) filters as described by Southern (48), except 1 M ammonium acetate was used as the neutralization buffer (49). After prehybridization for 1 h at 68°C, the filters were hybridized with nick-translated (50) probes for 12–14 h at 68°C in the presence of 10% dextran sulfate. The filters were then washed four times each for 30 min in 0.1× SSC with 0.1% SDS at 68°C, and after drying the filters were exposed to Kodak XR-70 film in the presence of intensifying screens. The approximate sizes of the hybridizing restriction fragments were estimated by comparison with the migration distances of Hind III restriction fragments of λ phage DNA. To quantitate the amount of gene rearrangement, autoradiograms were scanned in a Cary recording spectrophotometer (Varian Instruments, Palo Alto, CA), and hybridization bands were recorded as peaks of absorbance. The strength of the hybridization signal was quantified by measuring the peak areas using the Master Gelscan program from Varian Instruments and an Apple IIe computer. To obtain accurate quantitations, different amounts of each DNA sample were electrophoresed in adjacent lanes on the same gel, and after blotting and hybridization, the filters were exposed for varying time periods (24 h to 1 wk). To assure that exposures were within the linear range of the films, the signals from adjacent lanes of the gel containing different concentrations of the same sample were compared to one another. As an internal control for experimental artifacts related to the Southern transfer, the same lanes were also hybridized with a probe that detects a restriction fragment that does not undergo gene rearrangement. For the quantitative data shown, at least four separate determinations in the linear range were available.

Northern Blots. RNA was prepared from mouse livers and thymus tissue by homogenization of frozen tissue in guanidinium thiocyanate followed by centrifugation through a cushion of CsCl (51). Total RNA (up to 30 μ g/lane) was electrophoresed on 1% agarose-formaldehyde gels, transferred to nitrocellulose filters, and hybridized with nick-translated probe DNA according to published protocols (52, 53). The filters were processed as described above except that washing was carried out in 2× SSC with 0.1% SDS at 37°C.

Results

Experimental Strategy. Gene expression in differentiating T cells was tested by hybridizing Northern blots of total RNA from thymus or fetal liver with the appropriate probes. To determine the extent of gene rearrangement, DNA from whole organs or cell populations was analyzed on Southern blots. If an identical or similar rearrangement occurs in many cells in a population, this can be directly observed. Such prevalent rearrangements have been found for the γ genes, which apparently have a very limited germline V and J gene segment diversity

(31). For the β gene family, which contains multiple V_β , D_β , and J_β gene segments (reviewed in 25 and 54), individual rearrangements are present at too low a frequency to be detected on Southern blots of DNA from a heterogeneous lymphoid population. Nevertheless, every rearrangement alters the germline pattern, and therefore the degree of rearrangement can be estimated visually by assessing the loss of germline restriction fragments. In some instances, we quantitated the amount of β gene rearrangement by performing densitometry on the autoradiograms resulting from these Southern blots. Finally, to verify that the conclusions drawn from analysis of heterogeneous populations apply well to individual cells, we analyzed the DNA from hybridomas made by fusing fetal or adult cells to tumor cell lines. Germline J_α gene segments are spread out over at least 60 kb of DNA located 5' of the C_α gene (46). α gene segment rearrangements are therefore difficult to detect using a C_α probe, and we did not analyze the timing of rearrangement in this gene family.

Gene Rearrangement and Expression in Prethymic Cells. Because there are as yet no cell-surface markers that permit purification of T cell precursors that will migrate to the thymus, we analyzed nucleic acids extracted from whole fetal liver and from hybridomas made by fusing tumor cell lines with fetal liver cells. Previous work (29–31) has shown that there are three closely-related C_γ genes and at least one subfamily of three V_γ gene segments. Hybridization of Eco RI-digested C57BL/6J strain fetal liver DNA from day 12 of gestation with a γ probe that contains both V and C sequences, reveals the presumptive germline bands of ~14.0 kb (containing a C_γ gene), 11.0 kb (containing a C_γ gene on one restriction fragment and two V_γ gene segments on a separate restriction fragment at nearly identical size), 8.0 kb (containing a C_γ gene), and 5.2 kb containing a V_γ gene segment (30). Using the same probe, fetal liver DNA from day 12 of gestation also contains a single rearranged band of 9.5 kb (Fig. 1B, lane 1). A rearranged γ gene fragment of identical size can also be detected in C.AL-20 strain day 13 fetal liver DNA (day 12 fetal liver from this strain was not tested). Although the hybridization signal from this fragment varied, in most cases this 9.5 kb nongermline fragment hybridized much less intensely (<10%) than did the germline bands (Fig. 1A, lanes 1 and 3). This rearranged band of 9.5 kb is different in size from the predominant γ gene rearrangements (~16 and 22 kb) that have been observed in Eco RI-cut DNA from adult C57BL/6J and BALB/CJ thymocytes and mature T cells (30, 31) and the structure of the gene segments participating in this rearrangement have not yet been determined. However, the unique size of this rearranged fragment cannot be explained by interstrain restriction fragment length polymorphism, because the mice used in these experiments are from the same strains analyzed previously, or from the C.AL-20 congenic strain that differs from BALB/c only for the Ig H chain genes. In some cases, the presence of the 9.5 kb rearrangement in Eco RI-digested DNA was correlated with a diminution of hybridization signal from the 14 kb germline C_γ restriction fragment (Fig. 1B, lanes 1 and 4), indicating this C_γ gene may have been rearranged or deleted. Although γ gene rearrangement was observed in day 12 or in day 13 fetal liver, no rearrangement was observed in fetal liver DNA from later stages including days 14 through neonate (Fig. 1A, lane 2; B, lanes 2 and 3; and other data not shown).

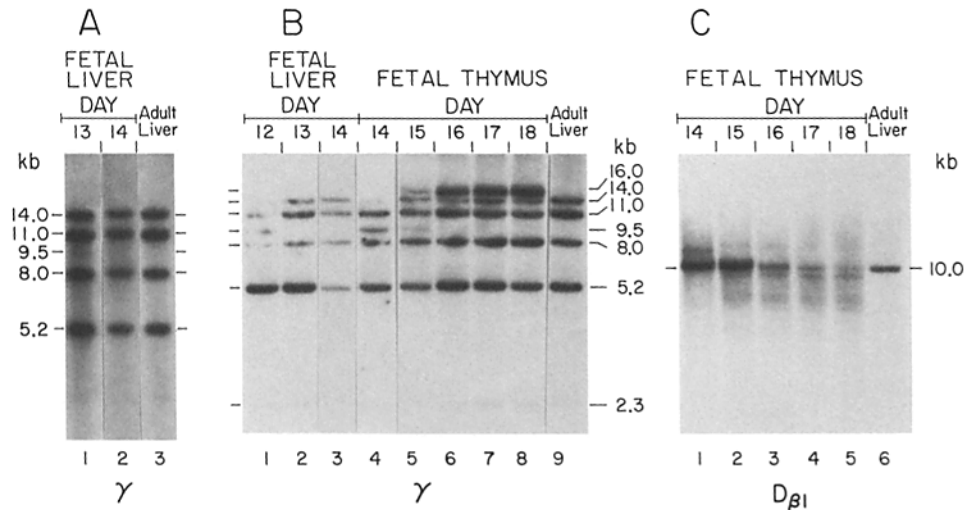


FIGURE 1. β and γ gene rearrangements during ontogeny. Analysis of gene rearrangement in DNA from adult liver and from fetal tissue of the indicated developmental stages obtained from C.AL-20 (A) or C57BL/6J strain mice (B and C). All DNA samples were digested with Eco RI. Filters were hybridized with a γ gene cDNA (A and B), and the $D_{\beta 1}$ probe (C), as indicated. Exposure times were 7 d (A), 5 d (B), and 1 d (C). The approximate sizes of the hybridizing restriction fragments are indicated.

