Identification of a Committed T Cell Precursor Population in Adult Human Peripheral Blood

By Ludovica Bruno,* Pieter Rees,† Mark Dressing,* Marina Cella,* and Hergen Spits‡

From the †Basel Institute for Immunology, 4005 Basel, Switzerland; ‡The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands

Summary

Here, we report data concerning the discovery in adult human peripheral blood of a precursor cell population able to differentiate into CD4+CD8+ mature T cells. These cells, which represent 0.1–0.5% of total peripheral blood mononuclear cells (PBMC), express substantial levels of CD4, but lack CD3 surface expression. At a molecular level, they express the pre-T cell receptor α (pTα) gene, CD3-γ, CD3-δ and CD3-ε, and RAG-1 recombination enzyme and have initiated rearrangements in the T cell receptor (TCR-β) locus (D-J). Moreover, low levels of CD3ε protein, but not of TCR-β chain, can be detected in their cytoplasm. Our results suggest that CD4+CD8+ cells identified in peripheral blood are different from CD3-CD4+CD8- thymocytes and may contain precursors of an extrathymic T cell differentiation pathway.

In murine T cell development, early thymocytes that productively rearrange the TCR-β locus are selected to continue maturation. This happens because of the coupling of the rearranged β chain with the invariant pre-TCR-α (pTα) protein (1, 2). This pre-TCR regulates early T cell development; later stages are under the control of the mature TCR, composed of the α and β chains (3). The TCR-β-pTα heterodimer is associated with CD3 molecules (4), and signals triggered by the pre-TCR induce expansion and differentiation of immature precursor cells (5).

Analysis of pTα gene-deficient mice provided formal proof that expression of the pre-TCR is required in the transition of CD25+ double-negative (DN) T cell precursors into small CD4+CD8+ thymocytes (5). This transition normally occurs through a stage in which maturing thymocytes proliferate vigorously and lose the expression of CD25. Nevertheless, low levels of mature αβ T cells can be detected in the periphery of pTα knockout mice, suggesting that the expression of pTα is necessary for quantitative expansion of maturing thymocytes, but not for crucial differentiation.

Using murine pTα cDNA as a probe, we were able to show that a comparable gene was expressed in human thymocytes (6). Amino acid sequence comparison between human and mouse pTα cDNAs revealed high sequence homology in the extracellular as well as the transmembrane region, but complete divergence in the cytoplasmic region. Recently, another group cloned human pTα cDNA and performed a comparison of the developmental regulation of pTα, TCR-β, TCR-α, and RAG-1 gene expression, providing a picture of the maturational progression of early human intrathymic stages (7).

We have previously shown that murine pTα expression is exquisitely T lineage specific and occurs in pre-T cells outside the thymus, in the earliest T cell precursors identified in the thymus, and in sites that support extrathymic T cell development (gut and liver), (8). Consistent with these observations, pTα RNA could not be detected in human non-T cells including B, NK, myeloid, and dendritic cells (7). Here, we have used the expression of the pTα gene as a tool to identify a human T cell precursor in the peripheral blood of adult donors.

Material and Methods

Antibodies. Cell staining was performed using the following mouse anti-human mAbs: phycoerythrin-conjugated (R-PE)-CD4, fluorescein-conjugated FITC-CD3, FITC-CD14, R-PE-CD5, biotin-CD10, R-PE-CD33, biotinylated HLA-DR, and RPE-CD34 (Becton Dickinson, San Jose, CA); tricolor CD4, biotinylated CD2 and RPE-CD7 (Caltag Laboratories, San Francisco, CA); CD45RA (IgG2b) and B7-2 (IgG2b) (PharMingen, San Diego, CA); FITC-pan-TCR-αβ (T Cell Diagnostic, Inc., Boston, MA). To deplete samples from CD3+ cells, we used TR 66 mAb (9) that was FITC conjugated according to standard procedures. For TCR-β cytoplasmic staining we used HB-9283 supernatant (Amer-
immature T cells in human peripheral blood
Results
Identification of a pTα+ Population in Peripheral Blood. By PCR analysis of retrotranscribed mRNA of PBMC of adult donors we were able to detect low levels of pTα message in all of the samples tested (data not shown; Fig. 1 B, lane 1). Depletion of CD3+ T cells from PBMC did not abrogate the detection of pTα expression by PCR. This result was expected, because mature T cells have completely lost expression of pTα (6–8). In view of our previously demonstration (6) that the thymic subset expressing the highest levels of pTα is CD3+CD4+CD8− (immature single positive, ISP), we investigated whether expression of CD4 was shared by pTα+ cells in PBMC. Fig. 1 demonstrates that this is indeed the case, because sorted CD4+, CD3− cells (Fig. 1 A, shown in the box, R2) expressed pTα (Fig. 1 B, lane 2). No pTα message could be detected in total PBMC depleted of CD4+CD3+CD14− cells (Fig. 1 B, lane 3). Monocytes, which also express low levels of CD4 but are CD3+, were excluded from the selection by staining with a specific mAb (CD14) and on the basis of their size (Fig. 1 A). Interestingly, the physical parameters (FSC versus SSC) of the pTα+ subset differ substantially from those of mature lymphocytes and monocytes, in that pTα+ cells have a size and a cell complexity intermediate between lymphocytes and monocytes (Fig. 1 A, bottom). This cell population represents, in different healthy adult individuals analyzed (25–35 yr old), 3–9% of gated cells (R 1) and 0.1–0.5% of the total PBMC, obtained by Ficoll preparation.

