Human $\gamma\delta$ T-Cell Receptor-Positive Cell-Mediated Inhibition of Erythropoiesis In Vitro in a Patient With Type I Autoimmune Polyglandular Syndrome and Pure Red Blood Cell Aplasia

By Toshiro Hara, Yumi Mizuno, Mari Nagata, Yasufumi Okabe, Shuichi Taniguchi, Mine Harada, Yoshiyuki Niho, Kazuo Oshimi, Shoichi Ohga, Yasunobu Yoshikai, Kiku Nomoto, Keiko Yumura, Keisei Kawa-Ha, and Kohji Ueda

The $\gamma\delta$ T-cell receptor-positive ($\gamma\delta$TCR+) lymphocytes were markedly expanded up to 68% of peripheral blood lymphocytes in a case with type I autoimmune polyglanular syndrome and pure red blood cell aplasia (PRCA). The $\gamma\delta$TCR+ cells showed CD4 negative, 16% dim-CD8 positive and 10% to 46% human leukocyte antigen-D-related (HLA-DR) positive, and exhibited no monoclonality as assessed by the patterns of TCR gene rearrangements. Functional studies revealed that the proliferative responses of the patient's peripheral blood mononuclear cells (PBMC) were severely depressed to candida antigen, alloantigens, and autoantigens (non-T cells). The $\gamma\delta$TCR+ cells had no suppressive effect on the proliferative response of the $\alpha\beta$TCR+ cells to candida. The patient's PBMC, isolated $\gamma\delta$TCR+ cells but not $\alpha\beta$TCR+ cells, exhibited non-major histocompatibility complex (MHC)-restricted cytotoxicity. Furthermore, the patient's PBMC and isolated $\gamma\delta$TCR+ cells inhibited burst-forming units-erythroid (BFU-E), but not colony-forming units/granulocyte-macrophage (CFU-GM). Supernatants derived from the patient's T cells similarly inhibited BFU-E but not CFU-GM. The clinical course of the patient also showed a close correlation between the decreased number of total lymphocyte counts, especially HLA-DR + $\gamma\delta$TCR+ cell counts, and recovery from PRCA. These observations suggest that the $\gamma\delta$TCR+ cells might be functional in vivo and involved in the pathogenesis of PRCA in this patient.

© 1990 by The American Society of Hematology.

MATERIALS AND METHODS

Case report. The patient was admitted to the hospital because of generalized seizures at the age of 12 years. Since early infancy, the patient had been suffering from mucocutaneous candidiasis. A diagnosis of idiopathic hypoparathyroidism was made by the findings of serum calcium (7.2 mg/dL) and phosphorus (10.6 mg/dL) levels, an undetectable level of serum parathormone (PTH), and a decrease of the tubular reabsorption of phosphorus after PTH injection. At age 14, he developed weakness, fatigue, nausea, vomiting, and increased skin pigmentation. A diagnosis of idiopathic adrenal insufficiency was made by serum sodium (127 mEq/L) and potassium (5.2 mEq/L) concentrations and no cortisol response in rapid corticotropin test.

From the Department of Pediatrics, and the First Department of Internal Medicine, the Faculty of Medicine, and the Department of Immunology, Medical Institute of Bioregulation, Kyushu University, Fukuoka; the Department of Internal Medicine, Tokyo Women's Medical College, Tokyo; and the Department of Pediatrics, Osaka University Hospital, Osaka, Japan.

Submitted July 17, 1989; accepted October 12, 1989.


Address reprint requests to Toshiro Hara, MD, Department of Pediatrics, Faculty of Medicine, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812, Japan.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1990 by The American Society of Hematology.