To study rearrangements in cloned cells that might be precursors to thymic T cells, we examined the DNA from six hybridomas that were made by fusion of fetal liver or adult bone marrow cells to HAT-sensitive tumor cells (38). Southern blot analysis of these hybridomas revealed that in some cases they have a hybridization pattern identical to the tumor cell line, indicating that they deleted all the β and/or the γ gene loci derived from the fetal cell parent (Table I and data not shown). This is most likely due to the loss of entire chromosomes from these cells. No β or γ gene rearrangements were observed in hybridomas that retained some of this DNA from the normal parent cell, and therefore we have no examples of a hybridoma with the 9.5 kb Eco RI fragment. However, for the γ genes, it is theoretically possible that a rearrangement was not detected because it generated a restriction fragment identical in size to the common 16 kb rearrangement that is present in the DNA of the BW5147 fusion partner.

Despite the presence of novel rearrangements in 5–10% of the chromosomes present in day 12 or 13 fetal liver, no transcripts were detected when poly(A)⁺ RNA from fetal liver days 12–18 of gestation was hybridized with the α , β , and γ gene probes (data not shown). T cell antigen receptor and γ gene transcripts could, however, be detected in total adult thymus RNA, which was present on the same filters as a positive control. In addition, β gene segment rearrangements were not observed in total fetal liver DNA from any stage tested. The absence of detectable γ gene transcripts, despite observable rearrangements in day 12 or 13 fetal liver, could be due to the relatively small number of cells that rearrange these genes. Alternatively, it is possible that transcriptional activation of the γ genes does not take place until well after rearrangement, when the putative thymic precursors in the fetal liver reach the thymus.

TABLE I
 β and γ Rearrangement in Fetal and Adult Hybridomas

Hybridoma	Stage	β		γ
		$J_{\beta 1}$	$J_{\beta 2}$	
Fetal thymus				
FTT17.1	d17	1D $_{\beta 1}$ -J $_{\beta 1}$, 1g	ND	$\geq 1r$
FTT17.2	d17	1D $_{\beta 1}$ -J $_{\beta 1}$, 1g	2g	$\geq 1r$
FTT19.4	d19	1D $_{\beta 1}$ -J $_{\beta 1}$	1D $_{\beta 2}$ -J $_{\beta 2}$	$\geq 1r$
Fetal liver				
FBL12.3	d12	$\geq 1g$	$\geq 1g$	$\geq 1g$
FTL12.3	d12	$\geq 1g$	$\geq 1g$	$\geq 1g$
FTL17.2	d17	2d	ND	$\geq 1g$
FTL19.2	d19	2d	ND	$\geq 1g$
Bone marrow				
A3	Adult	2d	2d	$\geq 1g$
B6	Adult	2d	2d	$\geq 1g$

Hybridomas are described in Materials and Methods and more fully by Owen (38). All the fetal liver and thymus hybridomas express the parental Thy-1 allele, Thy-1.2, and are Lyt-2⁻, L3T4⁻, except FTL 17.2, which does not express any of these three markers. g, germline; r, rearranged, d, deleted; Chromosome loss is common in these cells, and therefore, in cases where there are germline genes or very common rearrangements, it is difficult to determine whether one or two chromosomal homologs are present. We therefore used the designation ≥ 1 for these cases.

Gene Rearrangement and Expression in Fetal Thymocytes. We examined the kinetics of γ and β gene rearrangement in the fetal thymus days 14–18 of gestation. Mice have a gestation period of 19–20 d. Although immature lymphocytes first infiltrate the thymus on day 11 or 12 of gestation (1), immunocompetent T cells are not present until day 18 (55, 56). During the intervening period, the thymus increases from a few thousand to a few million cells (57 and data not shown), probably as a result of both some cell migration of precursors into the thymus and the extensive intrathymic proliferation of the migrants.

When Eco RI-digested C57BL/6J or C.AL-20 strain DNA was hybridized with a γ cDNA probe, in addition to the germline bands, one predominant rearranged fragment of 9.5 kb, identical in size to the one observed in day 12 or 13 fetal liver, was present in day 14 fetal thymus (Fig. 1B, lanes 1 and 4). On day 15 of gestation, a faint 16 kb rearranged band, which is known to include some productive rearrangements of a particular V $_{\gamma}$ with a particular J $_{\gamma}$ gene segment (30) could be detected in the C57BL/6J or C.AL-20 thymus DNA (Fig. 1B, lane 5). In addition, the 9.5 kb rearranged band described above (Fig. 1B, lanes 4 and 5) was still present. By day 16, the phenotype of the γ genes in total thymus DNA was quite similar to that present in adult thymus (Fig. 1B, lane 6, and data not shown); the 16 kb band was very intense and the 9.5 kb rearranged fragment could no longer be detected.

Rearrangement of the β genes in fetal thymocytes days 14–18 of gestation was examined by hybridization of Eco RI-digested C57BL/6J DNA with probes containing the two known germline D $_{\beta}$ gene segments, D $_{\beta 1}$ and D $_{\beta 2}$. The D $_{\beta 1}$ and D $_{\beta 2}$ gene segments are only 12 and 14 nucleotides long, respectively (44).

However, each probe also contains at least several hundred nucleotides of 5' and 3' germline sequence flanking the D_β gene segments (45). Therefore, although these probes do not detect V_β - D_β - J_β rearrangements which delete nearly all the sequences they can anneal with, they can detect rearrangements such as those involving only D_β and J_β gene segments that do not form a complete V_β gene. Several conclusions can be drawn from the hybridizations with the D_β probes (Fig. 1C and data not shown). First, most of the β -locus DNA is in the germline configuration on day 14 (Fig. 1C, lane 1), and there is an increasing amount of rearrangement in fetal thymocytes as development progresses to day 18 of gestation (Fig. 1C, lanes 1-5). The nearly complete loss of the germline 10.0 kb Eco RI fragment that hybridizes with a $D_{\beta 1}$ probe is also characteristic of adult T cells (see below). Second, on day 14, there are a few faint rearranged bands that have a size ~ 0.5 - 1.5 kb smaller than the germline fragment, consistent with rearrangement of D_β to J_β gene segments (Fig. 1C, lane 1). The intensity of these bands increases markedly on day 15, and slightly thereafter (Fig. 1C, lanes 2-5). Similar results were obtained when the same filters were hybridized with probes specific for the two J_β gene segment clusters (data not shown), demonstrating that these visible rearranged bands are derived from D_β to J_β as opposed to V_β - D_β or other rearrangements. The very early appearance of these rearranged bands suggests that D_β - J_β rearrangements are the first step in V_β gene assembly.

