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 Vδ1 or Vδ2 rearrangements. Five of six fetal liver γ/δ T cell clones had a Vδ2-Dδ3-Jδ3 gene rearrangement with limited junctional diversity; three of these clones had an unusual CD4+CD8- phenotype. Vδ2-Dδ3-Jδ3 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 Vγ1-Jγ2.3, Vγ2-Jγ1.2, and Vγ3-Jγ1.1 rearrangements with minimal incorporation of template-independent N region nucleotides. TCR δ 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.
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 fetal 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), Vv9; 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, L42B, L7FII, 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 (decellularized supernatant from PHA-stimulated blood mononuclear cells) at one cell/well in U-bottomed microtiter plates using 2 x 10\(^{5}\) irradiated mononuclear cells and 10\(^{5}\) 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 x 10\(^6\) cells using the RNAzol B method (Cinna/Biotech). 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\(_2\)O 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\(_2\)O 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 γ 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 for amplification of TCR γ chain were: V\(_{\gamma}1\) to V\(_{\gamma}5\) chains and used in combination with a C\(_{\gamma}\) primer (0.5 μg of each primer per reaction). Sequences of oligonucleotides used for PCR or for Southern blot hybridization were: V\(_{\gamma}1\), 5' gggggctacagttgcgctcatata-3'; V\(_{\gamma}2\), 5' gggggtcacccctgcgtctgca-3'; V\(_{\gamma}3\), 5' ggggcgtactaatgatcca-3'; V\(_{\gamma}4\), 5' ggggcgtactaatgatcca-3'; V\(_{\gamma}5\), 5' ggggcgtactaatgatcca-3'; V\(_{\alpha}1\), 5' gggggataagcttgaagtttag-3'; C\(_{\alpha}1\), 5' gggggataagcttgaagtttag-3'; C\(_{\alpha}2\), 5' gggggataagcttgaagtttag-3'; C\(_{\alpha}3\), 5' gggggataagcttgaagtttag-3'; C\(_{\alpha}4\), 5' gggggataagcttgaagtttag-3'; C\(_{\alpha}5\), 5' gggggataagcttgaagtttag-3'; C\(_{\alpha}6\), 5' gggggataagcttgaagtttag-3'; C\(_{\alpha}7\), 5' gggggataagcttgaagtttag-3'; C\(_{\alpha}8\), 5' gggggataagcttgaagtttag-3'; C\(_{\alpha}9\), 5' gggggataagcttgaagtttag-3'; C\(_{\alpha}10\), 5' gggggataagcttgaagtttag-3'; C\(_{\alpha}11\), 5' gggggataagcttgaagtttag-3'.

**cDNA Sequence Analysis.** V\(_{\alpha}\)C\(_\gamma\) PCR products, M13 mp19 plasmid DNA, and pUC-18 DNA were digested with EcoRI and Sall restriction endonucleases to generate compatible ends for

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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 cDNA synthesized from fetal liver RNA (using primers specific for Vδ1 to Vδ5 gene segments in combination with a Cδ primer). RNA was extracted from one fetal liver tissue sample (FL 2/27) 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 Cδ 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 V~2. Trace amounts of Vδ3, Vδ4, and Vδ5 were amplified from one fetal liver sample (FL 2/27) (Fig. 1).

To determine Jδ gene usage of fetal liver TCR δ chains, Vδ5 gene segments were amplified from cDNA using Vδ1 to Vδ5 primers in combination with a Cδ primer followed by Southern blot hybridization with probes for Jδ1, Jδ2, and Jδ3 gene segments. Hybridization of Vδ2-Cδ and Vδ1-Cδ 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-Cδ 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.

