Human Fetal Liver γ/δ T Cells Predominantly Use Unusual Rearrangements of the T Cell Receptor δ and γ Loci Expressed on Both CD4+CD8− and CD4−CD8− γ/δ T Cells

By Kai W. Wucherpfennig,*‡ Y. Joyce Liao,* Margaret Prendergast,* John Prendergast,* David A. Hafler,‡ and Jack L. Strominger*

*From the *Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138; and ‡Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115*

Summary

Substantial numbers of both α/β and γ/δ T cells are present in human fetal liver, which suggests a role of the fetal liver in T cell development. The diversity of fetal liver T cell receptor (TCR) γ and δ chain rearrangements was examined among both CD4+CD8− and CD4−CD8− γ/δ T cell clones. In addition, TCR δ chain transcripts from three fetal livers were sequenced after polymerase chain reaction amplification of TCR δ chains with $V_\delta1$ or $V_\delta2$ rearrangements. Five of six fetal liver γ/δ T cell clones had a $V_\delta2$-$D_\delta3$-$J_\delta3$ gene rearrangement with limited junctional diversity; three of these clones had an unusual CD4+CD8− phenotype. $V_\delta2$-$D_\delta3$-$J_\delta3$ gene rearrangements were also common among both in-frame and out-of-frame transcripts from three fetal livers, indicating that they are the result of an ordered rearrangement process. TCR γ chain sequences of the fetal liver γ/δ T cell clones revealed $V_\gamma1$-$J_\gamma2.3$, $V_\gamma2$-$J_\gamma1.2$, and $V_\gamma3$-$J_\gamma1.1$ rearrangements with minimal incorporation of template-independent N region nucleotides. TCR γ chain rearrangements found in these fetal liver T cell clones were different from those that have been observed among early thymic γ/δ T cell populations, while similar TCR δ chain rearrangements are found among γ/δ T cells from both sites. These data demonstrate that the fetal liver harbors γ/δ T cell populations distinct from those found in the fetal thymus, suggesting that the fetal liver is a site of γ/δ T cell development in humans. These unusual T cell populations may serve a specific function in the fetal immune system.

γ/δ T cells are a distinct subset of mature T cells defined by the expression of rearranged TCR γ and δ genes (1, 2). A number of studies performed in mice have demonstrated a developmental pattern of γ/δ TCR rearrangements as well as localization of γ/δ T cells with defined TCR rearrangements to specific organs. For example, γ/δ T cells in mouse intestinal mucosa and mesenteric lymph nodes preferentially use $V_\gamma7$, while γ/δ T cells in skin use the $V_\gamma3$ gene segment (3, 4). Such epithelial γ/δ T cells are thought to have specific functions in the immune surveillance of epithelial tissues, such as the lysis of infected or transformed cells (5).

In humans, the developmental pattern of γ/δ TCR rearrangements is not as well understood as it is in mice. Nevertheless, there is evidence that the occurrence of human γ/δ T cell populations is also developmentally regulated. In fetal thymus, the major population of TCR δ chains is rearranged to the $V_\delta2$ gene segment, while $V_\delta1^+$ T cells represent the major population of γ/δ T cells in postnatal thymus and blood of newborns (6–9). However, 6 mo after birth $V_\delta2^+$ γ/δ T cells become the predominant γ/δ T cell population in blood while $V_\delta1^+$ T cells continue to be the major γ/δ T cell population in the thymus (10, 11).

Murine fetal liver is not only a site at which T cell precursors develop from immature cells but also an organ in which γ/δ T cells can mature. Nude mice were found to have substantial numbers of intestinal intraepithelial γ/δ T cells despite the almost complete absence of α/β T cells. Furthermore, intraepithelial γ/δ T cells could be reconstituted in lethally irradiated mice by injection of fetal liver precursors, even in thymectomized animals (12). Since at least a subset of γ/δ T cells is thymus independent, fetal liver is a likely site of extrathymic γ/δ T cell maturation.

The presence of substantial numbers of γ/δ T cells in human fetal liver as well as the unusual CD4+ phenotype of a subpopulation of fetal liver γ/δ T cells support the hypothesis that fetal liver is also a site of extrathymic γ/δ T cell maturation in humans. Both α/β and γ/δ T cells have been cultured from human fetal liver and represent ~63 and ~32%

J. Exp. Med. © The Rockefeller University Press • 0022-1007/93/02/0425/08 $2.00
Volume 177 February 1993 425–432

of $CD3^+$ T cells, respectively. By surface expression of CD4 and CD8 molecules, three subsets of $CD3^+$ T cells in human fetal liver were identified. Approximately 20% of fetal liver γ/δ T cells have a $CD4^+CD8^-$ phenotype that is infrequent among γ/δ T cells in thymus or blood. In contrast to $CD4^-CD8^-$ and $CD4^-CD8^+$ γ/δ T cells, $CD4^+CD8^-$ γ/δ T cells from fetal liver were found to lack cytotoxic activity (13–15). Together, these data suggest that fetal liver γ/δ T cells represent a distinct T cell population. In the present paper, TCR γ and δ chain rearrangements of $CD4^+CD8^-$ and $CD4^-CD8^-$ fetal liver γ/δ T cell clones were examined. The data indicate that fetal liver γ/δ T cells represent T cell populations distinct from thymic γ/δ T cells.

Materials and Methods

Cell Preparations. Fetal liver samples FL 2/9, FL 2/27, FL 1/9, and FL 5/27 were obtained at the time of postmortem examination from electively aborted fetuses after 20–22 wk of gestation (FL 2/9, 21 wk; FL 2/27, 20 wk; FL 1/9, 22 wk; FL 5/27, 20 wk). The consent forms and collecting practices were approved by the Committee for the Protection of Human Subjects from Research Risks (Boston, MA). FL 2/9 liver cell suspension was prepared by gently teasing tissue, and cells were frozen down immediately in 90% FCS, 10% DMSO at −80°C. FL 2/27 and FL 1/9 single cell suspensions were prepared in a similar fashion, stimulated with PHA for 14 d to enrich for T cells, and frozen in 90% FCS, 10% DMSO at −80°C. PBL were isolated by Ficoll density gradient centrifugation from blood of a normal adult volunteer.

FACS® Analysis. Antibodies used for immunofluorescence analysis were: T3b (anti-CD3), OKT4 (anti-CD4), Leu2A (anti-CD8), WT31 (TCR α/β >> TCR γ/δ; Becton Dickinson & Co., Mountain View, CA), TCR-δ1 (anti-TCR δ chain; 16), δTCS1 (anti-TCR $V_\delta1$-$J_\delta1$; 17), BB3 (anti-TCR $V_\delta2$; 18), and TiγA (anti-TCR $V_\gamma9$; 19). Cells were incubated with saturating amounts of primary antibodies or isotype-matched control antibodies in PBS/5% normal human serum for 30 min at 4°C and washed in PBS, 1% BSA. Cells were then incubated with FITC-labeled goat anti-mouse antibody (Cappel Laboratories, Malvern, PA) for 30 minutes, washed, and fixed in 1% formaldehyde, PBS. Fluorescence staining was examined using an Epics C cell sorter (Coulter Electronics, Inc., Hialeah, FL).