DNA prepared from hybridomas made by fusing fetal thymocytes from day 17 or 19 of gestation was also analyzed for β and γ gene rearrangements. Hybridization patterns that are consistent with either $D_{\beta 1}$ - $J_{\beta 1}$ or $D_{\beta 2}$ - $J_{\beta 2}$ gene rearrangements could be detected in all three fetal thymus hybridomas that retained at least one β gene locus from the fetal thymocyte parent fusion partner (Table I). No rearrangements involving joining of V_β gene segments to D_β - J_β rearrangements have occurred in these cells.

As noted above, rearrangement of the γ genes in the thymocyte hybridomas is difficult to determine because the BW5147 fusion partner has the common, nongermline, 16 kb Eco RI fragment that hybridizes with the γ gene probe. A similar rearrangement in the hybridomas would therefore be obscured by this BW5147 rearrangement. Rearrangement in the thymocyte hybridomas was, however, inferred from their retention of some, but not the full complement of, germline γ gene restriction fragments. The simplest interpretation of this result is that the fetal thymocyte fusion partner deleted some of its γ genes as a consequence of rearrangement on both homologs, or on the one remaining homolog. It is however also possible, but less likely, that these cells suffered partial deletions of their γ gene loci that are unrelated to the normal rearrangement process.

Total RNA prepared from fetal thymus tissue on days 14-18 of gestation was analyzed for the presence of α , β , γ , and T3 δ gene transcripts. To obtain a semiquantitative comparison of the level of RNA present in these tissues with that present in adult thymus, three different concentrations of total adult thymus RNA were also present on each Northern blot (Fig. 2, A-D, lanes 1-3). α gene transcripts first appear on day 16 of gestation in the fetal thymus (Fig. 2A, lane 6). At this stage, most of the α transcripts are 1.4 kb long, although very small amounts of a 1.7 kb RNA band are present. The 1.7 kb transcript is similar in

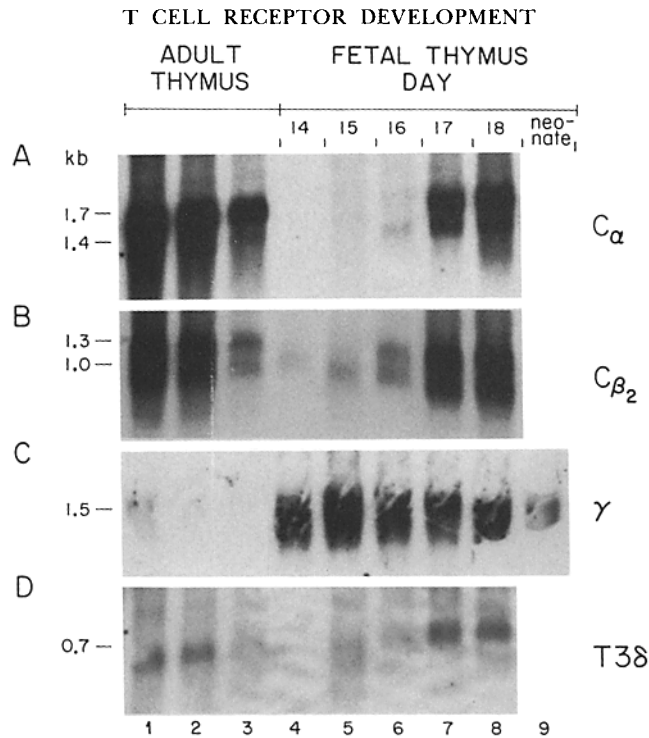


FIGURE 2. Expression of T cell antigen receptor, γ , and T3 δ genes during ontogeny. Northern blots of total cellular RNA obtained from C57BL/6J thymus tissue were performed as described in Materials and Methods. Hybridization probes were C_α (A); 3' $C_{\beta 2}$ (B); γ (C); T3 δ (D). Each filter contained 30 μg , 15 μg , and 7.5 μg of total adult thymus RNA in lanes 1, 2, and 3, respectively. Lanes 4-9 each contained 15 μg of total fetal thymus RNA from the indicated developmental stages. The same filter was used for hybridization with the C_α and T3 δ probes. After C_α hybridization and exposure to film, the probe was removed by stringent washing before it was used for rehybridization. The approximate sizes of the hybridizing RNA species are indicated. These were estimated using *E. coli* ribosomal (1.6 and 2.9 kb) and mouse ribosomal RNA (1.9 and 4.8 kb) as molecular size markers. Exposure times were 2 d (A), 4.5 h (B), 4 d (C), and 5 d (D), respectively.

size to the transcript found in mature T cells, and it is presumed to encode a functional α polypeptide. The structure of the 1.4 kb transcript has not been determined, although by analogy with the β gene locus it may be derived from a hypothetical D_α - J_α rearrangement, or it may originate from a promoter located in front of a germline J_α gene segment. There is a large increase in the amount of the 1.7 kb α transcript on day 17 of gestation; at this stage it is present at ~50% of the level detected in the adult thymus. The expression of this RNA increases steadily after day 17 until adult levels are reached. After day 16, the shorter, 1.4 kb C_α message is much less abundant than the 1.7 kb C_α transcript in total thymus RNA.

Levels of β gene expression in fetal thymus tissues were examined using probes specific for either one of the two closely related C_β genes (45). Results using a probe specific for the downstream gene, $C_{\beta 2}$, are shown in Fig. 2B. Expression of small amounts of a 1.0 kb $C_{\beta 2}$ RNA can be detected on days 14 and 15 (Fig. 2B, lanes 4 and 5). All or most of this 1.0 kb transcript is derived from a partial D_β - J_β gene rearrangement (44). Very low levels of the 1.3 kb $C_{\beta 2}$ RNA, presum-

ably derived from fully assembled V_{β} - D_{β} - J_{β} gene segments, are not observed until day 15 of gestation (Fig. 2B, lane 5). There is a large increase in the level of the 1.3 kb RNA on the 16th day of gestation (Fig. 2B, lane 6), one day earlier than the similar increase observed for the α transcript. On day 16, the 1.3 kb $C_{\beta 2}$ RNA is present at ~50% of the adult level. Analogous to the kinetics of α gene expression, β gene RNA levels increase steadily from this day until the adult level is reached. In contrast to α gene expression, nearly equal amounts of both the shorter 1.0 kb and the full-length, functional 1.3 kb species can be detected in thymus RNA on day 17 of gestation and thereafter, including the adult thymus (Fig. 2B, lanes 1-3, 6 and 7). Using a $C_{\beta 1}$ -specific probe, similar results were obtained. However, there are more $C_{\beta 2}$ than $C_{\beta 1}$ transcripts in the fetal thymus at all stages of gestation (data not shown).