Partial DJβ Rearrangement and β Gene Transcription in pTα+ Cells in Peripheral Blood. Rearrangement of B cell (13) and T cell (14) antigen receptor gene loci to form functional VDJ gene segments is a key molecular event in lymphocyte ontogeny. In the thymus, DJβ rearrangements precede V(D)β rearrangements (15). Therefore, the pattern of TCR β gene rearrangements represents a helpful marker to assess the maturational stage of a given population and its possible commitment to the T cell lineage. To analyze the rearrangement status of the TCR-β locus (DJβ versus germline configuration) in the pTα+ population observed in human peripheral blood, genomic DNA was isolated from CD4+CD3+CD14− pTα+ cells, and a recently described (10) PCR-based method was used, to amplify the DJβ1 gene region specifically. Relevant controls in which

![Figure 1](https://example.com/figure1.png)
Figure 2. Detection of partial (D–J) rearrangements of the TCR-β locus. (A) At DNA level. Genomic DNA was isolated from sorted CD4+CD3−CD14−, CD4+CD3+, CD14+, and total PBMC and amplified by PCR using TBF1 and TBR1 primers to detect Dβ1-Jβ1 rearrangements. Normal PBMC and monocytes (CD14+) were used as positive and negative controls, respectively. PCR products were blotted and hybridized with TBR3 probe. PCR products, ranging from 200 to 3000 bp, and the Jβ segment used are indicated. GL, germline. (B) At RNA level. Total RNA was first isolated from sorted CD4+CD3−CD14−, CD14+, and from total PBMC, and subsequently, RT–PCR using TBF1 and Cβ-specific primer. PCR products were blotted and probed with a Cβ-specific primer. PCR products were analyzed by Southern blotting with an internal oligo specific for the Cβ region. All cDNA were controlled for integrity with β-actin primers before D–J–C amplification (data not shown). Results are shown in Fig. 2 B, in which the presence of a transcript of 600 bp, corresponding to partial Dβ rearrangement, is detected both in unfractionated PBMC and sorted CD4+CD3−CD14− cells, but not in CD14+ monocytes. Taken together, these results show that both rearrangement of, and transcription from TCR-β loci are present in a fraction of the pTo+ cell population present in peripheral blood.

The CD4+CD3+CD14− cell population expresses RAG-1 gene and is not cycling. Previous studies have shown that analysis of the expression of RAG-1 and RAG-2 genes and of TCR transcription are useful to trace early events during T cell development both in murine and in human T cell development (7, 16). As independent confirmation of Dβ rearrangement of the TCR-β locus of CD4+CD3+CD14− cells, we tested for RAG-1 gene expression in two different preparations of CD4+CD3−CD14− cells from human peripheral blood. RAG-1 message could indeed be detected in both preparations (Fig. 3A, lanes 1 and 2), as well as in DN cells from thymus, but not in mature CD4+CD3+ T cells.

Because CD3+CD4−CD8− immature thymocytes are actively cycling, we then asked whether peripheral pTo+CD4−CD3−CD14− cells were also characterized by active proliferation. Analysis of DNA content in the potential T cell precursor population revealed that all of the cells were in G2-M phase of the cell cycle, while TCR-αβ+ Jurkat T cell line showed also G2-M phases (Fig. 3B). Thus, CD4−CD3−CD14− cells are in a resting state, express RAG-1 and pTo, and carry Dβ rearrangements at the TCR-β locus.