T Cell Cloning. Fetal liver T cell clones L3, L6, L7, L25, and L38 were isolated from FL 5/27 by direct single cell cloning on day 0, while fetal liver clones L2G9, L4B2, L7F11, L4G1, and L7F5 were generated from FL 2/9 after enrichment of TCR δ^+ T cells by magnetic bead separation. For cloning, T cells were grown in RPMI, 10% human serum (Biocell), 10–20% conditioned media (delectinized supernatant from PHA-stimulated blood mononuclear cells) at one cell/well in U-bottomed microtiter plates using 2×10^4 irradiated mononuclear cells and 10^4 irradiated JY cells (an EBV-transformed B cell line) per well. After 2 wk, growth-positive wells were expanded with fresh feeder cells and IL-2.

For bead selection, fetal liver cells were incubated with TCR-δ1 mAb (1:500 dilution of ascites) in RPMI, 2% FCS for 30 min at 4°C and washed in RPMI, 3% BSA. Antibody-coated cells were incubated with magnetic goat anti-mouse beads (Dynal) for 15 min at 37°C and TCR-δ1-positive cells recovered by magnetic bead separation and repeated washing of beads. Cells were cultured for 1–3 wk and cloned as described above.

RNA Preparation and cDNA Synthesis. RNA was prepared from $0.5–10 \times 10^6$ cells using the RNAzol B method (Cinna/Biotecx). Cells were washed twice with PBS and then homogenized in 1 ml of RNAzol B. 100 μl of chloroform was added and the sample centrifuged at 4°C. The upper, clear phase was transferred to a clean tube and RNA was precipitated by addition of an equal volume of isopropanol. When small numbers of cells were available for RNA preparation, 10 μg of tRNA was added as a carrier in the isopropanol precipitation step. After a 15-min incubation on ice, samples were centrifuged at 4°C. The RNA pellet was washed with 1 ml of cold 70% ethanol and air dried. RNA was resuspended in 10 μl of autoclaved H_2O and stored at −80°C. cDNAs were synthesized from 1–2 μg of RNA using oligo(dT) as a primer and AMV-reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD) (20). cDNAs were diluted to 200 μl with autoclaved H_2O and stored at −80°C. 5–10 μl of diluted cDNA was used for each PCR amplification.

PCR Amplification and Southern Blot Analysis. cDNAs from PBL,FL 2/9, FL 2/27, and FL 1/9 samples were amplified for 35 cycles in 50-μl reactions using AmpliTaq polymerase as recommended by the manufacturer (Perkin Elmer Cetus, Norwalk, CT). PCR cycles were 94°C denaturation (1 min), 55°C annealing (2.0 min), and 72°C extension (3.0 min). A different PCR program was used for amplification of the TCR δ chain from fetal liver T cell clones L3, L25, L2G9 (30 cycles of 94°C denaturation [1.5 min], 48°C annealing [1.5 min], and 72°C extension (1.5 min]). Primers used were specific for $V_\delta1$ to $V_\delta5$ chains and used in combination with a C_δ primer (0.5 μg of each primer per reaction). Sequences of oligonucleotides used for PCR or for Southern blot hybridization were: $V_\delta1$, 5′ ggggtcgacaagttggtggtcatatta-3′; $V_\delta2$, 5′ ggggtcgacctcaggtgctccatgaa-3′; $V_\delta3$, 5′ ggggtcgacactgtatattcaaatc-3′; $V_\delta4$, 5′ ggggtcgaccagcaagttaagcaa-3′; $V_\delta5$, 5′ ggggtcgacatatgacaccagtgatc-3′; C_δEcoRI, 5′ ggggtagaattccttcacca-3′; C_δ, 5′ gcaaacagcattcgtagcccaagcactgtg 3′; C_δ probe, 5′ gtcatgaaaaatggaacaaatgtcgcttgt-3′; $J_\delta1$, 5′ ccacagtcacacgggttcct 3′; $J_\delta2$, 5′ ccacgatgagttgtgttccc 3′; $J_\delta3$, 5′ ccgaaaaacatctgtcgggt 3′; $V_\gamma1$, 5′ tacatccactggtacctacaccagga 3′; $V_\gamma2$, 5′ ggggtcgacctggtgaagtcatacagt 3′; $V_\gamma3$, 5′ ggggaattcacaaggtttgaaacagat g 3′; $V_\gamma4$, 5′ ggggaattccaaggctttagcagtaaaa 3′; C_γ-BamHI, 5′ tcctgggatcccagaatcgtgttgct 3′; C_γ-PstI, 5′ tgttgtgagctgcagcagtagtgta 3′.

$V_\delta1$ to $V_\delta5$ primers have a SalI restriction site for cloning of PCR products into M13 vectors, while one of the C_δ primers has an EcoRI restriction site to facilitate cloning. For amplification of TCR γ chains, V_γ primers were used in combination with either C_γ primer (C_γ-BamHI is located closer to the 5′ end of C_γ). To prevent contamination of samples, reagents used for cDNA synthesis and PCR amplification were tested for possible contamination. Negative controls (all primer combinations, no cDNA) were included in experiments in order to detect accidental contamination.

PCR products were analyzed on 1% agarose gels stained with ethidium bromide. For Southern blot analysis, gels were denatured, neutralized, and PCR products transferred to nitrocellulose paper (Schleicher & Schuell, Inc., Keene, NH). Amplified DNA was hybridized to an internal C_δ oligonucleotide probe endlabeled using γ-[^{32}P]ATP and T4 polynucleotide kinase (Bethesda Research Laboratories). Hybridizations were performed for 18 h at 37°C in a buffer containing 6× SSC, 0.05% pyrophosphate, 5× Denhardt's, 0.5% SDS, 0.1 mg/ml of denatured salmon sperm DNA. After hybridization at 37°C for 18 h, filters were washed at a final stringency of 6× SSC, 70°C (C_δ probe) or 6× SSC, 50°C (J_δ probes) and processed for autoradiography (20).

DNA Sequence Analysis. V_δ-C_δ PCR products, M13 mp19 plasmid DNA, and pUC-18 DNA were digested with EcoRI and SalI restriction endonucleases to generate compatible ends for

cloning. The digested plasmid was treated with bacterial alkaline phosphatase (Bethesda Research Laboratories). Digested PCR products were gel purified, ligated to plasmid DNA, and used to transform JM101-competent cells. PCR products from fetal liver T cell clones were cloned into pUC-18 while TCR δ chain amplification products from fetal liver were cloned into M13 mp19. DNA was sequenced by the dideoxy method (21) using the M13 universal primer and α-^{35}S-ATP as radiolabeled nucleotide. For sequence analysis of TCR γ chains, PCR products were cloned into the pCRII vector (TA cloning system; Invitrogen, San Diego, CA) double-stranded plasmid DNA was sequenced by the dideoxy method using C_γ-BamHI as a primer.

