RERF TR 5-90
Research Project 3-87

Technical Report Series

Isolation and Partial Characterization of Peripheral Blood $CD4^+$ T Cell Clones Expressing $\gamma\delta$T Cell Receptors

Seishi Kyoizumi, Ph.D; Mitoshi Akiyama, M.D.; Yuko Hirai, Ph.D.; Yoichiro Kusunoki, Ph.D.

放影研 RERF **Radiation Effects Research Foundation**
A Cooperative Japan–United States Research Organization

RERF Technical Report Series

Technical reports are the basic medium for reporting of original research carried out at the Radiation Effects Research Foundation. Reports in this series receive both internal and external peer review and may serve as the basis for publication in the open scientific literature, in part or in toto. Although they may be quoted and cited, these reports are considered to be internal publications of the Foundation. Copies are available upon request from: Editorial Office, RERF, 5-2 Hijiyama Park, Minami-ku, Hiroshima, 732 Japan.

The Radiation Effects Research Foundation (formerly ABCC) was established in April 1975 as a private nonprofit Japanese Foundation, supported equally by the government of Japan through the Ministry of Health and Welfare, and the Government of the United States through the National Academy of Sciences under contract with the Department of Energy.

財団法人　放射線影響研究所
業　績　報　告　書

RERF TR　5-90
研究課題　3-87

T cell recepter γδ を発現する末梢血 CD4+ T cell clone の分離と部分的解析§

Isolation and Partial Characterization of Peripheral Blood CD4+ T Cell Clones Expressing γδT Cell Receptors

京泉誠之，秋山實利，平井裕子，楠　洋一郎

放射線生物学部

要　約

ヒトのT細胞抗原受容体（TCR）は2種類報告されている．一つはα鎖とβ鎖，他方はγ鎖とδ鎖のポリペプチドからなるヘテロダイマーである．γ鎖とδ鎖で構成されたTCR（TCRγδ）を細胞膜表面に発現しているT細胞は，末梢血T細胞の1％～10％を占めている．そしてTCRγδを発現しているT細胞のほとんどはT細胞の機能的マーカーであるCD4とCD8分子の両方を欠いている．最近になって，TCRγδ型のT細胞のうち非常にわずかの割合（5％以下）で，CD4分子をもつT細胞が末梢血中に存在することが報告された．しかしながら，これらの細胞の性状については何も分かっていない．更に，昨年9月のJ. Exp. Med. に胎児肝（胎児では肝臓は造血臓器である）に，このTCRγδ型のCD4分子を発現するT細胞が，TCRγδ型細胞の20％も存在することが発表されたが，免疫学的な機能については何も報告されていない．我々は原爆被爆者の末梢血リンパ球の亜群の調査の中で，このようなTCRγδ型でCD4分子を発現するという非常にまれな型のT細胞を分離し，クローン化することに成功した．成人末梢血中でもこのようなまれな型のT細胞が存在することを確認できたわけであるが，我々は，更に，これらの幾つかの株を用いて，TCRγδ型CD4+ T細胞の分子生物学的な解析と免疫学的機能について調べるとともに，これらの細胞の分化過程についても考察した．

健康成人数名の末梢血リンパ球から，抗CD4分子抗体と抗TCRδ鎖抗体を用いてTCRγδ鎖陽性CD4分子陽性のリンパ球をフローサイトメトリーで分離した．1）TCRγδ型CD4+ T細胞

§本報告にはこの要約以外に訳文はない．

承認1990年3月5日

印刷1990年6月

を培養しクローン化できたものは，すべて抗 TCR$\gamma\delta$ 鎖抗体と反応した．2）TCR$\gamma\delta$ 鎖ポリペプチドの免疫沈降法による解析では，調べたどのクローンにおいても，S-S結合か非S-S結合のいずれかをもつ分子量37-44 Kd の TCRγ 及びβ鎖が認められた．3）一方 TCRγ とδ鎖遺伝子のサザンブロット法による DNA レベルでの解析から，幾つかのクローンは同じ遺伝子再構成を示していることが分かり，この種の細胞は末梢血中での異形性は少ないと考えられた．4）そして通常の TCR$\alpha\beta$型 CD 4^+ T細胞と異なり，すべてのクローンはレクチン依存性並びに抗 CD 3 分子（すべてのT細胞が発現している）抗体依存性の逆方向細胞障害活性を示した．更に，クローン細胞株の60％はナチュラル細胞傷害活性も示した．

このように健康成人の末梢血でも，従来証明されていなかったユニークなT細胞集団を確認した．更に，これらは末梢血中に大多数を占める TCR$\alpha\beta$型 CD 4^+ 細胞とは機能を全く異にしていることが分かり，今後T細胞の分化過程を研究する過程に貴重な指標となると考えられる．

RERF TR 5-90
Research Project 3-87

Technical Report Series

Isolation and Partial Characterization of Peripheral Blood CD4$^+$ T Cell Clones Expressing $\gamma\delta$T Cell Receptors[§]

Seishi Kyoizumi, Ph.D; Mitoshi Akiyama, M.D.; Yuko Hirai, Ph.D.; Yoichiro Kusunoki, Ph.D.