To ascertain whether the presence of Dβ rearrangements was paralleled by transcription of the rearranged genes, we tested for the presence of transcripts corresponding to the partial Dβ rearrangements by RT–PCR. Total RNA was isolated from sorted peripheral populations (CD4+CD3−CD14− and CD14+) or total PBMC and reverse transcribed into cDNA before amplification with a primer specific for the upstream Dβ1 sequence together with a Cμ-specific primer. PCR products were analyzed by Southern blotting with an internal oligo specific for the Cμ region. All cDNA were controlled for integrity with β-actin primers before D–J–C amplification (data not shown). Results are shown in Fig. 2 B, in which the presence of a transcript of 600 bp, corresponding to partial Dβ rearrangement, is detected both in unfractionated PBMC and sorted CD4+CD3−CD14− cells, but not in CD14+ monocytes. Taken together, these results show that both rearrangement of, and transcription from TCR-β loci are present in a fraction of the pTo+ cell population present in peripheral blood.

The CD4+CD3+CD14− cell population expresses RAG-1 gene and is not cycling. Previous studies have shown that analysis of the expression of RAG-1 and RAG-2 genes and of TCR transcription are useful to trace early events during T cell development both in murine and in human T cell development (7, 16). As independent confirmation of Dβ rearrangement of the TCR-β locus of CD4+CD3+CD14− cells, we tested for RAG-1 gene expression in two different preparations of CD4+CD3−CD14− cells from human peripheral blood. RAG-1 message could indeed be detected in both preparations (Fig. 3A, lanes 1 and 2), as well as in DN cells from thymus, but not in mature CD4+CD3+ T cells.

Because CD3+CD4−CD8− immature thymocytes are actively cycling, we then asked whether peripheral pTo+CD4−CD3−CD14− cells were also characterized by active proliferation. Analysis of DNA content in the potential T cell precursor population revealed that all of the cells were in G2-M phase of the cell cycle, while TCR-αβ+ Jurkat T cell line showed also G2-M phases (Fig. 3B). Thus, CD4−CD3−CD14− cells are in a resting state, express RAG-1 and pTo, and carry Dβ rearrangements at the TCR-β locus.
Expression of CD3 Subunits but Not TCR-β Chain in CD4<sup>+</sup>CD3<sup>e</sup>-CD14<sup>-</sup> PBMC. Although the pT<sub>α</sub> peripheral blood cell population does not express CD3 on the cell surface, as assessed by FACS<sup>®</sup> analysis, we investigated whether transcripts for the different components of this TCR-associated signaling molecule could be detected. For this purpose, cDNA from CD4<sup>+</sup>CD3<sup>-</sup>CD14<sup>-</sup> sorted cells was amplified with three different sets of primers specific for CD3-γ, CD3-ε, and CD3-δ transcripts, respectively. Analysis of the PCR products showed that CD4<sup>+</sup>CD3<sup>-</sup>CD14<sup>-</sup> cells expressed all three CD3 components, as can be observed in the T cell line MOLT 4 (Fig. 5A); CD3-γ was found to be expressed in low amount, because it appeared barely detectable on agarose gels after staining with ethidium bromide; nonetheless, the presence of the message was readily detectable after hybridization with a specific internal probe of the blotted PCR products (data not shown).

The concomitant expression of transcripts of pT<sub>α</sub> and CD3 components in the CD4<sup>+</sup>CD3<sup>-</sup>CD14<sup>-</sup> cell population, together with the described rearrangement of TCR-β genes, raised the possibility that, at this stage, such cells might assemble in their cytoplasm a putative human pre-TCR complex consisting of a TCR-β chain associated with the pT<sub>α</sub> chain. To test this possibility we performed cytoplasmic staining to detect the presence of TCR-β and CD3-ε proteins in CD4<sup>+</sup>CD3<sup>-</sup>CD14<sup>-</sup> cells. Flow cytometric analysis shown in Fig. 5B revealed that while pT<sub>α</sub> cells do not express TCR-β chain in the cytoplasm, all cells within this population express CD3-ε protein, but at lower levels than in a mature human T cell clone. Specificity of the CD3-ε cytoplasmic staining was confirmed by relevant controls, and in particular by the lack of any FACS<sup>®</sup> profile shift in similarly stained B lymphocytes and dendritic cells (data not shown). The absence of TCR-β chain in the cytoplasm of CD4<sup>+</sup>CD3<sup>-</sup>CD14<sup>-</sup> cells is consistent with lack of VDJβ rearrangements at DNA level (data not shown). These data indicate that while some components of such putative human pre-TCR, and in particular CD3-ε, are not only transcribed, but also translated into proteins, others, like the TCR-β chain are only present in the form of D-J<sup>β</sup> transcript, while no translation can be detected.