Results

Analysis of TCR δ Chain Rearrangements in Human Fetal Liver. The TCR δ chain repertoire in human fetal liver was examined by PCR amplification of cDNAs synthesized from fetal liver RNA (using primers specific for Vδ1 to Vδ5 gene segments in combination with a C_δ primer). RNA was extracted from one fetal liver tissue sample (FL 2/9) and from two samples (FL 2/27 and FL 1/9) that had been enriched for T cells by PHA stimulation. Amplification of Vδ1 to Vδ5 gene segments from fetal liver cDNA resulted in bands for Vδ1 and Vδ2 reactions on ethidium bromide–stained agarose gels that hybridized to an internal C_δ oligonucleotide probe. Among all three fetal liver samples as well as in a PBL sample from an adult volunteer, Vδ2 amplifications gave the strongest signal indicating that the majority of TCR δ chains in fetal liver and adult blood (10, 11, 22) are rearranged to Vδ2. Trace amounts of Vδ3, Vδ4, and Vδ5 were amplified from one fetal liver sample (FL 2/9) (Fig. 1).

To determine J_δ gene usage of fetal liver TCR δ chains, V_δ gene segments were amplified from cDNA using Vδ1 to Vδ5 primers in combination with a C_δ primer followed by Southern blot hybridization with probes for Jδ1, Jδ2, and Jδ3 gene segments. Hybridization of Vδ2-C_δ and Vδ1-C_δ reactions to a Jδ3 probe gave strong signals for all fetal liver samples while weaker signals were detected using probes for Jδ1 and Jδ2 (Fig. 2). In contrast, products from a Vδ2-C_δ amplification using cDNA from adult blood T cells gave a strong hybridization with a Jδ1 probe; only trace amounts of Vδ2-Jδ3 rearrangements were detected in adult blood. This was expected as the majority of TCR δ chains in adult blood are rearranged to the Jδ1 gene segment (10). These data indicate that Vδ2-Jδ3 is the predominant TCR δ chain rearrangement in human fetal liver and that Vδ2 as well as Vδ1 are preferentially rearranged to the Jδ3 gene segment.

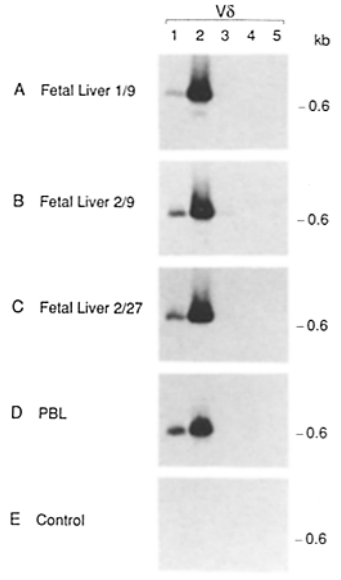

Figure 1. Southern blot analysis of the TCR δ chain repertoire in human fetal liver. TCR δ chains were amplified from fetal liver cDNA using primers specific for Vδ1 to Vδ5 gene segments in combination with a C_δ primer. Southern blots were hybridized to an internal ^{32}P-labeled C_δ oligonucleotide probe. (*A*) Fetal liver 1/9; (*B*) fetal liver 2/9; (*C*) fetal liver 2/27; (*D*) blood cDNA from an adult subject; (*E*) negative control (no cDNA).

Sequence Analysis of Vδ1 and Vδ2 Rearrangements from Human Fetal Liver Samples. In both fetal livers (FL 2/27 and FL 2/9), the majority of Vδ2 gene segments were rearranged to Jδ3 (22/24 sequences, 91.6%). Also, 21 of 22 TCR δ chains with a Vδ2-Jδ3 rearrangement carried the Dδ3 but not the Dδ1 gene segment (Fig. 3). Only few, if any, N region nucleotides were present at the VDJ junctions. An exception to this rule was sequence 9 (fetal liver 2/27), which contained an unusually long N region between Dδ3 and Jδ3 consisting of a repeated TGAAACC(T) sequence. All four Vδ1 gene segments sequenced were also rearranged to Dδ3 and Jδ3 (Fig. 3). Vδ-Jδ rearrangements in fetal liver are therefore almost exclusively rearranged to Dδ3 and Jδ3 with limited junctional diversity. In contrast, TCR δ chains from mature γ/δ T cells are most commonly rearranged to Jδ1, use Dδ1, Dδ2, and Dδ3 (frequently in tandem), and have extensive N region diversity (22–25).

The predominant Vδ2-Jδ3 gene rearrangement observed in these two fetal livers could result from a regulated rearrangement process or from positive selection of γ/δ T cells bearing specific TCR chains. Since 7 of 22 Vδ2-Jδ3 sequences were in frame, the predominance of Vδ2-Dδ3-Jδ3 rearrangements is most likely due to the presence of a regulated TCR δ chain rearrangement process in human fetal liver. The relative proportion of in-frame and out-of-frame rearrangements is consistent with the theoretical prediction that one-third of rearrangements should lead to an in-frame sequence.

Cloning of α/β and γ/δ T Cells from Human Fetal Liver. Since fetal liver γ/δ T cells with an unusual $CD4^+CD8^-$ phenotype have been described (13, 14), it was of interest to determine TCR δ chain rearrangements in fetal liver T cell clones with defined phenotypes. Fetal liver T cells were cloned by direct single cell cloning from fetal liver 5/27 (clones L3, L6, L7, L25, L38) using IL-2 as well as irradiated mononuclear cells and an irradiated EBV-transformed B cell line (JY) as a feeder layer. T cell clones were also generated from fetal liver 2/9 (clones L2G9, L4B2, L7F11, L4G1, L7F5) by magnetic bead selection of T cells expressing the TCR δ chain followed by in vitro expansion and single cell cloning. Of the 10 clones generated (Fig. 4), six had surface expression of the TCR δ chain (mAb TCR-δ1). Three of these γ/δ T cell clones (L3, L7, and L2G9) had moderate levels of CD4 expression, while clones L4B2, L25, and L7F11 were $CD4^-CD8^-$. Some of the γ/δ T cell clones also had weak staining with the WT31 mAb; however, it is unlikely that these clones contained a second α/β T cell population since >99% of cells from each clone were strongly stained by the