Department of Radiobiology

Summary

Rare T cell clones bearing both CD4 and T cell receptors (TCRγ and TCRδ) were obtained from human peripheral blood by cell sorting using anti-CD4 and anti-TCRδ1 antibodies. All the clones established were reactive with anti-TCR$\gamma\delta$1 antibody, whereas only about 20% of the clones showed reactivity with anti-δTCS1 antibody. Unlike CD4$^+$ T cells bearing TCR$\alpha\beta$, all the clones tested were lectin-dependent and showed CD3 antibody-redirected cytolytic activity. About 60% exhibited natural killer cell-like activity. Immunoprecipitation analysis of TCR$\gamma\delta$ showed that each clone expressed either a disulfide-linked or nondisulfide-linked heterodimer consisting of 37–44 kilodalton TCRγ and TCRδ chains. Southern blot analyses of TCRγ and TCRδ genes revealed some identical rearrangement patterns, suggesting the limited heterogeneity of CD4$^+$TCR$\gamma\delta^+$ T cells in peripheral blood.

Introduction

The second class of T cell antigen receptors (TCR) in humans is comprised of γ and δ chains, and it is expressed in a small subpopulation (1%–10%) of peripheral blood (PB) T cells.[1–3] The majority of T cells bearing TCR$\gamma\delta$ ($\gamma\delta$T cells) lack expression of both CD4 or CD8 accessory molecules which are involved in the recognition of major histocompatibility complex (MHC) antigens by mature T cells bearing TCR$\alpha\beta$ heterodimer ($\alpha\beta$T cells). Flow cytometric analyses of PB T cells have shown the presence of a minor fraction of $\gamma\delta$T cells (<10%) that express the CD8 molecules,[1–3] and these CD8$^+\gamma\delta$T cells have been isolated from intestinal intraepithelial lymphocytes[4] and PB T cells.[5,6] Furthermore, expression of the CD4 molecules in a small fraction of $\gamma\delta$T cells has been inferred from flow

[§]**The complete text of this report will not be available in Japanese.**

Approved 5 March 1990

Printed June 1990

cytometry studies of PB lymphocytes (PBL), although the frequency of such cells in PBL appears to be less than 5% of total $\gamma\delta$T cells[1–3] and no characterization has been done. Recently, it was reported that 20% of $\gamma\delta$ positive cells in fetal liver express the CD4 molecule, but the immunological function of these cells is unknown.[7] In the present study, we confirmed the presence of PB $CD4^+\gamma\delta$T cells by isolating and cloning them, as well as by characterizing their molecular nature. The possible differentiation pathways of PB $CD4^+\gamma\delta$T cells will also be discussed.

Materials and Methods

Monoclonal antibodies

Anti-TCRδ1,[8] fluorescein-labeled anti-TCRδ1 (FL-anti-TCRδ1), and FL-anti-δTCS1 antibodies[9] were purchased from T Cell Science (Cambridge, Mass). Anti-TCRδ1 antibody reacts with an invariant portion of the TCRδ chain,[8] whereas anti-δTCS1 antibody is believed to recognize the Vδ1 determinants expressed in a subpopulation of $\gamma\delta$T cells.[9,10] Anti-TCR$\gamma\delta$1 antibody[2] reacting with the TCRγ chain or the $\gamma\delta$ complex was kindly provided by Dr. J. Borst, Netherlands Cancer Institute, Amsterdam. FL-anti-Leu2a (CD8), phycoerythrin-labeled anti-Leu3a (CD4) (PE-anti-Leu3a), PE-anti-Leu4 (CD3), PE-anti-Leu11c (CD16), FL-anti-Leu7 (CD57), and PE-anti-Leu19 (CD56) antibodies were obtained from Becton Dickinson (Mountain View, Calif). NU-T3 (CD3) antibody[11] was purchased from Seikagaku Kogyo (Tokyo, Japan).

Cell sorting and culture

Human PB mononuclear cells from a healthy normal donor were isolated using Ficoll/Hypaque density centrifugation and were stained with FL-anti-TCRδ1 and PE-Leu3a antibodies, as described by the supplier. For two-color fluorescence analysis, the lymphocyte fraction was gated by forward and right-angle light scatter. Approximately 200 cells in the window for the $CD4^+TCR\delta1^+$ fraction were sorted (see Figure 1A). Immediately after sorting, cells were distributed into 96-well plates at a frequency of about one cell per well, and they were cultured with GIT medium (Wako Pure Chemical Industry, Osaka, Japan) containing fetal calf serum (FCS), phytohemagglutinin (PHA), recombinant IL-2, and feeder cells for 3–4 weeks.[12] Clones derived from these cultures were expanded with feed for further analysis.