Only a Fraction of CD4<sup>+</sup>CD3<sup>-</sup>CD14<sup>-</sup> Expresses pT<sub>α</sub>. To define whether all CD4<sup>+</sup>CD3<sup>-</sup>CD14<sup>-</sup> cells or, on the other hand, only a fraction of them expressed pT<sub>α</sub>, we performed PCR-based analysis of pT<sub>α</sub> expression on discrete numbers of sorted CD4<sup>+</sup>CD3<sup>-</sup>CD14<sup>-</sup> PBMC obtained with the ACDU technique. In brief, 50, 20, 10, or 5 CD4<sup>+</sup>CD3<sup>-</sup>CD14<sup>-</sup> cells were directly sorted in 96-well plates and cDNA was synthesized directly in the plates. Two rounds of PCR followed, a first one on the total cDNA obtained and a second one on a fraction of the first

**Figure 4.** Cell surface analysis of pT<sub>α</sub><sup>+</sup> cells. Mononuclear cells were separated with standard Ficoll gradient and depleted of CD3<sup>+</sup> cells with FITC-TR66 mAb. Three-color analysis was then performed using CD4- and CD14-specific mAbs in combination with a third antibody of interest (histograms of which are shown in the figure): in the case of biotinilated antibodies (CD2, CD10, and HLA-DR), CD4–R–PE and CD14–FITC and, subsequently, APC were used; in the case of directly PE-labeled antibodies (CD2, CD10, and HLA-DR), CD4–R–PE and CD14–FITC were used. When staining with CD33 or B7-2 mAbs we had to sort CD4<sup>+</sup>CD3<sup>-</sup>CD14<sup>-</sup> cells, as shown in Fig. 1, and restain them with one of the two antibodies that were then detected with mouse anti-human IgG2.
PCR product (2 μl). For both PCRs, the same pTα-specific primers were used.

When we initially restricted our analysis to duplicates of each sorted cell aliquots (50, 20, 10, 5 cells; Fig. 6, top left), a positive reaction could be detected in all the wells, down to the ones that contained 5 cells/well. To try and get a more accurate and reliable estimate of the frequency, ten separate PCR reactions were run, each on a well containing 5 cells (Fig. 6, top right). The analysis of the results showed a positive signal in 50% of the samples. Assuming that our PCR was 100% efficient on these 5-cell samples, a frequency of pTα+ cells of 1/7 can be calculated using Poisson's equation.

Next, we asked whether differences existed in the frequency of pTα+ cells in the two subsets that could be identified by differential expression of CD2. Therefore, we separated our starting population into CD2+ and CD2− (see Fig. 4) subsets and performed the same PCR-based analysis. This showed an enriched pTα+ cell frequency in the CD2− subset where all samples containing 5 cells well

Figure 5. (A) CD3-γ, CD3-ε, and CD3-δ transcripts are expressed in pTα+ cells. RT–PCR analysis with primers specific for three components (γ, ε, and δ) of the CD3 signaling molecule was performed on CD4+ CD3+ CD14− cell population. The T cell line MOLT4 and water were used as positive and negative controls, respectively. (B) CD3-ε molecule, but not TCR β chain, is present in the cytoplasm of pTα+ cells. Cytoplasmic staining was performed with a TCR-β-specific (top right and left) or a CD3-ε-specific (bottom right and left) mAbs on a T cell clone (right top and bottom) and on CD4+ CD3+ CD14− sorted PBMC (left top and bottom). In the case of TCR-β cytoplasmic staining, mouse anti-human IgG1 was subsequently used. Dotted histogram lines represent negative controls that were the following: for β-cytoplasmic, mouse anti-human IgG1 alone and for CD3-cytoplasmic, CD20–FITC.
scored positive, whereas only 50% of the samples containing 10 cells/well showed a positive PCR reaction in the CD2\(^+\) subset (Fig. 6, bottom).

**T Cell Differentiation Potential.** The fact that the CD4\(^+\)CD3\(^-\)CD14\(^-\) PB cell population was found to express CD3-\(\gamma\), CD3-\(\delta\), and CD3-\(\epsilon\), pT\(\alpha\), and RAG-1 transcripts, and has partially rearranged the TCR-\(\beta\) locus strongly suggested that committed T cell precursors were present. Moreover, the phenotype of these cells resembles that of the CD3\(^+\)CD4\(^+\)CD8\(^-\) ISP thymocytes. These latter cells differentiate into DP and CD4\(^-\)TCR-\(\alpha\beta\) cells in mouse FTOC. To analyze the T cell developmental potential, CD4\(^+\)CD3\(^-\)CD14\(^-\) peripheral blood cells were introduced into FTOC. After 3 wk of incubation only few human cells were recovered from the FTOC with CD4\(^+\)CD3\(^-\)CD14\(^-\) peripheral blood cells. To determine accurately the phenotype of these cells, we stained with anti-CD45, anti-CD4, and anti-CD3 or anti-CD8. Fig. 7 shows that almost all human cells expressed high levels of CD3 and CD4. The CD4\(^+\) cells completely lack CD8. This pattern of differentiation is distinct from what we observe with CD3\(^+\)CD4\(^-\)CD8\(^-\) ISP thymocytes, which give rise to a majority of DP and SP cells (Fig. 7).