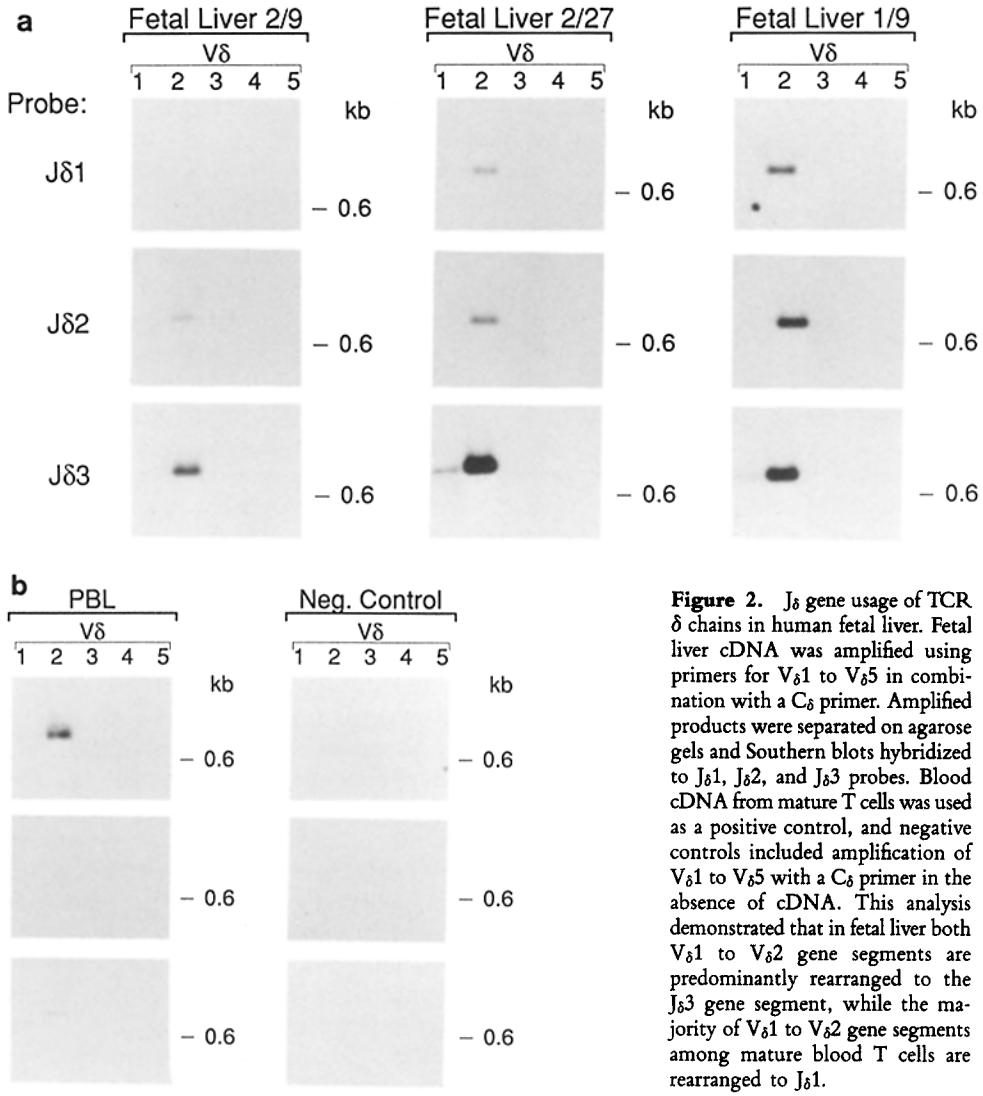

Figure 2. J_δ gene usage of TCR δ chains in human fetal liver. Fetal liver cDNA was amplified using primers for $V_\delta1$ to $V_\delta5$ in combination with a C_δ primer. Amplified products were separated on agarose gels and Southern blots hybridized to $J_\delta1$, $J_\delta2$, and $J_\delta3$ probes. Blood cDNA from mature T cells was used as a positive control, and negative controls included amplification of $V_\delta1$ to $V_\delta5$ with a C_δ primer in the absence of cDNA. This analysis demonstrated that in fetal liver both $V_\delta1$ to $V_\delta2$ gene segments are predominantly rearranged to the $J_\delta3$ gene segment, while the majority of $V_\delta1$ to $V_\delta2$ gene segments among mature blood T cells are rearranged to $J_\delta1$.

TCR-δ1 mAb. Four clones (L6, L38, L4G1, L7F5) expressed the TCR α/β as indicated by surface staining with the WT31 mAb. Two of the α/β T cell clones were $CD4^+$ (L6, L38), while two other clones were $CD4^+CD8^+$ (L4G1, L7F5). Thus, two unusual phenotypes were observed among these clones: three of six clones that expressed the TCR δ chain had a $CD4^+CD8^-$ phenotype, while two of four clones that expressed the α/β TCR were $CD4^+CD8^+$.

Three of the T cell clones positive for the TCR-δ1 mAb were further characterized for surface expression of TCR $V_\delta1$-$J_\delta1$ gene segments (mAb δTCS1), the TCR $V_\gamma2$ gene segment (mAb TiγA), and the TCR $V_\delta2$ gene segment (mAb BB3). Three of four clones were found to express the TCR $V_\delta2$ gene segment, while only one clone was positive for expression of the $V_\gamma2$ gene segment. These data confirm that the TCR $V_\delta2$ gene segment is the most commonly used V_δ gene segment by fetal liver γ/δ T cells.

TCR γ and δ Chain Rearrangements in Fetal Liver T Cell Clones. Sequence analysis of the TCR δ chain from six fetal liver γ/δ T cell clones demonstrated that all but one had a $V_\delta2$-$D_\delta3$-$J_\delta3$ rearrangement with limited junctional diversity (Fig. 5). The $V_\delta2$-$J_\delta3$ rearrangement of these clones was confirmed by genomic Southern blot analysis using probes for $V_\delta2$ and $J_\delta3$ (data not shown). Some clones showed strikingly similar protein sequences at the $V_\delta2$-$D_\delta3$-$J_\delta3$ junction; clones L3 and L25 differed only by one residue at the $V_\delta2$-$D_\delta3$ junction, while clones L7 and L2G9 differed by two additional residues present at the D_δ-J_δ junction of clone L2G9. These results indicate that the majority of fetal liver γ/δ T cell clones with $CD4^+CD8^-$ and $CD4^-CD8^-$ phenotypes have $V_\delta2$-$D_\delta3$-$J_\delta3$ rearrangements with limited junctional diversity.

To determine rearrangements of the TCR γ locus among these T cell clones, cDNA samples were amplified using a C_γ oligonucleotide in combination with primers specific for $V_\gamma2$-$V_\gamma4$ gene segments and for the $V_\gamma1$ family, which has five functional members. Of the six clones studied, two had a single $V_\gamma1$-C_γ rearrangement (clones L4B2, L2G9) while

A. $V_\delta 2$-$J_\delta 3$ rearrangements

	$V_\delta 2$	N	$D_\delta 2$	N	$D_\delta 3$	N	$J_\delta 3$	In Frame
	tgtgcctgtgacacc		ccttcctac		actgggggatacg		ctcctgggacac	
Fetal liver 2/27								
1	tgtgcctgtgacac				ggggat		tgggacac	+
2	tgtgcctgtgacac				tgggggata		ctcctgggacac	-
3	tgtgcctgtgac	t	ctt		actgggggatacg	ggag	tcctgggacac	-
4	tgtgcctgtgacac				tgggggatacg	g	ctcctgggacac	+
8	tgtgcctgtgacacc				ggggata		ctcctgggacac	+
9	tgtgcctgtgacacc	gg			ctgggggg	tgaaacctgaaacct	gacac	+
10	tgtgcctgtgac	gt			actgg	ag	ctcctgggacac	-
11	tgtgcctgtgac	g	cct		actgggggat		ctcctgggacac	+
3a	tgtgcctgtgacacc	gt			actgggggatac		ctgggacac	-
3c	tgtgcctgtgac				tgggggga	g	ctcctgggacac	-
4c	tgtgcctgtgac				actgggggatacg	g	ctcctgggacac	-
5d	tgtgcctgtgacac	g			ctgggggata		ctcctgggacac	-
4d	tgtgcctgtgacac	g			ctgggggata		ctcctgggacac	-
Fetal liver 2/9								
3a	tgtgcctgtgac				actgggggata		ctcctgggacac	-
4a	tgtgcctgtgacac				gggat	t	ggacac	-
2b	tgtgcctgtgacac	attgc			ggatacg	gag	ctcctgggacac	-
3b	tgtgcctgtgac				actgggggata		ctgggacac	-
1d	tgtgcctgtgac	tct	ctt				ctcctgggacac	-
2d	tgtgcctgtgac				actgggggatac	aa	ctcctgggacac	-
4d	tgtgcctgtgac				actgggggata		ctcctgggacac	+
6d	tgtgcctgtgac	t			actgggga	g	ctcctgggacac	-
1	tgtgcctgtgac	t	ctt		actgggggatacg	ggga	tcctgggacac	+