Immunoprecipitation

T cell clones were surface-labeled with ^{125}I using a standard lactoperoxidase method.[13] After radioiodination, cells were lysed in extraction buffer (10 mM triethanolamine, 0.15 M NaCl, pH 7.8, with the protease inhibitors) containing 1% digitonin or 0.5% NP-40.[14] Immunoprecipitations were performed by incubating the detergent lysates with 2 μg of NU-T3 (anti-CD3, IgG_{2a}), anti-TCRδ1 antibody (IgG_1), control mouse myeloma UPC-10 (IgG_{2a}), or MOPC21 (IgG_1) for three hours on ice, followed by the addition of protein A-Sepharose beads and further incubation of the mixture for one hour. For anti-TCRδ1 and

MOPC21 antibodies, protein A-Sepharose was precoated with rabbit-antimouse immunoglobulins. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 10%–20% linear gradient gels either under nonreduced or reduced conditions.

Chromium-51 release assays

Cytotoxicity assays were carried out for four hours using the ^{51}Cr release method as previously described.[12] For the assay of a redirected lysis and lectin-mediated lysis, anti-CD3 antibody (0.5 μg/ml) or PHA (1:1600) was added at the beginning and was present throughout the assay.

Southern blotting

High-molecular-weight DNA samples extracted from CD4$^+\gamma\delta$T cell clones were cleaved with *Bam*HI restriction enzyme, electrophoresed in 0.7% agarose gel, transferred to nitrocellulose filters[12] and probed with ^{32}P-labeled Jγ genomic[15] and Jδ cDNA[16] probes.

Results

To detect and isolate CD4$^+\gamma\delta$T cells, two-color flow cytometry of normal PBL stained with FL-anti-TCRδ1 and PE-anti-CD4 (Leu3a) antibodies was performed using a cell sorter. When a sorting window was set for the cell fraction expressing both anti-CD4 and anti-TCRδ1 reactive antigens (Figure 1A), CD4$^+$TCRδ1$^+$ cells were observed at a frequency of 0.05%–1% of TCRδ1$^+$ cells in five normal donors. This finding was in accord with previous reports.[1–3] Cells in the window were sorted from PBL of a normal donor and cloned in 96-well plates by limiting dilution. A total of 17 clones were found to be CD4$^+$TCRδ1$^+$ (Figure 1B), and they were expanded for further analyses. Although these data are not shown, expression of both TCRγ and CD4 mRNA was detected by Northern blot analyses for all the CD4$^+$TCRδ1$^+$ clones examined.

These CD4$^+$TCRδ1$^+$ cell clones were analyzed by cell surface immunofluorescence and cell-mediated cytolytic assay. All of these clones showed positive staining with anti-TCR$\gamma\delta$1 antibody, therefore they expressed both TCRγ and TCRδ chains (Figure 1C, Table 1). Among these, only four clones (about 20%) were positively stained with anti-δTCS1 antibody, supposedly specific to Vδ1 determinant (Figure 1D, Table 1). Levels of surface CD3 expression were found to be 1.5- to 4-fold higher in CD4$^+\gamma\delta$T cell clones than in ordinary CD4$^+\alpha\beta$T cell clones (data not shown). In addition, most of these clones weakly expressed CD56, but not CD16, CD57 (Table 1), or CD8. These clones exhibited variable levels of non-MHC-restricted cytolytic activity; 11 clones lysed the K562 tumor cells without externally added ligands and all the clones tested effectively lysed U937 cells coated with PHA or anti-CD3 (Leu4) antibody (Table 1).