As the CD2\(^-\) and CD2\(^+\) fractions were differentially enriched in pT\(\alpha\)-expressing cells, we asked whether either population could give rise to mature T cells when cultured in the presence of irradiated feeder cells, IL-2, IL-7, and PHA. CD3\(^-\)CD4\(^+\)CD8\(^-\) thymocytes are completely unable to grow under these conditions (Spits, H., unpublished data). However, when \(1 \times 10^6\) sorted potential precursor cells were cultured under these conditions, we observed that, while CD2\(^+\) cells developed into either CD4\(^+\)CD3\(^+\) resembling mature T cells or CD4\(^+\)CD3\(^-\) cells (Fig. 8 A), CD2\(^-\) cells were not able to expand. CD4\(^+\)CD3\(^-\) cells developing from the CD2\(^+\) fraction were found to have CD3 and TCR-\(\beta\) proteins in the cytoplasm and, after restimulation, gradually acquired CD3 surface expression (data not shown). pT\(\alpha\) expression was not present in both CD4\(^+\)CD3\(^-\) and CD4\(^+\)CD3\(^-\) phenotypes obtained (data not shown).

To characterize further the progeny obtained from CD4\(^+\)CD3\(^-\)CD14\(^-\) starting population, we cloned it by limiting dilution. Cloning frequencies ranging from 5 to 10% were observed in five different experiments performed; in addition, according to what was previously shown in bulk culture experiments, all clones were CD4\(^+\), but expressed different levels of CD3 molecule on the cell surface, vary-
Immature T Cells in Human Peripheral Blood

...ing from CD3^high to CD3^low. In Fig. 8 B, some representative clones are shown; interestingly, some of them were characterized by a smear in CD3 expression. All the different clones obtained, independently from their level of CD3 expression, became, after repeated stimulations, CD3^high and TCR-αβ^+, suggesting that stimulation favored the assembling of a functional TCR on the cell surface.

Discussion

In the present work, we describe a novel population of T cell progenitors present in human peripheral blood. This cell population lacks CD3 on the cell membrane, expresses CD4, but not the early hematopoietic progenitor cell marker CD34. The latter molecule has been shown to be expressed on all of the early T cell progenitor populations described until now, both inside (19) and outside the thymus (17, 20–22). Despite the absence of CD34, these cells have characteristics of committed T cell progenitors. Specifically, these cells show partial TCR-β locus rearrangements (DJ but not VDJ) and have transcripts of genes of pTα and RAG-1. Moreover, these cells express CD3-δ, CD3-γ, and CD3-ε genes. The fact that these CD4^+CD3^- cells do not express CD34 and that they were found to be the only fraction in total PBMC positive for pTα, is in contrast with some recently published data (7) in which CD34^+ cells isolated from both adult and cord blood (CB) were found to be pTα^+. We failed to detect pTα message in CD34^+ cells present in either PB (data not shown) and CB (Blom, B., and H. Spits, manuscript in preparation). This discrepancy may be due to differences in the purification of CD34^+ cells; moreover, CD34^+ cells were obtained with G-CSF treatment (7); it is possible that in this way pTα^+ cells are mobilized from bone marrow, where we can detect pTα RNA in CD34^+ cells (data not shown).

Cells within this population can develop into CD4^+ TCR-αβ^+ cells in both FTOC as well as with IL-2, IL-7, and PHA. This latter development is not due to selective outgrowth of mature T cells, because the cloning efficiency (5–10%) is much higher than the theoretical contamination of cell surface CD3^+ cells (0.1–0.5%). In addition, the possibility that CD4^+CD3^-CD14^- cells are derived from activated mature CD4^+ T cells that have downregulated the TCR seems to be highly unlikely because, in contrast with mature T cells, these cells do not express the TCR-β protein in the cytoplasm. Moreover, also the relatively low levels of CD3-ε protein in the cytoplasm, size and cell complexity (FSC versus SSC) distinguish the CD4^+CD3^-CD14^- cells from mature T cells.