Relatively high levels of γ gene expression can be detected in the fetal thymus on day 14, the earliest day tested. This is surprising, because in total thymus DNA from this stage, the 16.0 kb Eco RI fragment known to contain a productive V-J rearrangement was not detected. The γ transcripts on this day could, however, be derived from a few cells (<5%) that have this rearrangement and contain an abundant γ gene transcript, they could be derived from germline γ genes, and/or they could be from cells having the 9.5 kb Eco RI fragment described above. Maximum expression of γ gene RNA was found on day 15 of gestation (Fig. 2C, lanes 4 and 5), followed by a slow decline until day 18. This decline is more obvious on shorter exposures than the one shown in Fig. 2. Finally, there is a significant decrease in the RNA level at birth (Fig. 2C, lane 9). γ gene expression at day 15 is at least 50-fold higher than in adult thymus tissues (Fig. 2C, lane 5).

Transcripts of the gene encoding the T3 δ subunit can be detected clearly as early as day 16, although a diffuse smear of hybridizing material was present one day earlier. There is a dramatic increase in the steady-state level of this RNA on day 17 (Fig. 2D, lanes 6 and 7). The kinetics of T3 δ and α gene expression are quite similar, and expression of this T3 subunit is therefore not likely to be limiting for cell-surface expression of the T cell antigen receptor-T3 complex.

Rearrangement of β Genes in Adult Thymocyte Subpopulations. <10% of the T cells in the adult thymus have the ability to respond to antigens or mitogens (reviewed in 58). Thymocytes were separated into mature, immunocompetent, and less mature cells by two techniques, and the DNA from these cell populations was tested for gene rearrangement. Thymocytes were first fractionated according to their ability to bind PNA. Thymocytes that bind PNA well, (PNA^{hi} cells), are depleted of functional cells, whereas PNA^{lo} cells enriched for immunocompetent cells (59-61). Similarly, thymus cells that express high and low levels of the MEL-14 determinant, which is associated with the homing receptor for cells that migrate to the lymph nodes (40), were separated by FACS. <4% of adult thymocytes express high levels of the MEL-14 determinant, and this subpopulation is also enriched for immunocompetent cells and for cells which emigrate to the periphery (62, 63). When DNA from PNA^{hi}, PNA^{lo}, MEL-14^{hi}, and MEL-14^{lo} thymocytes was hybridized with β gene probes, the β genes were found to be nearly entirely rearranged on both chromosomal homologs in all four populations. For example, Fig. 3C shows hybridization of a $D_{\beta 1}$ probe to Eco RI-

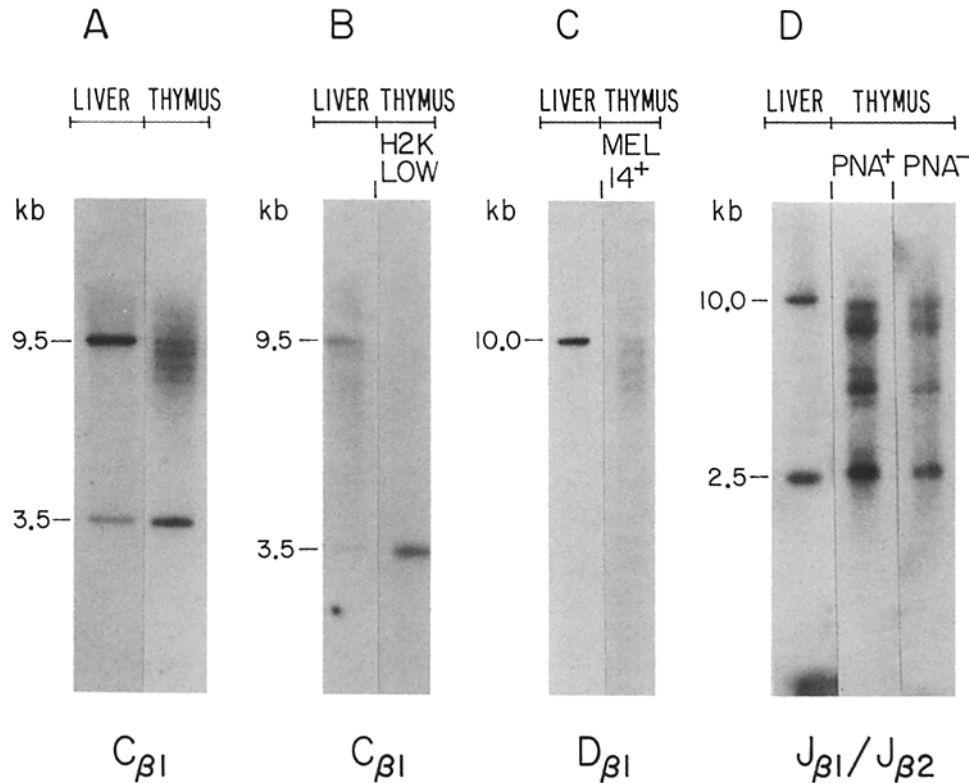


FIGURE 3. β gene rearrangement in adult thymocyte subpopulations. Thymus and liver tissue was obtained from BALB/cj strain mice (A and D), or C57BL/Ka strain mice (B and C). Thymocytes were selected according to the cell surface markers as indicated on top of each lane and as described in experimental procedures. DNA from adult liver and selected adult thymocyte subpopulations was digested with Hind III (A and B), or Eco RI (C and D). Hybridization probes are as indicated: $C_{\beta 1}$ (A and B); $D_{\beta 1}$ (C); a mixture of $J_{\beta 1}$ and $J_{\beta 2}$ (D). Exposure times were 4 d (A), 5 d (B), 3 d (C), and 2 d (D). The approximate sizes of the hybridizing restriction fragments are indicated.

digested liver DNA and to DNA prepared from MEL-14^{hi} thymocytes. Fig. 3D shows the result from hybridization of a combination of the $J_{\beta 1}$ and $J_{\beta 2}$ probes to DNA prepared from both PNA^{hi} and PNA^{lo} thymocytes. In all these cell populations, the hybridization signal from the 10 kb germline band that contains the $D_{\beta 1}$ and $J_{\beta 1}$ gene segments is much reduced, and nongermline restriction fragments having sizes consistent with $D_{\beta}-J_{\beta}$ rearrangement can be observed (Figs. 3C and D). High expression of the MEL-14 determinant is also found on nearly half of day 15 fetal thymocytes (64) which have much less β gene rearrangement (Fig. 1C, lane 2), and therefore expression of this marker is not well correlated with the rearrangement and expression of T cell receptor β genes.