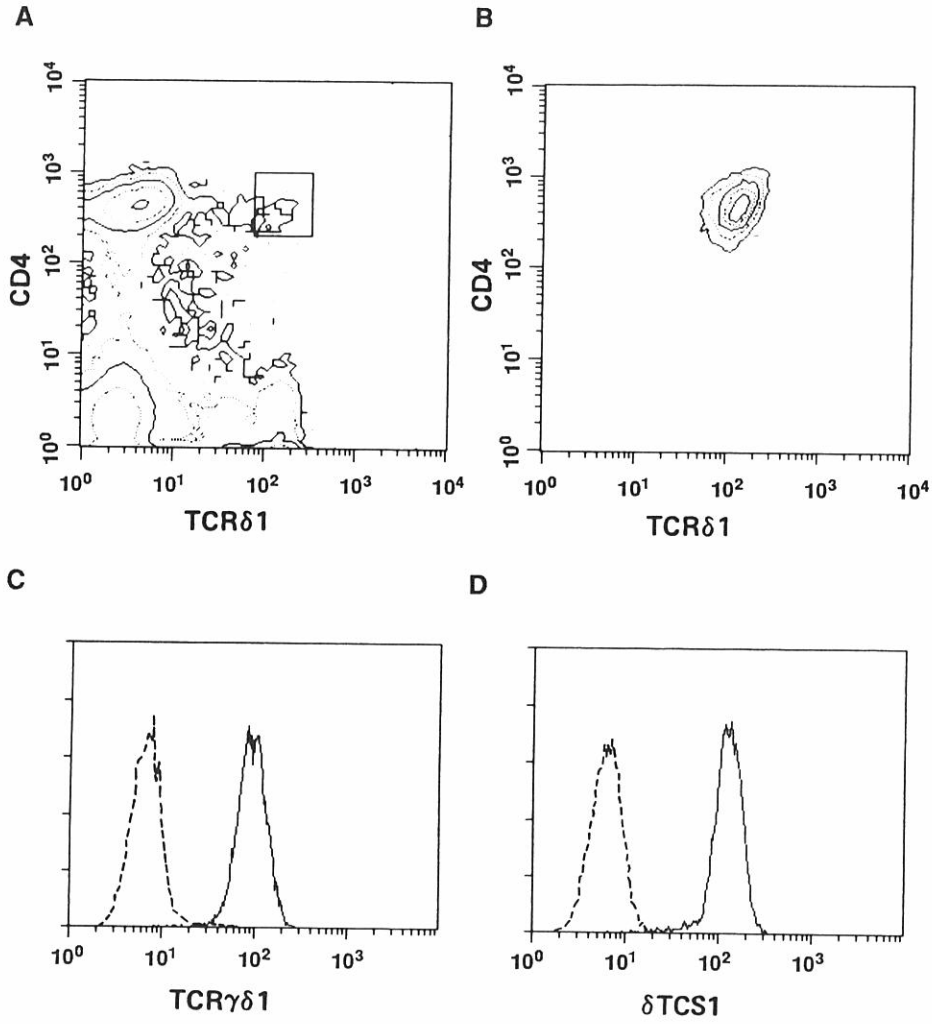

Figure 1. Flow cytometric analyses of TCR$\gamma\delta$ expression in peripheral blood (PB) lymphocytes and an isolated $\gamma\delta$T cell clone.
(A) Two-color distribution of 5 x 10^5 PB lymphocytes stained with FL-anti-TCRδ1 and PE-anti-CD4 (Leu3a) antibodies. Contours differ by a factor of 10 in events per channel with the lowest contour representing one event per channel. A sorting window for CD4$^+$TCRδ1$^+$ cells was set in the region shown in this figure.
(B) Expression of CD4 antigen and TCRδ in a T cell clone, T4D-51, stained with the same antibodies as shown in (A).
(C, D) Reactivity of anti-TCR$\gamma\delta$1 (C) and anti-δTCS1(D) antibodies with the T4D-51 clone. Dotted lines represent control staining with myeloma IgG_1 (MOPC21) antibody.

Table 1. Surface phenotypes and cytolytic activity of CD4$^+$$\gamma\delta$T cell clones

T cell clones	Surface phenotype					Cytolytic activity[a] (% ^{51}Cr release)		
								U937[b]
	TCR$\gamma\delta$1	δTCS1	CD16	CD56	CD57	Targets	K562	PHA	Anti-CD3
T4D-2	+	–	–	+	–		ND[c]	ND	ND
T4D-7	+	–	–	+	–		2	33	40
T4D-12	+	–	–	+	–		9	25	37
T4D-18	+	–	–	+	–		5	5	35
T4D-19	+	+	–	+	–		54	45	57
T4D-20	+	–	–	+	–		1	22	33
T4D-24	+	–	–	+	–		2	16	24
T4D-26	+	–	–	+	–		77	ND	ND
T4D-28	+	–	–	+	–		40	31	38
T4D-39	+	+	–	–	–		1	7	13
T4D-43	+	–	–	+	–		17	38	49
T4D-45	+	–	–	+	–		13	38	ND
T4D-46	+	+	–	–	–		ND	ND	ND
T4D-48	+	–	–	+	–		3	27	25
T4D-51	+	+	–	–	–		1	13	ND
T4D-55	+	–	–	+	–		69	63	70
T4D-60	+	–	–	+	–		18	53	65

[a] Effector cell-to-target cell ratio was 20:1.

[b] Cytolytic activity against ^{51}Cr-labeled U937 target cells was assayed in the presence of phytohemagglutinin (PHA) or anti-CD3 antibody (Leu4).