Blood, Vol 75, No 4 (February 15), 1990: pp 941-950
At age 27, he was admitted to the hospital because of general fatigue. There was no endocrinologic, immunologic, or hematologic disease in his family except for his sister, who developed chronic hepatitis at age 17 and insulin-dependent diabetes mellitus at age 33. Physical examination revealed marked pallor, alopecia, and oral candidiasis. There was no lymphadenopathy or hepatosplenomegaly. No thymoma was detected by chest X-rays and computed tomography scanning. Laboratory findings revealed red blood cells (RBCs) $1,420 \times 10^9/L$, hemoglobin (Hb) 4.8 g/dL, hematocrit 13.7%, reticulocyte $0.0\%$, platelets $463 \times 10^9/L$, white blood cells (WBCs) $10.4 \times 10^9/L$ with 1% band forms, 40% segmented neutrophils, 11% monocytes, and 48% lymphocytes. Seventy-three percent of lymphocytes in the peripheral blood were small to medium-sized granular lymphocytes. The surface marker analysis of the peripheral lymphocytes showed 94% CD3+, 6% CD4+ and 43% CD8+. The bone marrow aspirate showed normocellularity, a lack of erythroid precursors (0.5% of bone marrow nucleated cells), normal granulocyte precursors, and megakaryocytes with lymphocytosis (31%). The lymphocytes in the bone marrow were morphologically and phenotypically similar to those in the peripheral blood. Marrow cytogenetics were normal. Serum erythropoietin level was elevated to 685 mU/mL. The direct and indirect Coombs tests were negative. Antinuclear antibody, rheumatoid factor, lupus erythematosus (LE) test, anti-DNA antibody, thyroid test, microsome test, and antinuclear antibody were not detectable. The determination of serum immunoglobulin (Ig) levels showed IgG 830 mg/dL, IgA 105 mg/dL, and IgM 303 mg/dL. Antibody titers to human immunodeficiency virus (HIV) and human T-lymphotrophic virus type I (HTLV-1) were consistently negative. No serological evidence was found for recent or persistent infection with parvovirus, cytomegalovirus, Epstein-Barr virus, or hepatitis B virus. The patient was given several drugs, such as prednisolone, high-dose intravenous gammaglobulin, and anabolic steroids, sequentially without improvement and required frequent blood transfusions. Subsequently, he was treated orally with cyclophosphamide (200 mg/d) and prednisolone (60 mg/d), which resulted in a marked leukopenia ($1.5 \times 10^9/L$) with lymphopenia ($0.15 \times 10^9/L$), followed by a reticulocytosis (10.0%) and a rise in the Hb level to 11.7 g/dL. A repeated bone marrow aspirate showed reappearance of erythroid precursors (34.5% of nucleated cells) and a decrease of lymphocytes (4%).

Cyclophosphamide was discontinued in 1 month, and the Hb level was maintained over 10 g/dL with prednisolone alone. When the patient was placed on a daily dose of 15 mg of prednisolone 6 months later (July, 1988), he had the first relapse (Hb, 7.7 g/dL) and was successfully treated with prednisolone (60 mg/d) and cyclophosphamide (100 mg/d, 4 weeks). The dose of prednisolone was subsequently tapered. He again developed anemia (Hb, 7.2 g/dL) 6 months after the first relapse (January, 1989), despite a continuous dosage of 15 mg of prednisolone. He refused to receive cyclophosphamide because he had developed severe hemorrhagic cystitis last time.

Abbreviations: CD, cluster of differentiation; LFA, leukocyte function-associated antigen; NK, natural killer; TCS, T Cell Science; OP, Ortho Pharmaceutical Co; BD, Beckton-Dickinson; CI, Coulter Immunology.

*Proposed gene assignments of the epitopes. 15

Fig 1. Demonstration of $\gamma\delta$TCR+ cells in the patient's PBL. Two-color flow cytometric analysis showed 7% CD3- $\alpha\beta$TCR (WT31) - cells and 2% CD3- $\gamma\delta$TCR (WTCS1) - cells in control PBL (C) and 68% CD3- $\alpha\beta$TCR (WT31) - cells and 67% CD3- $\gamma\delta$TCR (WTCS1) - cells in the patient's PBL (P).
and preferred lymphopheresis (15 times, twice a week) with a daily regimen of 60 mg of prednisolone. Lymphopheresis resulted in persistent lymphopenia (<1.0 x 10^9/L), followed by a reticulocytosis (3.7%) and an improvement of anemia (March, 1989). After cessation of lymphopheresis, the patient was given cyclosporin A (4 mg/kg) and a reducing dose of prednisolone, which further raised the Hb level to 12.2 g/dL (May, 1989). Each time anemia improved, the dose of 25-hydroxyvitamin D3 was reduced to lower the elevated levels of serum calcium. Informed consent to the present study was obtained.