The majority of thymocytes never emigrate to the spleen and lymph nodes; instead, they die in the thymus (65–66). Cells that are destined to die have a characteristic phenotype that includes a low level of expression of MHC-encoded H-2K molecules (59–61, 67, 68). To determine whether these cells have β gene segment rearrangement, we analyzed DNA from thymocytes that were selected

for their low expression of H-2K. FACS analysis of the H-2K^{lo} cells confirmed that >95% of them also express both Lyt-2 and L3T4 (data not shown). When Hind III-digested DNA from these cells was tested for β gene rearrangement, it was clear that these cells have also undergone gene rearrangement on both chromosomes. The germline DNA contains two fragments that hybridize with a $C_{\beta 1}$ probe, a 9.5 kb fragment containing both the $C_{\beta 1}$ gene and $J_{\beta 1}$ gene segments, and a 3.5 kb fragment containing the $C_{\beta 2}$ gene that hybridizes somewhat less intensely with this probe (Fig. 3, A and B). The $C_{\beta 2}$ -containing fragment has no J_{β} gene segments, and therefore is not altered by any gene segment rearrangements. While the $C_{\beta 2}$ band is present in the lane containing the H-2K^{lo} cells, the germline $C_{\beta 1}$ fragment is missing, indicative of rearrangements and/or deletions occurring on both chromosomes in most of these cells (Fig. 3B).

Quantitative Analysis of β Gene Rearrangement in Adult Thymocytes. Previous studies on various types of long-term T cell lines indicated that β gene rearrangement generally occurs on both chromosomal homologs (45). The hybridization results on DNA from normal fetal (Fig. 1) and adult thymocytes (Fig. 4) shows that this is also true for these normal cell populations. To gain a quantitative estimate of the fraction of β gene loci that remain in the germline configuration, and to determine the frequency of rearrangement to the $J_{\beta 1}$ and $J_{\beta 2}$ gene segment clusters, we performed densitometry on autoradiograms of Southern blots of adult thymus DNA (see Materials and Methods).

When 10 μ g of total thymus DNA was hybridized with a $J_{\beta 1}$ gene segment probe, densitometric measurements showed that the germline signal was <5% that of 10 μ g of adult liver DNA (Fig. 4B and Fig. 5). We therefore conclude that at least 95% of the $J_{\beta 1}$ gene segment clusters in this cell population have undergone either a rearrangement or, alternatively, a deletion as a consequence of rearrangement to the $J_{\beta 2}$ gene segment cluster. To distinguish deletion from rearrangement, we hybridized Southern blots with a $C_{\beta 1}$ probe, and measured the decrease in hybridization signal in adult thymus DNA of a 2.0 kb Eco RI fragment that contains most of the $C_{\beta 1}$ gene (Fig. 5A). This 2.0 kb germline restriction fragment does not contain any J_{β} gene segments, and therefore is not affected by rearrangement of V_{β} and $D_{\beta 1}$ gene segments to the $J_{\beta 1}$ gene segment cluster. Any decrease in hybridization signal of this C_{β} -containing fragment is therefore likely to be due to deletions of this gene as a consequence of rearrangement to one of the $J_{\beta 2}$ gene segments. ~45% of the germline $C_{\beta 1}$ hybridization signal was retained in the adult thymus DNA, indicating that 55% of the chromosomes have deleted $C_{\beta 1}$ and presumably the $J_{\beta 1}$ gene segments as well (Fig. 5B). Since >95% of the $J_{\beta 1}$ gene segment clusters are either deleted or rearranged, at least 40% of the $J_{\beta 1}$ gene segments must have rearranged,

A similar quantitative analysis was applied to the $J_{\beta 2}$ gene segment cluster. Because there are no J_{β} gene segments further 3' (Fig. 5A), we do not expect that entire $J_{\beta 2}$ gene segment clusters will undergo deletion, but rather that these gene segments either undergo rearrangement or remain in the germline configuration (Fig. 5B). We estimate that ~80% of the $J_{\beta 2}$ gene segment clusters have undergone rearrangement, while the remaining 20% are in the germline configuration. In contrast, only 40% of the $J_{\beta 1}$ gene segment clusters are rearranged, indicating that there is roughly a two-to-one bias in favor of rearrangement to

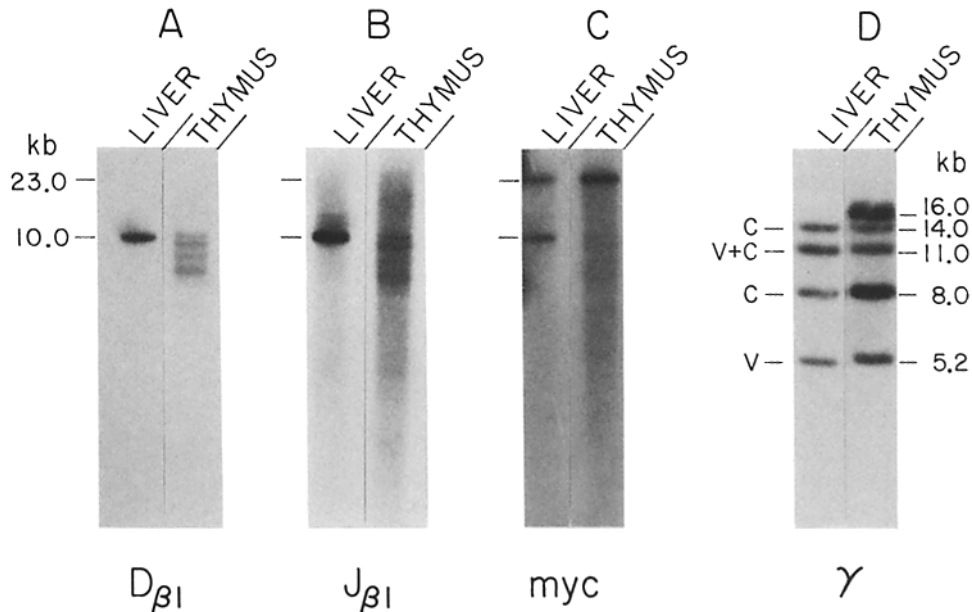


FIGURE 4. β and γ gene rearrangements in DNA prepared from unfractionated adult thymocytes. Liver and thymus tissue were obtained from young adult BALB/cJ strain mice (A–C) or C57BL/6J strain mice (D). All DNA samples were digested with Eco RI and hybridized with the indicated probes. There is ~ 2.5 times as much thymus as there is liver DNA in the adjacent lanes shown. Exposure times were 3 d (A), 2 d (B), 5 d (C), and 4 d (D). The same filter was used in B and C. After hybridization with the $J_{\beta 1}$ probe and exposure to film, the filter in B was rehybridized with the *c-myc* probe, to provide an internal standard for the hybridization signal obtained with a gene that does not rearrange in normal T cells. Leftover $J_{\beta 1}$ hybridization signal is evident. The sizes of the hybridizing restriction fragments are indicated. The exposures shown are derived from the set of exposures that was subjected to densitometry.

the $J_{\beta 2}$ gene segments in normal thymocytes. This bias may exist because one of the two known D_{β} gene segments, $D_{\beta 2}$, can only rearrange to $J_{\beta 2}$ gene segments; while $D_{\beta 1}$ can rearrange with approximately equal frequency to $J_{\beta 1}$ or $J_{\beta 2}$ gene segments. The inherent statistical bias in favor of rearrangement to $J_{\beta 2}$ gene segments may explain the greater abundance of $C_{\beta 2}$ transcripts that we have observed in thymocytes and other T cell populations (45 and data not shown).