[c] Not done

The nature of the TCR$\gamma\delta$/CD3 complex was analyzed by immunoprecipitation of ^{125}I-labeled extracts with anti-CD3 and TCRδ1 antibodies (Figure 2). SDS-PAGE of immunoprecipitates obtained from three δTCS1$^-$ clones (Figure 2, lanes 1–8) revealed TCR proteins of 37–43 kilodalton (kDa) under reduced conditions, and 82–84 kDa under nonreduced conditions, indicating disulfide-linked TCR$\gamma\delta$ dimers. In one clone, T4D-12, three discrete TCR bands were observed (lane 7). This is most probably due to differential glycosylation resulting in a doublet of TCRγ protein in SDS-PAGE, as reported by Hochstenbach et al.[17] Among the two δTCS1$^+$ clones tested, one clone exhibited disulfide-linked TCR$\gamma\delta$ (37–42 kDa) (lanes 9 and 10), whereas the other expressed nondisulfide-linked γ and δ chains (40–44 kDa) (lanes 11 and 12).

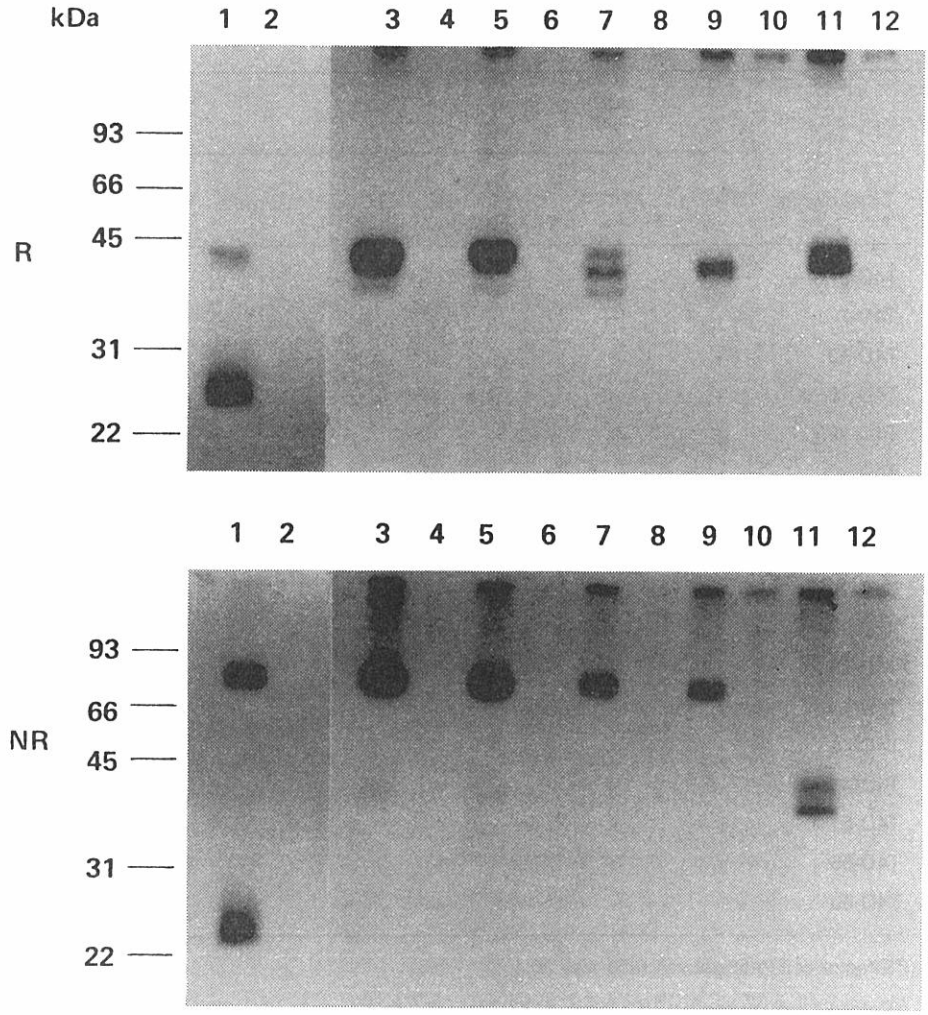

Figure 2. SDS-PAGE analyses of TCR$\gamma\delta$ molecules immunoprecipitated from CD4$^+\gamma\delta$T cell clones, T4D-12 (lanes 1–4), T4D-18 (lanes 5 and 6), T4D-7 (lanes 7 and 8), T4D-19 (lanes 9 and 10), and T4D-39 (lanes 11 and 12) under reduced (R) or nonreduced (NR) conditions. For T4D-12, 18 and 7 cells were δTCS1$^-$, and for T4D-19, 39 cells were δTCS1$^+$ (see Table 1). A total of 5 to 10 x 10^6 cells were labeled with ^{125}I and lysed in digitonin (lanes 1 and 2) or NP-40 (lanes 3–12) lysing buffer, and the lysates were immunoprecipitated with NU-T3 (CD3) (lane 1), UPC10 (lane 2), anti-TCRδ1 (lanes 3, 5, 7, 9, and 11) and MOPC21 (lanes 4, 6, 8, 10, and 12) antibodies.