B. $V_\delta 2$-$J_\delta 2$ rearrangement

	$V_\delta 2$	N	$D_\delta 2$	N	$D_\delta 3$	N	$J_\delta 2$	In Frame
	tgtgcctgtgacacc		ccttcctac		actgggggatacg		ctttgacagcacaa	
Fetal liver 2/9								
4b	tgtgcctgtgaca	t			actgggg	ataggggt	ttgacagcacaa	-

C. $V_\delta 2$-$J_\delta 1$ rearrangement

	$V_\delta 2$	N	$D_\delta 2$	N	$D_\delta 3$	N	$J_\delta 1$	In Frame
	tgtgcctgtgacacc		ccttcctac		actgggggatacg		acaccgataaactc	
Fetal liver 2/9								
3d	tgtgcctgtgac				tggggg		accgataaactc	-

D. $V_\delta 1$-$J_\delta 3$ rearrangement

	$V_\delta 1$	N	$D_\delta 2$	N	$D_\delta 3$	N	$J_\delta 3$	In Frame
	tgtgctcttggggaact		gaaatagt		actgggggatacg		ctcctgggacac	
Fetal liver 2/9								
1a	tgtgctcttggggaact		agt		actgggggata		cctgggacac	+
6	tgtgctcttggggaa	tc	agt		actgggggata		cctgggacac	-
Fetal liver 2/27								
1	tgtgctcttgggg	ctgt			actggggg	ttcag	ctcctgggacac	-
5	tgtgctcttggggaac	caatgt			actgggggata		cctgggacac	-

Figure 3. Junctional diversity of TCR δ chain. Amplified TCR δ chains ($V_\delta 1$ and $V_\delta 2$) from two fetal livers were cloned into M13 mp19 and sequenced by the dideoxy method. (*A*) TCR δ chain sequences with a $V_\delta 2$-$J_\delta 3$ rearrangement; (*B*) TCR δ chain sequences with a $V_\delta 2$-$J_\delta 2$ rearrangement; (*C*) TCR δ chain sequences with a $V_\delta 2$-$J_\delta 1$ rearrangement; (*D*) TCR δ chain sequences with a $V_\delta 1$-$J_\delta 3$ rearrangement. The plus and minus signs on the far right signify in-frame or out-of-frame rearrangement, respectively. For fetal liver 2/9, three M13 clones were found to have the $V_\delta 1$-$J_\delta 3$ junctional sequence of sample 1a. It is assumed that these sequences resulted from PCR amplification of the same cDNA since the stochastic nature of the exonuclease and terminal transferase activities rarely gives rise to TCR δ chains with identical junctional sequence.

the four remaining clones had $V_\gamma 1$-C_γ and $V_\gamma 2$-C_γ rearrangements (clones L3, L7F11) or $V_\gamma 2$-C_γ and $V_\gamma 3$-C_γ rearrangements (clones L7, L25) (Fig. 6 and Table 1). By sequence analysis of the V_γ-J_γ junction, all four $V_\gamma 1$ gene segments were found to be rearranged to $J_\gamma 2.3$; three members of the $V_\gamma 1$ family ($V_\gamma 1.3$, $V_\gamma 1.4$, $V_\gamma 1.8$) were represented among these four sequences (the $V_\gamma 1.8$-$J_\gamma 2.3$ rearrangement was out of frame). In contrast, three of four $V_\gamma 2$ gene segments were rearranged to $J_\gamma 1.2$; both $V_\gamma 3$ segments were rearranged to $J_\gamma 1.1$. Thus, there appears to be an ordered rearrangement process that results in preferential rearrangement of $V_\gamma 1$ segments to the $J_\gamma 2$ cluster (specifically $J_\gamma 2.3$) and of $V_\gamma 2$ and $V_\gamma 3$ segments to the $J_\gamma 1$ cluster ($J_\gamma 1.2$ and $J_\gamma 1.1$, respectively). While all clones had only one in-frame δ chain rearrangement, three of six clones were found to have two in-frame γ chain rearrangements, in apparent violation of allelic exclusion. However, other reports have indicated that two functional δ or α chain rearrangements can be found in some T cell clones and that allelic exclusion may not be complete (6, 26).

The TCR γ chain rearrangements observed among these fetal liver T cell clones are distinct from those that have been observed during thymic development. During early thymic development, both $V_\gamma 1$ and $V_\gamma 2$ were found to be rearranged to the $J_\gamma 1$ cluster ($V_\gamma 1.8$-$J_\gamma 1.1$ and $V_\gamma 2$-$J_\gamma 1.3$, neither of which was present in the fetal liver T cell clones), while γ/δ T cells that use $V_\gamma 2$-$J_\gamma 2.3$ rearrangements predominate in postnatal thymus (6, 10).

Discussion

In mice, the fetal liver appears to be an extrathymic organ of γ/δ T cell development and maturation since intestinal intraepithelial γ/δ T cells can be reconstituted in lethally ir-