Discussion

Developmental Control of T Cell Antigen Receptor and γ Gene Rearrangement and Expression. The analysis of fetal tissues and hybridomas presented here is consistent with an ordered expression of the genes encoded in the unlinked α , β , and γ gene families, namely that the γ gene family is expressed first, followed by the β and then the α genes. These results are summarized in Table II. γ gene transcripts can be detected in day 14 fetal thymus RNA, the earliest stage of thymus development tested (Fig. 2C, lane 4). At this time, most of the cells already in the fetal thymus are T cells, as evidenced by their expression of Thy-1 and IL-2-R (Table II) (69–72). Expression of β gene RNA precedes expression of α gene RNA by ~ 1 d in fetal development. A number of mouse thymomas

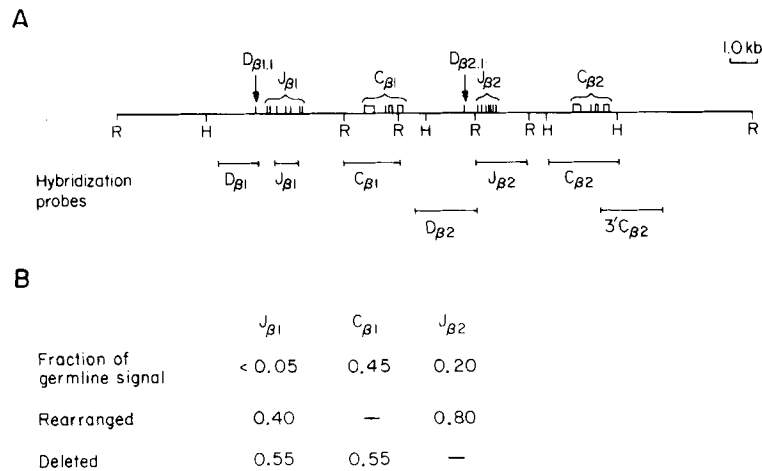


FIGURE 5. A. Germline T cell antigen receptor β gene locus. T cell antigen receptor β gene exons are indicated by vertical lines or boxes above the horizontal line. Restriction enzyme cleavage sites are indicated by vertical lines below the horizontal line. H, Hind III; R, Eco RI. The indicated hybridization probes were restriction enzyme fragments purified from subclones or from a germline cosmid clone. B. Quantitation of the extent β gene segment rearrangement. The top line shows the measured fraction of the germline signal, rounded off to the nearest 5%. The bottom two lines contain a likely interpretation of this quantitative data.

TABLE II
Characteristics of T Cell Development in Fetal Mice

Tissue	DNA rearrangements			RNA transcripts				Cell surface markers					
	γ	β		C_{γ}	C_{β} (1.3 kb)	C_{α} (1.7 kb)	T3 δ	Pgp-1	Thy-1	Lyt-2	Lyt-2/L3T4	IL-2-R	MEL14 (%)
		D-J	V-D-J										
Fetal liver, day 12 or 13	+/-	-	-	-	-	-	ND		ND			ND	ND
Fetal thymus													
day 14	+	+/-	-	+	-	-	-	+	+	-	-	ND	22
day 15	+	+	+/-	+	+/-	-	+/-	+/-	+(54%)	-	-	+(56%)	40
day 16	+	+	+	+	+	+/-	+/-	-	+(84%)	+(16%)	+(17%)	ND	28
day 17	+	+	+	+	+	+	+	-	+	+(54%)	+(77%)	ND	7
day 18	+	+	+	+	+	+	+	-	+(90%)	+(75%)	+(87%)	+(17%)	4

Information on gene rearrangements and transcripts are compiled from the data presented in the text. + means rearrangements, transcripts, or cell surface markers are present. For detectable transcripts, different levels of abundance are not distinguished. +/- means rearrangement is present in <10% of the chromosomes, the transcripts are barely detectable, or a low level of cell surface staining. - means rearrangements, transcripts, or cell surface markers are not detectable. The data on the expression of cell surface markers are from the following references: 55, 57, 64, 70-72, 75, 82, 88. Numbers in parentheses indicate the percent of cells that can be stained with mAb specific for the marker in question. Lyt-2/L3T4 includes cells expressing either or both markers.

that we have analyzed also transcribe β but not α genes (data not shown), consistent with the notion that β genes are expressed before α in T cell ontogeny. Substantial levels of both the 1.7 kb α and 1.3 kb β gene transcripts are not present until day 17 of gestation (Fig. 2, A and B). This first substantial expression of the α and β genes correlates not only with the first appearance of T cell receptor molecules on the cell surface (73, 74), but also with an increase in transcription of the δ subunit of the T3 molecule (Fig. 2D, lane 7), and the appearance of T cell markers such as Lyt-2 and L3T4 (Table II) (55, 57, 75). ~1 d later, immunocompetent cells can first be detected (55, 56). Furthermore,

soon after the occurrence of these events, there is a significant decrease in γ gene transcription (Fig. 2C, lane 9). Similar results on the ordered expression of α , β , and γ genes to some of those described above have been reported recently by two other groups (74, 76–77).

Studies of gene segment rearrangements indicate that the ordered appearance of transcripts from the α , β , and γ gene families in fetal cells is likely to be due to ordered rearrangements. The data presented here and elsewhere (77) suggest that rearrangement of V_γ gene segments occurs before V_β gene segments. This is supported by the presence of restriction fragments characteristic of V_γ gene segment rearrangement on day 15 before significant V_β gene segment rearrangement (Fig. 1B) (78), by the early maturation of the γ gene rearrangement pattern in total fetal thymus DNA (Fig. 1, B and C), and possibly by the analysis of fetal thymus hybridomas (Table I). Rearrangement of D_β gene segments also precedes rearrangement of V_β gene segments. This is supported by the data on fetal thymus hybridomas (Table I), by the early appearance of restriction fragments that are likely to be due to D_β - J_β gene segment rearrangement in total thymus DNA (Fig. 1C), and by the appearance of the 1.0 kb β gene transcript derived from such D_β - J_β rearrangements 1–2 d before the 1.3 kb transcript that contains V_β genes (Fig. 2B, lanes 4 and 5). The results from a previous analysis of the β gene segment rearrangements in a large panel of fetal thymus hybridomas is also consistent with the rearrangement of D_β to J_β before V_β gene segment rearrangement (78), suggesting that the fetal hybridomas analyzed in the previous study are representative of the bulk of the normal fetal thymocytes. We do not have sufficient data to determine whether V_γ gene segment rearrangement occurs before any D_β gene segment rearrangement, or whether these steps occur at the same time.