The DNA configuration at the TCRγ and TCRδ genes was analyzed for 13 CD4$^+\gamma\delta$T cell clones by Southern blotting using Jγ genomic DNA and Jδ cDNA as probes, respectively. Analysis using the *Bam*HI (Figure 3) and other restriction enzymes (*Eco*RI and *Hind*III) revealed that all of the CD4$^+\gamma\delta$T cell clones examined had rearrangements of both TCRγ and δ genes. Furthermore, three clones, T4D-39, 46, and 51, exhibited the same rearrangement patterns of both TCRγ and δ genes (Figure 3, lanes 4, 6, and 8) and two other clones, T4D-26 and 60 (Figure 3, lanes 3 and 9). Although these data are not shown, these

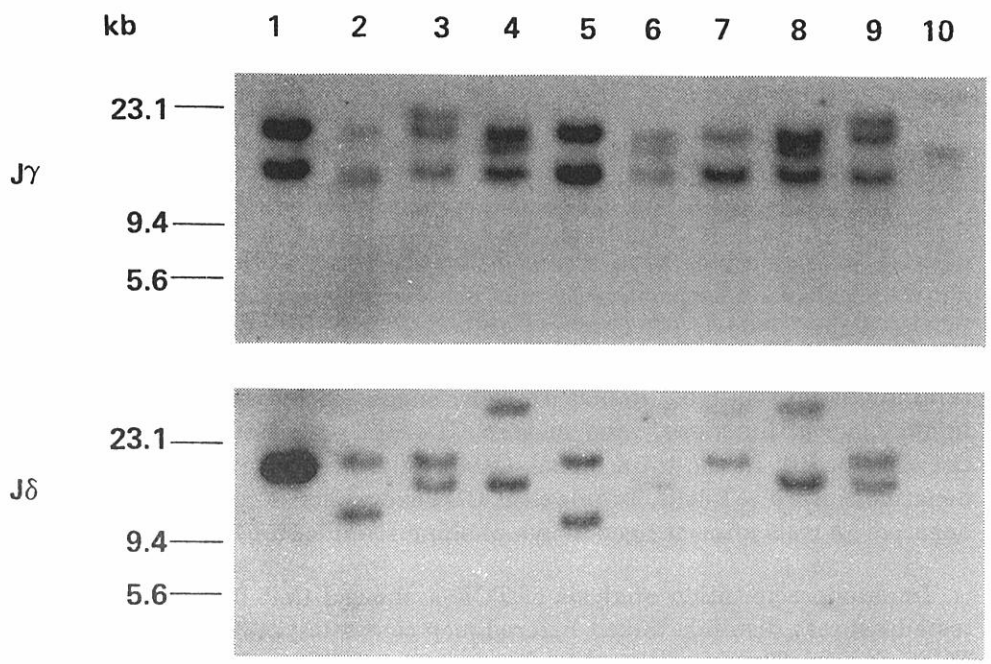

Figure 3. Southern blot analysis of TCRγ (upper) and TCRδ (lower) genes using Jγ and Jδ probes, respectively. *Bam*HI-digested DNA was prepared from T4D-24 (lane 2), T4D-26 (lane 3), T4D-39 (lane 4), T4D-43 (lane 5), T4D-46 (lane 6), T4D-48 (lane 7), T4D-51 (lane 8), T4D-60 (lane 9), and from a CD4$^+\alpha\beta$ T cell clone established from the same donor (lane 10). The germline configurations (15 kb and 12.5 kb for γ; 14 kb for δ) are shown in lane 1 (B cell line, Daudi). Both TCRδ alleles were deleted in the CD4$^+\alpha\beta$T cell clone (lane 10).

clones also had the same rearrangement patterns of TCRβ, γ, and δ genes in *Eco*RI and *Hind*III digestion. Since cell cloning had been performed immediately after sorting without preculture, these observations suggested in vivo clonality for each set of the CD4$^+\gamma\delta$T cell clones.