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 TGAACC(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 Jα1, use Dα1, Dα2, and Dα3 (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
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 Vβ1-Jα1 gene segments (mAb δTCS1), the TCR Vβ2 gene segment (mAb TrγA), and the TCR Vβ3 gene segment (mAb BB3). Three of four clones were found to express the TCR Vβ2 gene segment, while only one clone was positive for expression of the Vβ1 gene segment. These data confirm that the TCR Vβ2 gene segment is the most commonly used Vβ 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 Vβ2-Dβ3-Jδ3 rearrangement with limited junctional diversity (Fig. 5). The Vβ2-Jδ3 rearrangement of these clones was confirmed by genomic Southern blot analysis using probes for Vβ2 and Jδ3 (data not shown). Some clones showed strikingly similar protein sequences at the Vβ2-Dβ3-Jδ3 junction; clones L3 and L25 differed only by one residue at the Vβ2-Dβ3 junction, while clones L7 and L2G9 differed by two additional residues present at the Dβ-Jδ 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 Vβ2-Dβ3-Jδ3 rearrangements with limited junctional diversity.

To determine rearrangements of the TCR γ locus among these T cell clones, cDNA samples were amplified using a Cγ oligonucleotide in combination with primers specific for Vγ1-2-Vγ4 gene segments and for the Vγ1 family, which has five functional members. Of the six clones studied, two had a single Vγ1-Cγ rearrangement (clones L4B2, L2G9) while...
A. V62-J63 rearrangements

| V62 | N | D62 | N | D63 | N | J63 | In Frame |
|-----|---|-----|---|-----|---|-----|----------|
| 1   |   |     |   |     |   |     |          |
| 2   |   |     |   |     |   |     |          |
| 3   |   |     |   |     |   |     |          |
| 4   |   |     |   |     |   |     |          |
| 5   |   |     |   |     |   |     |          |
| 6   |   |     |   |     |   |     |          |

B. V62-J52 rearrangement

| V62 | N | D62 | N | D52 | N | J52 | In Frame |
|-----|---|-----|---|-----|---|-----|----------|
| 1   |   |     |   |     |   |     |          |
| 2   |   |     |   |     |   |     |          |
| 3   |   |     |   |     |   |     |          |
| 4   |   |     |   |     |   |     |          |
| 5   |   |     |   |     |   |     |          |
| 6   |   |     |   |     |   |     |          |

C. V62-J53 rearrangement

| V62 | N | D62 | N | D53 | N | J53 | In Frame |
|-----|---|-----|---|-----|---|-----|----------|
| 1   |   |     |   |     |   |     |          |
| 2   |   |     |   |     |   |     |          |
| 3   |   |     |   |     |   |     |          |
| 4   |   |     |   |     |   |     |          |
| 5   |   |     |   |     |   |     |          |
| 6   |   |     |   |     |   |     |          |

D. V61-J53 rearrangement

| V61 | N | D61 | N | D53 | N | J53 | In Frame |
|-----|---|-----|---|-----|---|-----|----------|
| 1   |   |     |   |     |   |     |          |
| 2   |   |     |   |     |   |     |          |
| 3   |   |     |   |     |   |     |          |
| 4   |   |     |   |     |   |     |          |
| 5   |   |     |   |     |   |     |          |