Analysis of adult thymocyte subpopulations indicates that most thymocytes have undergone β gene segment rearrangement on both chromosomal homologs, including populations that either do not contain immunocompetent cells or that are enriched for cells thought to die in the thymus. This suggests that these DNA rearrangements occur early in the thymus maturation process or before migration to the thymus. Recent results from other laboratories indicate that a small subpopulation of adult thymocytes that likely to be precursors of at least some of the other thymus cell populations (79, 80) have not completed rearrangement of their β gene segments and have little or no α gene transcripts (76, 81–82). These cells also have high levels of γ gene RNA (76), and they express neither Lyt-2 nor L3T4 (79, 80). With regard to expression of these and other cell surface markers (see Table II) and rearrangement and expression of the α , β , and γ genes, these adult thymocytes are similar to day 14–16 thymocytes. It is therefore possible that a single ordered pathway of rearrangement events is characteristic of all differentiating T cells, including both the majority of day 14–17 fetal thymocytes and the small fraction of adult, double negative, or Lyt-2⁻, L3T4⁻ thymocytes.

Studies on the ontogeny of Ig gene rearrangement and expression have shown that rearrangements in the three unlinked Ig gene families also proceed in an ordered fashion that can be divided into four separate stages (reviewed in 83). Based on these data, models have been proposed which maintain that Ig gene

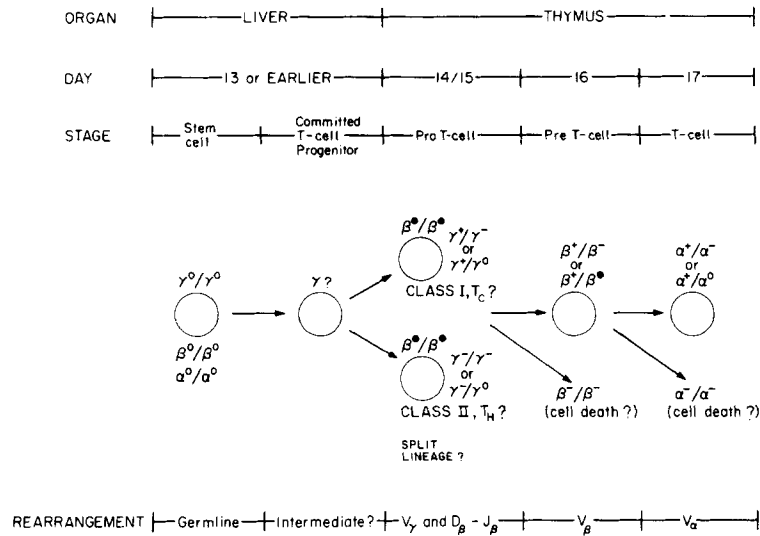


FIGURE 6. A model of the sequence of gene rearrangements and expression during T cell ontogeny. The top two horizontal lines show the location and timing respectively of the various rearrangement events in fetal mice only. The third horizontal line shows a proposed name for the various stages, as discussed in the text. Beneath this, is a diagrammatic representation of the various stages. The status of each chromosomal homolog is illustrated above or below the cells, which are represented as circles. The superscript 0 , as in α^0 , represents germline α genes. $\gamma^?$ marks the γ gene rearrangement, which has not yet been characterized. The + and - superscripts denote productive and nonproductive rearrangements, respectively. The filled circle superscripts, as in β^* represents a D-J rearrangement alone. Therefore, γ^+/γ^- represents a cell with one productive and one nonproductive γ gene rearrangement. *Class I, T_c* MHC class I-restricted cytotoxic T cell. *Class II, T_H* is an MHC class II-restricted helper T cell. The bottom horizontal line indicates the rearrangement(s) that characterize particular stages. Rearrangements and the stages they characterize are listed according to their first appearance. Therefore, while significant amounts of V_β gene segment rearrangement are first found on day 16, they continue to occur after this as well. *Intermediate?* refers to the 9.5 kb uncharacterized γ gene rearrangements found in fetal liver and thymus.

segment rearrangements are regulated by the products of rearranged genes. For example, according to this view, synthesis of an H chain polypeptide following productive V_H gene segment rearrangement inhibits further rearrangement at the H chain locus, and also activates κ L chain gene rearrangement (84). There is substantial evidence (26, 28, 54) suggesting that the rearrangement mechanism(s) in B and T lymphocytes are similar. Based on this similarity, and the data we and others (74, 76-78, 81, 82) have generated so far on the ordered expression of rearranging families in T cells, we believe that a version of the regulated model for gene segment rearrangement described above for Ig may also pertain to the α , β , and γ gene segment rearrangements in differentiating T cells. A diagram outlining one such model, with particular reference to fetal cells, is presented in Fig. 6. Some of the steps illustrated in the figure are well-supported by the available data, while others, discussed below, are speculative.

All lymphocytes arise from multipotent hematopoietic stem cells whose progeny at some point become committed to the T cell lineage. The early steps in this differentiation pathway are not well-defined, although in the pathway presented (Fig. 6), we speculate that this commitment can occur before migration

to the thymus, and that it is associated with the presence of the 9.5 kb rearranged γ gene restriction fragment, designated $\gamma?$. The timing of the disappearance of this band from fetal liver DNA, and its simultaneous appearance in fetal thymus DNA, is certainly consistent with the migration of a large number of cells with this rearrangement from the fetal liver to the fetal thymus at around days 12–13; suggesting that this rearrangement might be a marker for prethymic cells committed to the T cell lineage. However, although at least 1–5% of fetal liver cells have this rearrangement, some data suggest that the frequency of committed T cell progenitors in fetal liver is much lower (85).

Consistent with the names already in use for the different stages of B cell maturation (reviewed in 86), we propose that cells at the next stage be called pro-T cells. These cells have undergone V_γ gene segment rearrangements, which may be either productive or nonproductive. As indicated in Fig. 6, these pro-T cells are also likely to have undergone D_β - J_β gene segment rearrangements on both homologs. They are therefore typical of many day 14–15 fetal thymocytes, as well as the three fetal thymocyte hybridomas from later stages, which are described in Table I. The β gene rearrangements that are found on both chromosomal homologs of nearly all mature T cells reflect the fact that D_β - J_β gene segments occur first and are not subject to allelic exclusion, as is also true for Ig D_H - J_H rearrangements. Cells at the next stage, pre-T cells, have differentiated a further step by rearranging their V_β gene segments. These cells may constitute the majority of fetal thymocytes by day 16 or 17. Both pre- and pro-T cells are also found in the adult, double-negative thymocyte population (76, 81, 82). Finally, V_α gene segment rearrangement occurs, and T cells that have both V_α and V_β rearrangements begin to accumulate by the 17th day of gestation. According to the model, α gene segment rearrangement does not begin until productive β gene segment rearrangement has occurred, and the β polypeptide might be involved in the activation of α gene segment rearrangement.

In summary, considering just the α and β gene families, it appears that there is a striking symmetry in the progression of rearrangements in the Ig and T cell receptor gene families. In each case, rearrangements take place first in a gene family known to have D gene segments, and the very first rearrangements are the joining of D to J gene segments that are not subject to allelic exclusion. Temporally, there is also similarity between the two processes. H chain gene rearrangements occur at the same time or slightly earlier than β gene rearrangements. Furthermore, the first appearance of cell-surface Ig on fetal liver cells, presumed to follow directly from productive κ gene rearrangement, occurs on days 16–17 (86, 87), when the T cell antigen receptor is also first expressed. γ gene rearrangement and expression however, cannot be easily fitted into a model based upon the ontogeny of Ig gene segment rearrangement. Although γ gene segment rearrangement occurs earliest in ontogeny, a productive γ gene rearrangement may not be required for further T cell maturation. In the model shown in Fig. 6, we speculate that γ gene rearrangement is a developmental switch point, in that cells with productive γ gene rearrangements tend to become MHC class I-restricted, while cells with only nonproductive rearrangements tend to become MHC class II-restricted.