Discussion

In the present study, we isolated CD4$^+\gamma\delta$T cell clones from human PB, but both the immunological functions and the differentiation pathway of this T cell subset are not well understood. Similar to double negative $\gamma\delta$T cells, almost all CD4$^+\gamma\delta$T cell clones exhibit cytolytic activity. Although it is not clear whether such cytotoxicity is a result of either the in vitro culture condition or is a reflection of the in vivo activity of these cells, the functional properties of CD4$^+\gamma\delta$T cells are distinctively different from CD4$^+\alpha\beta$T cells that exhibit mostly helper activity but rarely cytolytic activity.[18] Moreover, PB CD4$^+\gamma\delta$T cell clones displayed another feature of fetal liver CD4$^+\gamma\delta$T cells which cannot lyse targets in lectin-mediated cytolytic assays.[7] It is not known whether such a functional difference between PB and fetal liver CD4$^+\gamma\delta$T cells is due to a differential T cell subset or a differential maturation stage.

There are three possible schemes for the differentiation pathway of PB $CD4^+\gamma\delta T$ cells. Described by Strominger,[19] one is analogous to the generally accepted model for that of $CD4^+$ or $CD8^+$ $\alpha\beta T$ cells; the majority of the $CD4^-8^-$ thymic cells expressing surface $TCR\gamma\delta$ exit the thymus and presumably appear in the periphery. A residual minor population of double-negative $\gamma\delta$ cells could express both CD4 and CD8 molecules, and these double-positive cells might be intermediate stages to peripheral single positive $\gamma\delta T$ cells. Although these double positive $\gamma\delta$ thymocytes have not been identified as yet, these hypothetical cells may be a substrate for positive thymic selection, as are $TCR\alpha\beta$-expressing cells.[19] In the second model, PB $CD4^+\gamma\delta T$ cells are differentiated directly from fetal liver $CD4^+\gamma\delta$ cells not via the thymus-dependent pathway. Fetal liver $CD4^+\gamma\delta$ cells might be relatively immature cells, suggested by their lack of detectable immunological functions,[7] and mature $CD4^+\gamma\delta T$ cells having cytolytic activity circulate in PB. In the third model, $CD4^+\gamma\delta T$ cells are derived from PB double-negative mature $\gamma\delta T$ cells. In this case, CD4 expression may be induced in double-negative $\gamma\delta$ cells after antigen or lymphokine stimulation.

Immunoprecipitation analysis of $TCR\gamma\delta$ showed that four out of five clones tested express disulfide-linked heterodimer consisting of 37–44 kDa $TCR\gamma$ and $TCR\delta$ chains. This suggests that the majority of $CD4^+\gamma\delta T$ cells in PB use $C\gamma1$ gene segments[17] for their $TCR\gamma$ chain expression. It is also noteworthy that the $\delta TCS1^+$ clones bear either a disulfide-linked or nondisulfide-linked receptor, a finding consistent with an observation by Seki et al,[20] but not with that reported by Bottino et al,[21] which described that $\delta TCS1^+$ clones express only nondisulfide-linked receptors.

The frequency of $CD4^+\gamma\delta T$ cells measured by flow cytometry appeared to be approximately 10^{-3} to 10^{-4} of total PB T cells. Among such rare T cells, in vivo clonality was suggested by Southern blot analyses of the rearrangement patterns of $TCR\gamma$ and δ genes for randomly selected clones from one normal donor. It is possible that $CD4^+\gamma\delta T$ cells in PB had multiplied in vivo probably as a result of clonal expansion by antigen-induced proliferation. Because of the limited number of clones analyzed, the total number of independent clones in PB $CD4^+\gamma\delta T$ cells cannot be evaluated at the present time. However, the present finding suggests that the heterogeneity of the $CD4^+\gamma\delta T$ cell population is not very great. It remains to be determined whether such a rare T cell subset with limited heterogeneity will respond to particular antigens and exhibit specific functions. As a first step toward answering this question, we hope to examine whether specific $V\gamma$ or $V\delta$ genes are preferentially used for these clones and whether CD4 molecules expressed on this rare T cell subset are involved in MHC recognition.

Acknowledgments

We thank M. Itoh and K. Tanabe for assistance with FACS analysis, K. Takahashi and K. Koyama for help with DNA analysis, Y. Watanabe for cell culture, M. Takagi for manuscript preparations, and N. Nakamura for helpful advice.

References

1. Jitsukawa S, Faure F, Lipinski M, Triebel F, Hercend T: A novel subset of human lymphocytes with a T cell receptor-γ complex. J Exp Med 166:1192–7, 1987

2. Borst J, Van Dongen JJM, Bolhuis RLH, Peters PJ, Hafler DA, De Vries E, Van de Griend RJ: Distinct molecular forms of human T cell receptor γ/δ detected on viable T cell by a monoclonal antibody. J Exp Med 167:1625–44, 1988

3. Groh V, Porcelli S, Fabbi M, Lanier LL, Picker LJ, Anderson T, Warnke RA, Bhan AK, Strominger JL, Brenner MB: Human lymphocytes bearing T cell receptor γ/δ are phenotypically diverse and evenly distributed throughout the lymphoid system. J Exp Med 169:1277–94, 1989