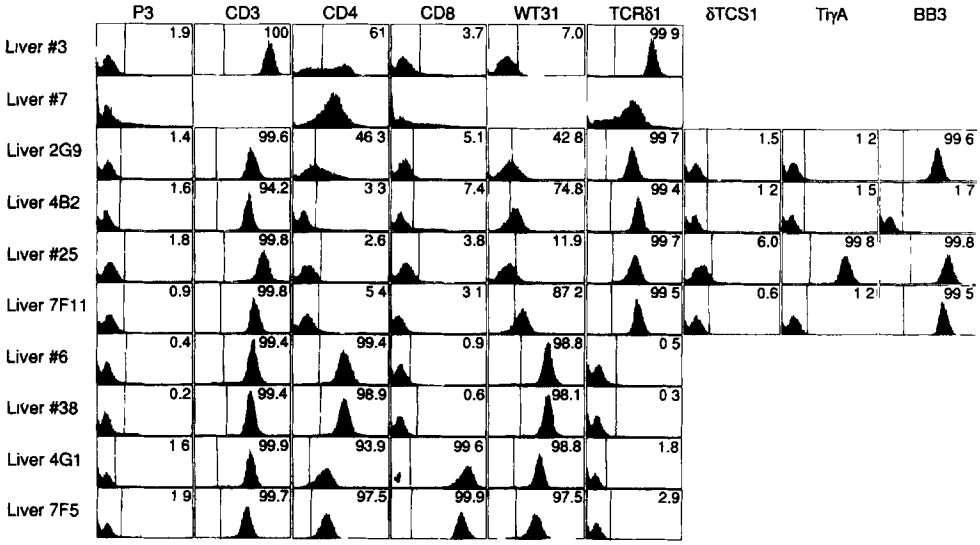

Figure 4. FACS® analysis of fetal liver T cell clones. T cell clones from fetal liver 5/27 (clones L3, L6, L7, L25, L38) and fetal liver 2/9 (clones L2G9, L4B2, L7F11, L4G1, L7F5) were examined for surface expression of CD4 and CD8 antigens as well as α/β and γ/δ TCRs by indirect immunofluorescence analysis. Antibodies used were: P3 (negative control), T3b (anti-CD3), OKT4 (anti-CD4), Leu2A (anti-CD8), WT31 (TCR α/β >> TCR γ/δ), TCR-δ1 (anti-TCR δ chain), δTCS1 (anti-TCR $V_\delta1$-$J_\delta1$), TiγA (anti-TCR $V_\gamma9$), and BB3 (anti-TCR $V_\delta2$). T cell clones L3, L7, L2G9, L4B2, L25, and L7F11 were positive for the TCR-δ1 mAb, while T cell clones L6, L38, L4G1, and L7F5 were positive for the WT31 mAb. Three of the γ/δ T cell clones (L3, L7, L2G9) expressed moderate levels of CD4, while three γ/δ T cell clones (clones L4B2, L25, L7F11) were $CD4^-CD8^-$. Also, two α/β T cell clones (L4G1, L7F5) had a $CD4^+CD8^+$ phenotype.

Nucleotide sequences

Clone	$V_\delta2$	N	$D_\delta3$	N	$J_\delta3$	Rearrangement	In Frame
	gcctgtgacacc		actggggatacg		ctcctgg		
Clone L3	gcctgtgacac		ctgggga	g	ctcctgg	$V_\delta2$-$D_\delta3$-$J_\delta3$	+
Clone L7	gcctgtgaca	t	actgggggatac		ctgg	$V_\delta2$-$D_\delta3$-$J_\delta3$	+
Clone L25	gcctgtgaca		actgggga	g	ctcctgg	$V_\delta2$-$D_\delta3$-$J_\delta3$	+
Clone L2G9	gcctgtgaca	t	actgggggatacg	ca	ctcctgg	$V_\delta2$-$D_\delta3$-$J_\delta3$	+
Clone L7F11	gcctgtgacacc	ggtgct	ctggg	tc	cctgg	$V_\delta2$-$D_\delta3$-$J_\delta3$	+

Clone	$V_\delta3$	N	$D_\delta3$	N	$J_\delta2$	Rearrangement	In Frame
	tactgtgcctt		actggggatacg		ctttgaca		
Clone L4B2	tactgtgcct		actgggga		ctttgaca	$V_\delta3$-$D_\delta3$-$J_\delta2$	+

Protein sequences

Clone L3	AlaCysAsp		ThrTrpGly		SerSerTrp
Clone L25	AlaCysAsp		AsnTrpGly		SerSerTrp
Clone L7	AlaCysAsp		IleLeuGlyAspThr		Trp
Clone L2G9	AlaCysAsp		IleLeuGlyAsp	Thr	HisSerTrp
Clone L7F11	AlaCysAspThr	GlyAla	LeuGly	Pro	Trp
Clone L4B2	TyrCysAla		TyrTrpGlyThr		LeuThr

Figure 5. Sequence analysis of the junctional region of the TCR δ chain among fetal liver γ/δ T cell clones. cDNA from fetal liver T cell clones was amplified using primers for $V_\delta2$ and C_δ. Amplified products were cloned into M13 mp19 and sequenced by the dideoxy method. Five of six clones were found to have a $V_\delta2$-$D_\delta3$-$J_\delta3$ rearrangement with little N region diversity, one clone had a $V_\delta3$-$D_\delta3$-$J_\delta2$ rearrangement.

Nucleotide sequences

Clone	V_γ	N	J_γ	Rearrangement	In Frame
Clone L3	tactgtgccacctg	ag	attataagaaactcttt	$V_\gamma1.8$ - $J_\gamma2.3$	-
Clone L2G9	tactgtgccacctgggga		ttattataagaaactcttt	$V_\gamma1.4$ - $J_\gamma2.3$	+
Clone L4B2	tactgtgccacctgggacaggc	c	ttataagaaactcttt	$V_\gamma1.3$ - $J_\gamma2.3$	+
Clone L7F11	tactgtgccacctgggatgggc	cc	aagaaactcttt	$V_\gamma1.4$ - $J_\gamma2.3$	+
Clone L3	tactgtgccttgtgggaggtg		caagagttgggcaaa	$V_\gamma2$ - $J_\gamma1.2$	+
Clone L7	tactgtgccttgtgggaggtg	c	agttgggcaaa	$V_\gamma2$ - $J_\gamma1.2$	+
Clone L25	tactgtgccttgtgggag		caagagttgggcaaa	$V_\gamma2$ - $J_\gamma1.2$	+
Clone L7F11	tactgtgccttgtgggaggtg	cgag	aactctttggcagtgg	$V_\gamma2$ - $J_\gamma2.3$	+
Clone L7	tactgtgctgcgtgg	gatt	ccactggttggtt	$V_\gamma3$ - $J_\gamma1.1$	+
Clone L25	tactgtgctgcgtgg	gatt	ataccactggttggtt	$V_\gamma3$ - $J_\gamma1.1$	+

Protein sequences

Clone L2G9	TyrCysAlaThrTrpAsp		TyrTyrLysLysLeuPheGlySerGly
Clone L4B2	TyrCysAlaThrTrpAspArg	Pro	TyrLysLysLeuPheGlySerGly
Clone L7F11	TyrCysAlaThrTrpAspGly	Pro	LysLysLeuPheGlySerGly
Clone L3	TyrCysAlaLeuTrpGluVal		GlnGluLeuGlyLysLysIleLysValPheGlyProGly
Clone L7	TyrCysAlaLeuTrpGluVal	Gln	LeuGlyLysLysIleLysValPheGlyProGly
Clone L25	TyrCysAlaLeuTrpGlu		GlnGluLeuGlyLysLysIleLysValPheGlyProGly
Clone L7F11	TyrCysAlaLeuTrpGluVal	ArgGlu	LeuPheGlySerGly
Clone L7	TyrCysAlaAlaTrp	AspSer	ThrGlyTrpPheLysIlePheAlaGluGly
Clone L25	TyrCysAlaAlaTrp	AspTyrThr	ThrGlyTrpPheLysIlePheAlaGluGly

Figure 6. Sequence analysis of the junctional region of the TCR γ chain among fetal liver γ/δ T cell clones. V_γ gene usage was determined by amplification of cDNAs with primers for $V_\gamma1$-$V_\gamma4$ in combination with a C_γ primer. Amplified products were cloned into pCRII (TA cloning system) and double-stranded plasmid DNA was sequenced by the dideoxy method. Note that clone L7F11 was found to have an in-frame $V_\gamma2$-$J_\gamma2.3$ sequence (in addition to an in-frame $V_\gamma1.4$-$J_\gamma2.3$ rearrangement) even though the T cell clone was not recognized by the mAb TiγA. It is therefore assumed that the $V_\gamma2$-$J_\gamma2.3$ sequence originated from a second T cell population present in the original clone.