### Antibodies
Specificities and sources of the monoclonal antibodies (MoAbs) used are shown in Table 1. Fluorescein isothiocyanate (FITC)-conjugated goat F(ab')2 anti-mouse IgG was purchased from Tago, Inc, Burlingame, CA. For two-color analysis, FITC and phycoerythrin (PE)-conjugated MoAb were used.

### Cell preparation
Purified αβ TCR (WT31)-CD3+ and γδ TCR (δTC51)-CD3+ subsets were obtained by solid-phase immunoadsorption (panning). Peripheral blood mononuclear cells (PBMC), isolated by density gradient centrifugation, were depleted of monocytes by plastic adherence. Monocyte-depleted PBMC were over 95% CD3+ in this patient. The T cells were cultured with saturating concentrations of WT31 or δTC51 MoAb, washed twice, and then added to petri dishes previously coated with affinity purified goat F(ab')2 anti-mouse IgG according to the method described by Young and Lehner. After incubation of MoAb-coated T cells on the petri dishes for 1 hour at room temperature, the nonadherent cells were gently washed with RPMI 1640. In some experiments, nonadherent cells were repanned to obtain over 95% pure cell population.

### Surface marker analysis
Single-color or two-color flow cytometric analysis of patient's PBL was done using an Ortho Cytoron (Ortho Diagnostic Systems, KK, Tokyo, Japan) as described previously. Background fluorescence was assessed by staining with control irrelevant isotype-identical MoAb.

### Proliferation assays
PBMC or separated T cells were cultured at 10^6 cells/mL in RPMI 1640 medium supplemented with 10% FCS, 1 mmol/L glutamine in the presence or absence of 10 ng/mL (final concentration) of phytohemagglutinin-P (PHA-P; Difco Laboratories, Inc, Detroit, MI), 5 μg/mL of concanavalin A (ConA; Pharmacia Fine Chemicals, Piscataway, NJ), 5 μg/mL of purified protein derivative (PPD; Japan BCG Laboratory, Tokyo, Japan), 1 μg/mL of candida (Torii Co, Ltd, Tokyo, Japan), 1 U/mL of recombinant human interleukin-2 (rIL-2; Takeda Chemical Industries, Ltd, Osaka, Japan), 10 ng/mL of 12-0-tetradecanoyl phorbol-13-acetate (TPA; Sigma Chemical Co, St Louis, MO); irradiated autologous non-T cells or allogeneic cells (5 x 10^6 cells/mL). Incorporated radioactivity was determined as described.

### Cytotoxicity assays
A 5-hour ^51Cr release test was used for the assay of non-MHC-restricted cytotoxicity. PBMC or isolated T cells were incubated in triplicate with 8 x 10^6 of ^51Cr-labeled target cells. After 5 hours' incubation, the supernatant fluid was harvested, and

<table>
<thead>
<tr>
<th>Table 2. Surface Marker Analysis of Patient's PBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
</tr>
<tr>
<td>WBC (μL)</td>
</tr>
<tr>
<td>Lymphocytes (μL)</td>
</tr>
<tr>
<td>CD2+</td>
</tr>
<tr>
<td>CD3+</td>
</tr>
<tr>
<td>aβTCR(WT31)+CD3+</td>
</tr>
<tr>
<td>γδTCR subset (δTC51)+</td>
</tr>
<tr>
<td>γδTCR subset (ββ3)+</td>
</tr>
<tr>
<td>pan γδTCR(ζ/δ)+</td>
</tr>
<tr>
<td>HLA-DR+CD3+</td>
</tr>
<tr>
<td>HLA-DR+γδTCR(δTC51)+</td>
</tr>
<tr>
<td>HLA-DR+αβTCR(WT31)+</td>
</tr>
<tr>
<td>CD4+CD8+</td>
</tr>
<tr>
<td>CD4+CD8—</td>
</tr>
<tr>
<td>IL2R+CD3+</td>
</tr>
</tbody>
</table>

Abbreviation: NT, not tested.
*Values represent percentage of total number of lymphocytes.
the radioactivity was determined with a gamma well counter. The specific $^{51}$Cr release was calculated as described.19 The results of cytolysis were expressed as the percent specific $^{51}$Cr release ± SD. The SD of the percent specific $^{51}$Cr release was less than 5% in most of the experiments.