Are T Cell Receptor and γ Gene Rearrangements Restricted to the Thymus?

Analysis of fetal tissues and hybridomas for T cell antigen receptor gene rearrangement and expression strongly suggests that thymic precursors do not express T cell antigen receptors, because complete α and β transcripts are not present in the fetal liver, and they are absent from the thymus until day 17 of gestation (Fig. 2, A and B) (76, 77). Furthermore, a gradient of increasing β gene rearrangement is observed in fetal thymocyte DNA (Fig. 1C), and in hybridomas derived thereof (78), as development progresses from days 14–18 of gestation. Similar conclusions have been drawn by others from the pattern of gene rearrangement present in double negative, adult thymocytes; namely that immature T cells arrive in the thymus with germline α and β genes, and these rearrange during thymus cell differentiation. However, other interpretations for these data are possible. For example, the observed changes in fetal thymus DNA could be caused by the seeding of this organ by waves of increasingly mature precursors. This seems unlikely, because culture of day 13, 14, and 15 thymus tissue has shown that thymocytes at this stage can differentiate and acquire immunocompetence in vitro (5, 55, 56, 75, 88). Alternatively, it is possible that a small minority of the cells in the thymus at day 14 arrived there having previously undergone α and β gene rearrangement, and that this cell population is selectively expanded during development. However, the rapid change in the hybridization pattern of total thymus DNA and RNA between days 14 and 17 (Figs. 1 and 2), combined with the \sim 20-fold increase in thymus cell number during this same period (57 and data not shown), make this selective expansion of a small subpopulation with previously rearranged genes unlikely.

The possibility of some prethymic α and β gene segment rearrangements cannot be formally ruled out, however, and some data are consistent with prethymic expression of T cell antigen receptors (89, 90). Perhaps most convincing in this regard is the demonstrated presence of small numbers of functional T cells in athymic *nu/nu* mice (91–93). However, it is possible that in *nu/nu* mice T cell receptor gene rearrangements take place in the thymic rudiment that is present in these animals. Alternatively, because there are relatively few functional T cells in these mice, and because the number of such cells tends to increase steadily until late in life (94), it is possible that extrathymic α and β gene segment rearrangements occur, albeit slowly and inefficiently in these animals. In contrast to the results using α and β gene probes, analysis of fetal tissues indicates that γ gene rearrangements do not occur solely in the thymus, but that they can also occur in the fetal liver.

Implications for Intrathymic T Cell Differentiation. It is generally believed that most thymocytes are destined to die in the thymus (65, 66). Although the reason(s) for this cell death remain unknown, it is tempting to connect it with the selection of a repertoire of self MHC-restricted T cell antigen receptors (95). However, if both the α and β genes rearrange in the thymus, then it is possible that a substantial fraction, although not necessarily all of the intrathymic cell death is associated with gene rearrangement, rather than the selection of receptor specificities.

Rearrangement in the α and β gene families leads to the random deletion, and at least for the β gene family, insertion of nucleotides from the junctions of the joined gene segments (reviewed in 26, 28, 54). If the addition/deletion of

junctional nucleotides is truly random, then two-thirds of all gene segment rearrangements will be nonproductive because they put the J and C genes in an inappropriate translational reading frame. For a population of developing T cells undergoing gene rearrangement in the thymus, $0.66 \times 0.66 = 0.44$ or 44% will have nonproductive β gene segment rearrangements on both chromosomal homologs. According to the regulated model proposed above, these cells will not go on to rearrange their α gene segments. Of the 56% of cells that do undergo α gene rearrangement, for the reasons already outlined, we expect that 44% will have nonproductive rearrangements on both homologs and will not be able to synthesize a T cell antigen receptor. Therefore, considering the progeny of a single committed T cell progenitor, or pro-T cell undergoing gene segment rearrangement in the thymus, no more than $0.56 \times 0.56 = 0.31$ or 31% of the cells will be able to produce a functional α - β heterodimer. If we assume that only those thymocytes that synthesize T cell antigen receptors emigrate from the thymus, then nearly 70% of the cells may die as a direct result of the imprecision of gene segment rearrangement.

This calculation provides only a crude estimate for the fraction of cells with nonproductive rearrangements on both homologs. Two-thirds of the gene rearrangements will be nonproductive if V gene segments join directly to J gene segments, as may be true for the α gene family, or if V-D-J rearrangements occur, but the D gene segment can be read in three reading frames, as has been shown for the β gene family (96, 97). If V-D-J rearrangements are required in the α gene family but the D gene segment can be read primarily in only one reading frame, as is true for the Ig H chain locus, then the actual proportion of productive rearrangements will be lower, and consequently the proportion of cells dying may be higher. On the other hand, the fraction of cells with only nonproductive rearrangements may be overestimated, because more than one V-J or V-D-J rearrangement may occur on a single chromosomal homolog. Therefore, although the fraction of cells having only nonproductive α or β gene rearrangements cannot be precisely estimated, the clear prediction is that a substantial fraction of thymocytes cannot express T cell receptors because they lack productive rearrangements. The finding that as few as 30% of human thymocytes express the T3 molecule, which is apparently required for cell surface expression of T cell receptors (98), is consistent with this hypothesis.

Because differentiating thymocytes rearrange and express their T cell receptor genes and attain immunocompetence, an autoreaction would occur if the thymus also did not eliminate strongly self-reactive cells. Therefore some cell death should also result from the elimination of these cells. However, the fraction of cells that die in the thymus has been estimated to be >95%. If this estimate is accurate, it remains possible that some substantial fraction of thymocytes are selected against for other reasons, possibly because their receptors cannot recognize antigens in the context of self MHC-encoded molecules.

Summary

Rearrangement and expression of the T cell antigen receptor and the γ genes during T cell ontogeny is a regulated process; the γ genes are rearranged and expressed first, followed by the β and then the α genes. Expression of both

functional α and β gene RNA first occurs at day 17 of gestation, along with the expression of T3 δ chain RNA. T cell antigen receptor gene rearrangements occur primarily or exclusively in the thymus, although some γ gene rearrangements occur outside the thymus in fetal liver cells that may be committed T cell progenitors. There is no gross difference in the extent of β and γ gene rearrangements in the adult thymocyte subpopulations that were analyzed, despite the fact that some of these populations cannot respond to antigen and never emigrate from the thymus. Quantitative analysis of rearrangements in total adult thymocyte DNA shows that β gene rearrangements generally occur on both chromosomal homologs, and that rearrangements occur preferentially to the $J_{\beta 2}$ gene segment cluster.

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