Table 1. *TCR γ/δ Rearrangements and Phenotype of Fetal Liver T Cell Clones*

Clone	Phenotype	TiγA/BB3 staining	V_γ-J_γ	In frame	V_δ-D_δ-J_δ	In frame
L3	$CD4^+CD8^-$	ND	$V_\gamma2$-$J_\gamma1.2$	+	$V_\delta2$-$D_\delta3$-$J_\delta3$	+
			$V_\gamma1.8$-$J_\gamma2.3$	−		
L7	$CD4^+CD8^-$	ND	$V_\gamma2$-$J_\gamma1.2$	+	$V_\delta2$-$D_\delta3$-$J_\delta3$	+
			$V_\gamma3$-$J_\gamma1.1$	+		
L2G9	$CD4^+CD8^-$	−/+	$V_\gamma1.4$-$J_\gamma2.3$	+	$V_\delta2$-$D_\delta3$-$J_\delta3$	+
L25	$CD4^-CD8^-$	+/+	$V_\gamma2$-$J_\gamma1.2$	+	$V_\delta2$-$D_\delta3$-$J_\delta3$	+
			$V_\gamma3$-$J_\gamma1.1$	+		
L7F11	$CD4^-CD8^-$	−/+	$V_\gamma1.4$-$J_\gamma2.3$	+	$V_\delta2$-$D_\delta3$-$J_\delta3$	+
			$V_\gamma2$-$J_\gamma2.3$	+		
L4B2	$CD4^-CD8^-$	−/−	$V_\gamma1.3$-$J_\gamma2.3$	+	$V_\delta3$-$D_\delta3$-$J_\delta2$	+

radiated mice after injection of fetal liver precursors, even in the absence of a thymus. Also, nude mice were found to have substantial numbers of intestinal intraepithelial γ/δ T cells despite the almost complete absence of α/β T cells. This demonstrates that at least a subset of γ/δ T cells is thymus independent and that the fetal liver is at least one likely site of extrathymic maturation (12). These results also suggest that the initial repertoire selection may take place in the fetal liver. Previous studies have demonstrated that thymic γ/δ T cells can undergo both positive and negative selection (27–29), and this selection may also take place in the fetal liver.

During γ/δ T cell development in mice, a sequential maturation of γ/δ T cells with defined TCR γ and δ chain rearrangements can be observed (4, 30). γ/δ T cells with invariant receptors mature early and migrate to specific epithelial organs, while γ/δ T cells with greater receptor diversity mature later and localize to the spleen (3–5). Even though the maturation process of human γ/δ T cells is not as well understood, there is evidence for a sequential appearance of γ/δ T cells bearing specific receptors during thymic development. $V_\gamma1.8$-$J_\gamma1.1$/$V_\delta2$-$J_\delta3$ rearrangements are present in early fetal thymus, while $V_\gamma2$-$J_\gamma2.3$/$V_\delta1$-$J_\delta1$ represent the most common γ and δ chain rearrangements in postnatal thymus (6, 10). Presumably due to extrathymic events, γ/δ T cells with $V_\gamma2$-$J_\gamma1.2$/$V_\delta2$-$J_\delta1$ rearrangements become the major γ/δ T cell population in blood during childhood (10, 11, 25).

A comparison of TCR δ chain sequences in human fetal thymus to the present results in human fetal liver demonstrates that similar TCR δ chain rearrangements are found at both sites of T cell maturation. In both fetal liver and thymus, a predominant $V_\delta2$-$D_\delta3$-$J_\delta3$ rearrangement with little N region diversity is seen (6, 7). In contrast, γ chain rearrangements of γ/δ T cells from fetal thymus (6) and fetal liver are different. During early thymic development, both $V_\gamma1$ and $V_\gamma2$ are rearranged to the $J_\gamma1$ cluster ($V_\gamma1.8$-$J_\gamma1.1$ and $V_\gamma2$-$J_\gamma1.3$), while $V_\gamma2$-$J_\gamma2.3$ rearrangements predominate during late stages of thymic development (6, 10). In fetal liver, however, an ordered rearrangement process results in preferential rearrangement of $V_\gamma1$ segments to the $J_\gamma2$ cluster (specifically $J_\gamma2.3$) and of $V_\gamma2$ and $V_\gamma3$ segments to the $J_\gamma1$ cluster ($J_\gamma1.2$ and $J_\gamma1.1$, respectively). Fetal liver γ/δ T cells may therefore have specificities different from γ/δ T cells that mature in the thymus.

A subset of human fetal liver γ/δ T cells (~20%) has an unusual CD4 phenotype (13, 14). Among the six fetal liver γ/δ T cell clones established in this study, three were found to express moderate levels of CD4 and to use TCR δ chains with a $V_\delta2$-$D_\delta3$-$J_\delta3$ rearrangement. Such fetal liver γ/δ T cells may have a specialized immune function as $CD4^+$ $CD8^-$ γ/δ T cells from fetal liver do not possess an NK-like cytotoxic activity observed among $CD4^-CD8^+$ and $CD4^-CD8^-$ γ/δ T cells (13). $CD4^+CD8^-$ γ/δ T cells from adult blood, which constitute a minor subpopulation of mature γ/δ T cells, were also found to be functionally different from $CD4^-CD8^-$ and $CD4^-CD8^+$ γ/δ T cells as they provide help for B cells but lack cytotoxic activity (15). Thus, fetal liver γ/δ T cells, in particular the $CD4^+CD8^-$ subset, may have specific functions in the fetal immune system. The identification of ligands for fetal liver γ/δ T cells would further our understanding of γ/δ T cells and their role in immune recognition during development.

This research was supported by a National Institutes of Health research grant (CA-47554).

Address correspondence to Jack L. Strominger, Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138.

Received for publication 20 November 1991 and in revised form 20 October 1992.

Note added in proof: T cell receptor γ and δ chain rearrangements similar to those found in fetal liver T cell clone L4B2 have been described for autoaggressive γ/δ T cells from a case of polymyositis. These muscle-infiltrating T cells use a Vδ2-Dδ3-Jδ3/Vγ1.3-Jγ1.3 (Jγ1.3 is identical to Jγ2.3 at the protein level) but have longer N regions than the fetal liver T cell clone (31).