**IL-2 and interferon-γ assays.** IL-2 was quantitatively determined with enzyme immunoassay using two kinds of anti-IL-2 antibodies.19 One unit of rIL-2 corresponds to 340 units of human IL-2 (Jurkat), the Biological Response Modifiers Program reference reagent.20 Interferon-γ (IFN-γ) was measured with Sucrose Interferon-γ immunoradiometric assay kit, Celltech Diagnostics, Ltd., Berkshire, UK.

**Hematopoietic progenitor cell assay.** Clonal assays for burst-forming units-erythroid (BFU-E), colony-forming units-erythroid (CFU-E), and colony-forming units-granulocyte-macrophage (CFU-GM) were performed as described.21 Bone marrow mononuclear cells (BMMCs), obtained by Ficoll-Hypaque density-gradient centrifugation, were washed three times in alpha minimum essential medium (α-MEM; Gibco, Grand Island, NY) and suspended in Iscove's modified Dulbecco medium (IMDM; Gibco) supplemented with 10% fetal calf serum (FCS). BMMC at a final concentration of 2.0 x 10^7 cells/ml were plated in a 35 mm plastic Petri dish (Lux 5221; Miles Laboratories, Inc, Naperville, IL) in 1 ml of IMDM or α-MEM containing 0.88% methicellulose, 30% FCS, 5 x 10^{-3} mol/L 2-mercaptoethanol (2-ME; Sigma Chemical Co, St Louis, MO) and 500 mU recombinant erythropoietin (rEpo; SNB 5001, Snow Brand Milk Products Co Ltd, Tokyo, Japan). Dishes were incubated at 37°C in a humidified 5% CO₂ atmosphere. BFU-E colonies were scored on day 7 and BFU-E and CFU-GM colonies, on day 14. For BFU-E assay, 1 U/ml of rEpo was used. To test the effects of the patient's PBMC or γδTCR+ cells on colony formation, various numbers of the patient's cells were co-cultured with normal BMMC for 18 hours in the absence or presence of MoAb, washed once and then clonal assays were performed. T cell supernatants were prepared after 3 day-culture at a concentration of 10^6 cells/ml without mitogen stimulation. The effect of T cell supernatants on colony formation was tested at final concentrations of 0% to 15% (vol/vol).

**Northern blot analysis.** RNA was extracted from human thymocytes, fetal liver, and the patient's PBMC by the guanidium thiocyanate and CsCl gradient centrifugation procedure.22 Same amounts of RNA (25 μg per lane) were electrophoresed on 0.8% agarose in 10 mmol/L sodium-phosphate buffer at pH 7.0 and transferred to Gene Screen Plus (New England Nuclear, Boston, MA). The filter was hybridized with 32P-labeled γ probe, which was derived from γcDNA (GP6; 1.0 kb BamHI-EcoRI fragment),23 and with 32P-labelled Cα genomic probe (1.0 kb EcoRI fragment),24 as described previously.23

**Analysis of gene rearrangements.** High molecular weight DNA prepared from the patient's T lymphocytes was digested with EcoRI or HindIII restriction endonuclease, size-fractionated by agarose-gel electrophoresis, and transferred to nylon filter paper, as described previously.25 These blots were hybridized with nick-translated, 32P-labeled DNA probes of the human TCRγ gene or the TCRδ gene.26 The blots were visualized on autoradiography. The γJ probe (0.7 kb HindIII-EcoRI fragment kindly provided by Dr T.H. Rabbitts, Laboratory of Molecular Biology, Cambridge, UK) was used to detect TCRγ gene rearrangements. The M6H probe (4.5 kb EcoRI genomic fragment) was used to detect rearrangements involving J61 locus.26