Figure 3. Junctional diversity of TCR δ chain. Amplified TCR δ chain (V61 and V62) from two fetal livers were cloned into M13 mp19 and sequenced by the dideoxy method. (A) TCR δ chain sequences with a V62-J63 rearrangement; (B) TCR δ chain sequences with V62-J52 rearrangement; (C) TCR δ chain sequences with V62-J53 rearrangement; (D) TCR δ chain sequences with a V61-J53 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 V61-J53 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 V61-1-Ca and V62-2-Ca rearrangements (clones L3, L7F11) or V62-Ca and V62-3-Ca rearrangements (clones L7, L2S) (Fig. 6 and Table 1). By sequence analysis of the V61-J5 junction, all four V61 gene segments were found to be rearranged to J5,2,3; three members of the V61 family (V61.1, V61.4, V61.8) were represented among these four sequences (the V61-1,1 rearrangement was out of frame). In contrast, three of four V62 gene segments were rearranged to J5,2,3; both V62 segments were rearranged to J5,1,1. Thus, there appears to be an ordered rearrangement process that results in preferential rearrangement of V61 segments to the J5,2 cluster (specifically J5,2,3) and of V62 and V63 segments to the J5,1 cluster (J5,1,2 and J5,1,1, 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 V61 and V62 were found to be rearranged to the J5,1 cluster (V61.8-J5,1,1 and V62-J5,1,3, neither of which was present in the fetal liver T cell clones), while γ/δ T cells that use V62-J5,2,3 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\textsuperscript{a} 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 \(\alpha/\beta\) and \(\gamma/\delta\) TCRs by indirect immunofluorescence analysis. Antibodies used were: P3 (negative control), T3b (anti-CD3), OKT4 (anti-CD4), Leu2A (anti-CD8), WT31 (TCR-\(\alpha/\beta\) >> TCR-\(\gamma/\delta\)), \(\delta TCS1\) (anti-TCR-\(\delta\)), TyA (anti-TCR-\(\gamma/\delta\)), and BB3 (anti-TCR \(\gamma/\delta\)). T cell clones L3, L7, L25, L4B2, L25, and L7F11 were positive for the TCR-\(\delta\) mAb, while T cell clones L6, L38, and L7F5 were positive for the WT31 mAb. Three of the \(\gamma/\delta\) T cell clones (L3, L7, L38) expressed moderate levels of CD4, while three \(\alpha/\beta\) T cell clones (clones L4B2, L25, L7F11) were CD4\textsuperscript{+}CD8\textsuperscript{+}. Also, two \(\alpha/\beta\) T cell clones (L4G1, L7F5) had a CD4\textsuperscript{+}CD8\textsuperscript{+} phenotype.

Figure 5. Sequence analysis of the junctional region of the TCR \(\delta\) chain among fetal liver \(\gamma/\delta\) T cell clones. cDNA from fetal liver T cell clones was amplified using primers for V\(\delta\)2 and C\(\delta\). Amplified products were cloned into M13 mp19 and sequenced by the dideoxy method. Five of six clones were found to have a V\(\delta\)2-D\(\delta\)3-J\(\delta\)2 rearrangement with little N region diversity, one clone had a V\(\delta\)3-D\(\delta\)3-J\(\delta\)2 rearrangement.

Figure 6. Sequence analysis of the junctional region of the TCR \(\gamma\) chain among fetal liver \(\gamma/\delta\) T cell clones. V\(\gamma\) gene usage was determined by amplification of cDNAs with primers for V\(\gamma\)1-V\(\gamma\)4 in combination with a C\(\gamma\) primer. Amplified products were cloned into p_CRII (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\(\gamma\)2-J\(\gamma\)2,3 sequence (in addition to an in-frame V\(\gamma\)1.4-J\(\gamma\)2,3 rearrangement) even though the T cell clone was not recognized by the mAb TyA. It is therefore assumed that the V\(\gamma\)2-J\(\gamma\)2,3 sequence originated from a second T cell population present in the original clone.
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. Vv1.8-Jv1.1/Vs2-Jv3 rearrangements are present in early fetal thymus, while Vv2-Jv2.3/Vs1-Jv1 represent the most common γ and δ chain rearrangements in postnatal thymus (6, 10). Presumably due to extrathymic events, γ/δ T cells with Vv2-Jv1.2/Vs2-Jv1 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 Vv2-Ds3-Js3 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 Vv1 and Vv2 are rearranged to the Jv1 cluster (Vv1.8-Jv1.1 and Vv2-Jv1.3), while Vv2-Jv2.3 rearrangements predominate during late stages of thymic development (6, 10). In fetal liver, however, an ordered rearrangement process results in preferential rearrangement of Vv1 segments to the Jv2 cluster (specifically Jv2.3) and of Vv2 and Vv3 segments to the Jv1 cluster (Jv1.2 and Jv1.1, 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 (3–20%) 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 Vv2-Ds3-Js3 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.

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

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Note added in proof: T cell receptor γ and δ chain rearrangements similar to those found in fetal liver T cell clones 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).

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