The development of CD4^+CD3^-CD14^- peripheral blood cells into CD4^+ TCR-αβ^+ cells in both a human/mouse hybrid FTOC (Blom, B., and H. Spits, unpublished) and in human PBMC (Blom, B., and H. Spits, manuscript in preparation) is contrast with some recently published data (7) in which CD34^+ cells isolated from both adult and cord blood (CB) were found to be pTα^+. We failed to detect pTα message in CD34^+ cells present in either PB (data not shown) and CB (Blom, B., and H. Spits, manuscript in preparation). This discrepancy may be due to differences in the purification of CD34^+ cells; moreover, CD34^+ cells were obtained with G-CSF treatment (7); it is possible that in this way pTα^+ cells are mobilized from bone marrow, where we can detect pTα RNA in CD34^+ cells (data not shown).

Cells within this population can develop into CD4^+ TCR-αβ^+ cells in both FTOC as well as with IL-2, IL-7, and PHA. This latter development is not due to selective outgrowth of mature T cells, because the cloning efficiency (5–10%) is much higher than the theoretical contamination of cell surface CD3^+ cells (0.1–0.5%). In addition, the possibility that CD4^+CD3^-CD14^- cells are derived from activated mature CD4^+ T cells that have downregulated the TCR seems to be highly unlikely because, in contrast with mature T cells, these cells do not express the TCR-β protein in the cytoplasm. Moreover, also the relatively low levels of CD3-ε protein in the cytoplasm, size and cell complexity (FSC versus SSC) distinguish the CD4^+CD3^-CD14^- cells from mature T cells.

The development of CD4^+CD3^-CD14^- peripheral blood cells into CD4^+ TCR-αβ^+ cells in both a human/mouse hybrid FTOC (Blom, B., and H. Spits, manuscript in preparation) and in human PBMC (Blom, B., and H. Spits, manuscript in preparation) is contrast with some recently published data (7) in which CD34^+ cells isolated from both adult and cord blood (CB) were found to be pTα^+. We failed to detect pTα message in CD34^+ cells present in either PB (data not shown) and CB (Blom, B., and H. Spits, manuscript in preparation). This discrepancy may be due to differences in the purification of CD34^+ cells; moreover, CD34^+ cells were obtained with G-CSF treatment (7); it is possible that in this way pTα^+ cells are mobilized from bone marrow, where we can detect pTα RNA in CD34^+ cells (data not shown).
thymus and to give rise to DP and SP thymocytes (23). A fraction (~65%) of these fetal blood murine cells also expressed CD4 (Rodewald, H.R., personal communication). It is possible that the CD4+CD3-CD14+ PBMC are the direct precursors of the CD3-CD4+CD8-ISP cells in the thymus. These latter cells are actively dividing, express CD1, CD5, and CD7, and develop into DP and SP cells in an FTOC and in human thymic fragments transplanted SCID mice. However, although CD4+CD3-CD14- cells and CD3-CD4+CD8-ISP thymocytes share expression of CD4 and of pTα, the two populations are distinct both with respect to phenotypic and differentiation characteristics (Table 1). The most conspicuous phenotypic difference concerns the expression of CD1a, which is present on all ISP thymocytes but not on the CD4+CD3-CD14- PBMC and CD45RA, which has an opposite expression pattern. Moreover, CD4+CD3-CD14+ PBMC are not able to develop in the hybrid human/mouse FTOC in double positive (DP) cells, but only into single-positive (SP) CD4+CD3+ cells, whereas DP and few CD3+ SP are obtained from ISP thymocytes in the same experimental conditions. Moreover, it is impossible to expand ISP thymocytes with IL-2, IL-7, PHA, and feeder cells as observed for CD4+CD3-CD14- PBMC both in bulk culture and in limiting dilution (results not shown). If these CD3-CD4+ PBMC are prothymocytes, one should assume that cells of different developmental stages can enter the thymus, because the thymus also contains CD3+CD14+ progenitor cells with the TCR in germline configuration and the capacity to develop into T cells, DC, and NK cells (18). One should also expect to find a population that is intermediate between CD3-CD4+ PBMC and ISP thymocytes. Such a population has not yet been described in the human thymus. Based on the fact that the thymus in adults is severely involuted, it may be reasoned that there is not an influx of new progenitors into the thymus in adults. This notion, together with the observations pointing to substantial differences with ISP thymic precursors, in particular with respect to the capacity to develop into DP cells in an FTOC, could raise the alternative possibility that CD4+CD3-CD14- cells represent an extrathymic pathway of T cell development. Studies with nude mice have shown that mature T cells can develop in aged mice in the absence of a thymus (24), indicating that this organ is not required for development of some T cells CD3-Thy-1low cells have been found in the bone marrow of nude mice that have partial DJ rearrangements of the TCR-β locus (25). A transcript containing TCR-β constant region sequences but not variable region sequences was amplified, suggesting that an unarranged TCR-β gene locus is transcriptionally active in this bone marrow population. These characteristics of Thy-1lowCD3- cells in nude bone marrow cells are very similar to those of the CD3-CD4+ PBMC population described here. T cells that develop in nude mice have a different TCR-β repertoire than euthymic controls (26) and some TCR-β can be expressed on extrathymically developed cells that are deleted in euthymic controls (27). The TCR-β repertoire of the CD3+ cells that develop from the CD4+CD3-CD14- has not yet been investigated in detail. However, we can exclude that these circulating PBMC are the precursors of those T cells that express a monomorphic TCR (Vα24, Vβ11) (28, 29), because some of the CD4+CD3+ cells developing from CD4+CD3-CD14- PBMC cells were found to be Vα24+Vβ11+ (data not shown).