References

1. Brenner, M.B., J. McLean, D.P. Dialynas, J.L. Strominger, J.A. Smith, F.L. Owen, J.G. Seidman, S. Ip, F. Rosen, and M.S. Krangel. 1987. Identification of a putative second T-cell receptor. *Nature (Lond.).* 322:145.
2. Loh, E.Y., L.L. Lanier, C.W. Turck, D.R. Littman, M.M. Davis, Y.-H. Chien, and A. Weiss. 1987. Identification and sequence of a fourth human T cell antigen receptor chain. *Nature (Lond.).* 330:569.
3. Takagaki, Y., A. DeCloux, M. Bonneville, and S. Tonegawa. 1989. Diversity of T-cell receptors in murine intestinal intraepithelial lymphocytes. *Nature (Lond.).* 339:712.
4. Asarnow, D.M., W.A. Kuziel, M. Bonyhadi, R.E. Tigelaar, P.W. Tucker, and J.P. Allison. 1988. Limited diversity of $\gamma\delta$ antigen receptor genes of Thy-1 dendritic epidermal cells. *Cell.* 55:837.
5. Janeway, C.A., Jr., B. Jones, and A. Hayday. 1988. Specificity and function of T cells bearing $\gamma\delta$ receptors. *Immunol. Today.* 9:73.
6. Krangel, M.S., H. Yssel, C. Brocklehurst, and H. Spits. 1990. A distinct wave of human T cell receptor γ/δ lymphocytes in the early fetal thymus: evidence for controlled gene rearrangement and cytokine production. *J. Exp. Med.* 172:847.
7. van der Stoep, R. de Krijger, J. Bruining, F. Koning, and P. van den Elsen. 1990. Analysis of early fetal T-cell receptor δ chain in humans. *Immunogenetics.* 32:331.
8. Carding, S.R., J.G. McNamara, M. Pan, and K. Bottomly. 1990. Characterization of γ/δ T cell clones isolated from human fetal liver and thymus. *Eur. J. Immunol.* 20:1327.
9. McVay, L.D., S.R. Carding, K. Bottomly, and A.C. Hayday. 1991. Regulated expression and structure of T cell receptor γ/δ transcripts in human thymic ontogeny. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:83.
10. Casaroti, G., G. De Libero, A. Lanzavecchia, and N. Migone. 1989. Molecular analysis of human γ/δ^+ clones from thymus and peripheral blood. *J. Exp. Med.* 170:1521.
11. Parker, C.M., V. Groh, H. Band, S.A. Porcelli, C. Morita, M. Fabbi, D. Glass, J.L. Strominger, and M.B. Brenner. 1990. Evidence for extrathymic changes in the T cell receptor γ/δ repertoire. *J. Exp. Med.* 171:1597.
12. Bandeira, A., S. Itohara, M. Bonneville, O. Burlen-Defranoux, T. Mota-Santos, A. Coutinho, and S. Tonegawa. 1991. Extrathymic origin of intestinal intraepithelial lymphocytes bearing T-cell antigen receptor γ/δ. *Proc. Natl. Acad. Sci. USA.* 88:43.
13. Aparicio, P., J.M. Alonso, M.L. Toribio, M.A.R. Marcos, L. Pezzi, and C. Martinez-A. 1989. Isolation and characterization of (γ, δ) CD4$^+$ T cell clones derived from human fetal liver cells. *J. Exp. Med.* 170:1009.
14. Strominger, J.L., M. Fabbi, M. Prendergast, R.T. Maziarz, S.J. Burakoff, and V. Groh. 1989. Novel subsets of human T cells (CD4$^+$CD8$^-$TCR $\gamma\delta$ and CD4$^-$CD8$^-$ TCR $\alpha\beta$) and T-cell development. *Int. J. Cancer.* 4(Suppl.):43.
15. Spits, H., X. Paliard, Y. Vandekerckhove, P. van Vlasselaer, and J.E. de Vries. 1991. Functional and phenotypic differences between CD4$^+$ and CD4$^-$ T cell receptor-$\gamma\delta$ clones from peripheral blood. *J. Immunol.* 147:1180.
16. Borst, J., J.J.M. van Dongen, R.L.H. Bolhuis, P.J. Peters, D.A. Hafler, E. de Vries, and R.J. van de Griend. 1988. Distinct molecular forms of human T cell receptor γ/δ detected on viable T cells by a monoclonal antibody. *J. Exp. Med.* 167:1625.
17. Wu, Y.-J., W.-T. Tian, R.M. Snider, C. Rittershaus, P. Rogers, L. LaManna, and S.H. Ip. 1988. Signal transduction of γ/δ T cell antigen receptor with a novel mitogenic anti-δ antibody. *J. Immunol.* 141:1476.
18. Ciccone, E., S. Ferrini, C. Bottino, O. Viale, I. Prigione, G. Pantaleo, G. Tambussi, A. Moretta, and L. Moretta. 1988. A monoclonal antibody specific for a common determinant of the human T cell receptor γ/δ directly activates CD3$^+$ WT31$^-$ lymphocytes to express their functional programs. *J. Exp. Med.* 168:1.
19. Triebel, F., F. Faure, M. Graziani, S. Jitsuhansa, M.P. Lefranc, and T. Hercend. 1988. A unique V-J-C rearranged gene encodes a γ protein expressed on the majority of CD3$^+$ T cell receptor α^-/β^- circulating lymphocytes. *J. Exp. Med.* 167: 694.
20. Wucherpfennig, K.W., K. Ota, N. Endo, J.G. Seidman, A. Rosenzweig, H.L. Weiner, and D.A. Hafler. 1990. Shared human T cell receptor V_β usage to immunodominant regions of myelin basic protein. *Science (Wash. DC).* 248:1016.
21. Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463.
22. Loh, E.Y., S. Cwirla, A.T. Serafini, J.H. Phillips, and L.L. Lanier. 1988. Human T-cell-receptor δ chain: genomic organization, diversity, and expression in populations of cells. *Proc. Natl. Acad. Sci. USA.* 85:9714.
23. Hata, S., M. Clabby, P. Devlin, H. Spits, J.E. De Vries, and M.S. Krangel. 1989. Diversity and organization of human T cell receptor δ variable gene segments. *J. Exp. Med.* 169:41.
24. Hata, S., K. Satyanarayana, P. Devlin, H. Band, J. McLean, J.L. Strominger, M.B. Brenner, and M.S. Krangel. 1988. Extensive junctional diversity of rearranged human T cell receptor δ genes. *Science (Wash. DC).* 240:1541.
25. Tamura, N., K.J. Holroyd, T. Banks, M. Kirby, H. Okayama, and R.G. Crystal. 1990. Diversity in junctional sequences associated with the common human $V_\gamma 9$ and $V_\delta 2$ gene segments in normal blood and lung compared with the limited diversity in a granulomatous disease. *J. Exp. Med.* 172:169.
26. Malissen, M., J. Trucy, F. Letourneur, N. Rebai, D.E. Dunn, F.W. Fitch, L. Hood, and B. Malissen. 1988. A T cell clone expresses two T cell receptor α genes but uses one $\alpha\beta$ heterodimer for allorecognition and self MHC-restricted antigen recognition. *Cell.* 55:49.
27. Wells, F.B., S.-J. Gahm, S.M. Hedrick, J.A. Bluestone, A. Dent, and L.A. Matis. 1991. Requirement for positive selection of $\gamma\delta$ T cells receptor-bearing T cells. *Science (Wash. DC).* 253:903.
28. Itohara, S., and S. Tonegawa. 1990. Selection of $\gamma\delta$ T cells with canonical T-cell antigen receptors in fetal thymus. *Proc. Natl. Acad. Sci. USA.* 87:7935.
29. Dent, A.L., L.A. Matis, F. Hooshmand, S.M. Widacki, J.A. Bluestone, and S.M. Hedrick. 1990. Self-reactive $\gamma\delta$ T cells are eliminated in the thymus. *Nature (Lond.).* 343:714.
30. Strominger, J.L. 1989. Developmental biology of T cell receptors. *Science (Wash. DC).* 244:943.
31. Pluschke, G., D. Rüegg, R. Hohlfeld, and A.G. Engel. 1992. Autoaggressive myocytotoxic T lymphocytes expressing an unusual γ/δ T cell receptor. *J. Exp. Med.* 176:1785.