4. Goodman T, Lefrancois L: Expression of the γ-δ T cell receptor on intestinal $CD8^+$ intraepithelial lymphocytes. Nature 333:855–8, 1988

5. Moretta A, Bottino C, Ciccone E, Tambussi G, Mingari MC, Ferrini S, Casorati G, Varese P, Viale O, Migone N, Moretta L: Human peripheral blood lymphocytes bearing T cell receptor γ/δ. Expression of CD8 differentiation antigen correlates with the expression of the 55-kD, Cγ2-encoded γ chain. J Exp Med 168:2349–54, 1988

6. Kozbor D, Trinchieri G, Monos DS, Isobe M, Russo G, Haney JA, Zmijewski C, Croce CM: Human TCR-γ^+/δ^+, $CD8^+$ T lymphocytes recognize tetanus toxoid in an MHC-restricted fashion. J Exp Med 169:1847–51, 1989

7. Aparicio P, Alonso JM, Toribio ML, Marcos MAR, Pezzi L, Martinez-A C: Isolation and characterization of (γ, δ)$CD4^+$T cell clones derived from human fetal liver cells. J Exp Med 170:1009–14, 1989

8. Band H, Hochstenbach F, McLean J, Hata S, Krangel MS, Brenner MB: Immunochemical proof that a novel rearranging gene encodes the T cell receptor δ subunit. Science 238:682–4, 1987

9. Wu YJ, Tian WT, Snider RM, Rittershaus C, Rogers P, LaManna L, Ip Sh: Signal transduction of γ/δ T cell antigen receptor with a novel mitogenic anti-δ antibody. J Immunol 141:1476–9, 1988

10. Faure F, Jitsukawa S, Triebel F, Hercend T: Characterization of human peripheral lymphocytes expressing the CD3-γ/δ complex with anti-receptor monoclonal antibodies. J Immunol 141:3357–60, 1988

11. Knapp W (Ed): Abstracts from 4th International Conference on Human Leukocyte Differentiation Antigens. Tissue Antigens 33:62, 1989

12. Hakoda M, Hirai Y, Shimba H, Kusunoki Y, Kyoizumi S, Kodama Y, Akiyama M: Cloning of phenotypically different human lymphocytes originating from a single stem cell. J Exp Med 169:1265–76, 1989 (RERF TR 11-88)

13. Jones PP: Analysis of radiolabeled lymphocyte proteins by one- and two-dimensional polyacrylamide gel electrophoresis. In *Selected Methods in Cellular Immunology.* Ed by B.B. Mishell, S.M. Shiigi. San Francisco, Freeman and Company, 1980. pp 398–407

14. Oettgen HC, Pettey CL, Maloy WL, Terhorst C: A T3-like protein complex associated with the antigen receptor on murine T cells. Nature 320:272–5, 1986

15. Lefranc M-P, Rabbitts TH: Two tandemly organized human genes encoding the T-cell γ constant region sequences show multiple rearrangement in different T-cell type. Nature 316:464–6, 1985

16. Tkachuk DC, Griesser H, Takihara Y, Champagne E, Minden M, Feller AC, Lennert K, Mak TW: Rearrangement of T-cell δ locus in lymphoproliferative disorders. Blood 72:353–7, 1988

17. Hochstenbach F, Parker C, McLean J, Gieselmann V, Band H, Bank I, Chess L, Spits H, Strominger JL, Seidman JG, Brenner MB: Characterization of a third form of the human T cell receptor γ/δ. J Exp Med 168:761–76, 1988

18. Moingeon P, Jistukawa S, Faure F, Troalen F, Triebel F, Graziani M, Forestier F, Bellet D, Bohuon C, Hercend T: A γ-chain complex forms a functional receptor on cloned human lymphocytes with natural killer-like activity. Nature 325:723–6, 1987

19. Strominger JL: Developmental biology of T cell receptors. Science 244:943–50, 1989

20. Seki H, Nanno M, Chen PF, Itoh K, Ioannides C, Good RA, Platsoucas CD: Molecular heterogeneity of $\gamma\delta$ T-cell antigen receptors expressed by $CD4^{-}8^{-}$T cell clones from normal donors: Both disulfide- and non-disulfide-linked receptors are $\delta TCS1^{+}$. Proc Natl Acad Sci USA 86:2326–30, 1989

21. Bottino C, Tambussi G, Ferrini S, Ciccone E, Varese P, Mingari MC, Moretta L, Moretta A: Two subsets of human T lymphocytes expressing γ/δ antigen receptor are identifiable by monoclonal antibodies directed to two distinct molecular forms of the receptor. J Exp Med 168:491–505, 1988