We thank H. von Boehmer for advice and support; K. Hafen for expert technical assistance; H.P. Strahlberger for art work; L. Inverardi, A. Kruisbeek, and H.R. Rodewald for critical reading of the manuscript; E. Ten Boeckel for suggestions concerning the PCR, T. Goebel, T. Winkler, G. Wiedle, A. Young, J. Bachl, J. Kirberg, T. Borgreffe, C. Schwarzerler, C. Schaniel, R. Ceredig, M. Kopf, R. M. usman, M. Colonna, and volunteers working at the Basel Institute for Immunology for providing their blood.

Table 1. Phenotypic and Functional Comparison between CD3-CD4+CD8- Immature Single-positive Cells (CD4+CD3-ISP) Present in Thymus and CD4+CD3- Cells Identified in Peripheral Blood

|                       | Thymus CD4+CD3-, ISP | Peripheral blood CD4+CD3+ |
|-----------------------|----------------------|--------------------------|
| pTα                   | +                    | +                        |
| cCD3                  | +                    | +                        |
| cTCR-β                | –                    | –                        |
| CD1a                  | +                    | –                        |
| CD2                   | 100%                 | 15-40%                   |
| CD7                   | 100%                 | 2-5%                     |
| CD5                   | 100%                 | 2-5%                     |
| CD10                  | nt                   | –                        |
| CD45Rα                | ±                    | +                        |
| CD33                  | –                    | ±                        |
| B7-2                  | nt                   | +                        |
| HLA-DR                | +                    | +                        |
| Cell cycle            | +++                  | –                        |
| TCR-β locus           |                      |                          |
| DJβ rearrangement     | +                    | +                        |
| FTOC                  | DP, SP               | SP                       |
| PHA, IL-2, IL-7       | –                    | +                        |

nt, not tested. ++++, a lot.
References

1. Groettrup, M., and H. von Boehmer. 1993. T cell receptor beta chain dimers on immature thymocytes of normal mice. Eur. J. Immunol. 23:1393–1396.

2. Saint-Ruf, C., K. Ungewiss, M. Groettrup, L. Bruno, H.J. Fehling, and H. von Boehmer. 1994. Analysis and expression of a cloned pre-T cell receptor gene. Science (Wash. DC). 266:1208–1212.

3. von Boehmer, H. 1990. Developmental biology of T cells in the T-cell receptor transgenic mice. Annu. Rev. Immunol. 8:531–556.

4. Groettrup, M., A. Baron, G. Griffith, R. Palacios, and H. von Boehmer. 1992. T cell receptor (TCR) beta chain homodimers on the surface of immature but not mature alpha, gamma, delta chain deficient T cell lines. EMBO (Eur. Mol. Biol. Organ.) J. 11:2735–2745.

5. Fehling, H.J., A. Krotkova, C. Saint-Ruf, and H. von Boehmer. 1995. Crucial role of the pre-T cell receptor a gene in development of alpha beta but not gamma T cells. Nature (Lond.). 375:795–798.

6. Porte, P., L. Bruno, M.G. Mattei, H. von Boehmer, and C. Saint Ruf. 1995. Cloning and comparative analysis of the human pre-T-cell receptor alpha chain gene. Proc. Natl. Acad. Sci. USA . 92:12105–12109.

7. Rimjito, A.R., C. Trigueros, C. Marchon, J.L. San Millan, and M.L. Toribio. 1996. Regulation of pre-T cell receptor (pTalpha–TCRbeta) gene expression during human thymic development. J. Exp. Med. 178:501–509.

8. Bruno, L., B. Rocha, A. Rolink, H. von Boehmer, and H.R. Rodewald. 1995. Intracellular and extracellular expression of the pre-T cell receptor alpha gene. Eur. J. Immunol. 25:1877–1882.

9. Lanzavecchia, A., and D. Schleidinger. 1987. The use of hybrid hybridomas to target human cytotoxic T lymphocytes. Eur. J. Immunol. 17:105–111.

10. Ktorza, S., C. Blanc, C. Laurent, S. Sarun, M.P. Verpilleux, P. Debere, and C. Schmitt. 1996. Complete TCR-5 rearrangements and partial (D-J) recombination of the TCR-beta locus in CD34+7a precursors from human cord blood. J. Immunol. 156:4120–4127.

11. Fisher, A.G., L. Larson, L.K. Goff, D.E. Restall, L. Happerfield, and M. Merkenschlager. 1991. Human thymocytes development in organ cultures. Int. Immunol. 3:1–5.

12. Yssel, H., J.E. de Vries, M. Koken, W. van Blitterswijk, and H. Spits. 1984. A serum-free medium for the generation and propagation of functional human cytotoxic and helper T cell clones. J. Immunol. Meth. 72:219–225.

13. Tonegawa, S. 1983. Somatic generation of antibody diversity. Nature (Lond.). 302:575–581.

14. Davis, M.M. 1985. Molecular genetics of the T cell–receptor beta chain. Annu. Rev. Immunol. 3:537–560.

15. Kronenberg, M., G. Suiu, L.E. Hood, and N. Shastri. 1986. The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. Annu. Rev. Immunol. 4:2736–2742.

16. Wilson, A., H. Werner, and R. Macdonald. 1996. Two waves of recombination in expression in developing thymocytes. J. Exp. Med. 179:1355–1360.

17. Galy, A., M. Travis, D. Chen, and B. Chen. 1995. Human T, B, natural killer, and dendritic cells arise from a common bone marrow progenitor cell subset. Immunity. 3:459–473.

18. Rest, P., A.C. Jaleco, E. Noteboom, K. Wijer, and H. Spits. 1996. CD34+CD38dim cells in the human thymus can differentiate into T, natural killer and dendritic cells but are distinct from stem cells. Blood. 87:5196–5206.

19. Galy, A., S. Verma, A. Barcelo, and H. Spits. 1993. Precursors of CD3+CD4+CD8- cells in the human thymus are defined by the expression of CD34: delineation of early events in human thymic development. J. Exp. Med. 178:391–399.

20. Galy, A., S. Webb, D. Chen, L.J. Murray, J. Condino, R.S. Negrin, and B.P. Chen. 1994. Generation of T cells from cytokine-mobilized peripheral blood and adult bone marrow CD34+ cells. Blood. 84:104–111.

21. Galy, A., D. Chen, M. Travis, S. Chen, and B.P. Chen. 1995. Delineation of T-progenitor activity within the CD34+ compartment of adult bone marrow. Blood. 85:2770–2779.

22. Spits, H. 1994. Early stages in human and mouse T-cell development. Curr. Opin. Immunol. 6:212–221.

23. Rodewald, H.R., K. Kretzschmar, S. Takeda, C. Hohl, and M. Desing. 1994. Identification of pro-thymocytes in murine fetal thymus: T lineage commitment can precede thymus colonization. EMBO (Eur. Mol. Biol. Organ.) J. 13:4229–4240.

24. Rocha, B., P. Vassalli, and D. Guy-Grand. 1992. The extrathymic T-cell development pathway. ImmunoL. Today. 13: 449–454.

25. Soloff, R.S., T.G. Wang, L. Lymbarger, D. Dempsey, and R. Chervenak. 1995. Transcription of the TCR-beta locus initiates in adult murine bone marrow. J. Immunol. 154:3888–3901.

26. Rocha, B. 1990. Characterization of V beta–bearing cells in athymic (nu/nu) mice suggests an extrathymic pathway for T cell differentiation. Eur. J. Immunol. 20:919–925.

27. Hodes, R.J., S.O. Sharrow, and A. Solomon. 1989. A failure of T cell receptor V beta negative selection in an athymic environment. Science (Wash. DC). 246:1041–1044.

28. Lanzott, O., and Bendelac A. 1994. An invariant T cell receptor alpha chain is used by a unique subset of major histocompatibility complex class I-specific CD4+ and CD4+8+ T cells in mice and humans. J. Exp. Med. 180:1097–1106.

29. Della Bonafé, P., E. Padovan, G. Casarab, M. Brockhaus, and A. Lanzavecchia. 1994. An invariant Valpha24-Jalpha/Q/Vbeta11 T cell receptor is expressed in all individual by clonally expanded CD4+8+ T cells. J. Exp. Med. 180:1171–1176.