**Statistics.** Comparisons were made with the Student's t test.

**RESULTS**

**Surface marker analysis.** The surface phenotypes of fresh peripheral blood lymphocytes (PBL) were examined at onset of PRCA, at relapses, and at remissions. Before therapy, the patient's PBL were 94% CD3+, 6% CD4+, and 43% CD8+, suggesting the presence of double negative (CD4−CD8−) CD3+ cells. At the first relapse, the surface marker analysis of the patient's PBL revealed that 68% of CD3+ cells were αδTCR(WT31)− and 67% of the whole PBL were γδTCR(δTCS1)+ (Fig 1), demonstrating a prominent expansion of γδTCR+ cells in the peripheral blood. The γδTCR+ cells expressed a Vβ1/J61-encoded determinant (δTCS1+) and no Vβ2-encoded determinant (BB3−). The patient's γδTCR(δTCS1)− cells always carried higher proportions of HLA-DR antigen, a marker of in vivo T cell activation, than the αδTCR(WT31)− cells (Table 2, Fig 2). Longitudinal studies showed that, as the hemoglobin level declined, total lymphocyte counts and proportions of γδTCR+ cells and HLA-DR+CD3+ cells, especially HLA-DR+γδTCR+ cells, increased. Further characterization of γδTCR(δTCS1)− cells shows CD4 negative, 16% dim CD8+, 1% CD11+, 4% CD16+, 4% CD56+, and IL-2R gp55 negative (Fig 2), whereas αδTCR(WT31)− cells were 11% CD4+, 29% CD8+, 1% CD11+, 3% CD16+, 5% CD56, and IL-2R gp75 was 15% weakly present on γδTCR(δTCS1)+ cells.

![Fig 3. Detection of γ and δ transcripts in the patient's lymphocytes by Northern blot analysis. Total RNA preparations (25 μg/lane) from human thymus (T), fetal liver (L), and the patient's lymphocytes (P) were analyzed on Northern blots using TCRγ and TCRδ cDNA probes.](image-url)
Both parents and his sister had normal proportions (<5%) of γδTCR+ cells in the peripheral blood.

Analysis of TCR transcripts and gene rearrangements. Since the MoAbs (δTCS1, TCRδ1) used recognize epitopes on δ chain of T cell receptor, northern blot analysis was performed to determine whether or not γ chain of T cell receptor was associated with δ chain. As shown in Fig 3, abundant quantities of both TCRγ and δ transcripts were detected in the patient's T lymphocytes, suggesting that the T cell receptor of this patient consisted of γ and δ subunits.

Rearrangements of the TCR γ and δ genes in the DNA from the patient's T lymphocytes (>95% CD3+, ~60% δTCS1-) were examined by hybridization with TCR γ (Jγ) or δ (MH6) probe. As shown in Fig 4A, Jγ probe detected two faint rearranged bands in addition to the 3.3 and 1.8 kb germline bands after EcoRI digestion. MH6 probe also showed two faint rearranged bands and the 3.3 and 2.7 kb germline bands (Fig 4B). These results suggested a non-monoclonal expansion of the γδTCR+ cells in the patient.

Functional studies. The responses of the patient's PBMC were severely depressed to mitogens and candida and moderately to anti-CD3 MoAb and PPD. His T cells were non-reactive in allogeneic and autologous mixed lymphocyte culture (MLC) (Table 3). To investigate a possible involvement of γδTCR+ cells in the pathogenesis of mucocutaneous candidiasis, the suppressive effect of γδTCR+ cells on the proliferative response of αβTCR+ cells to candida was studied. As shown in Table 4, neither the patient's whole PBMC, isolated αβTCR+ (δTCS1-) nor γδTCR+ (WT31-) cells responded to candida antigen. Both αβTCR+ and γδTCR+ cells showed similar proliferative responses to anti-CD3 MoAb and rIL-2. These data suggest that the patient's unresponsiveness to candida was not ascribed to the presence of γδTCR+ cells in the patient's PBMC. The patient's γδTCR+ (WT31- cells produced similar quantities of IL-2 and IFN-γ as the αβTCR+ (δTCS1-) cells (Table 5). No detectable IL-2 (<0.001 U/mL) of IFN-γ (<6 U/mL) was demonstrated in the patient’s serum at relapse and at remission.

Cytotoxic function of the patient’s PBMC and isolated T cells was assessed using various target cells. As shown in Fig 5, the patient’s PBMC exhibited strong cytotoxicity to K562 and less strongly to MOLT-4. Addition of anti-CD3 MoAb or anti-γδTCR MoAb strongly blocked the cytotoxicity against K562 and MOLT-4, whereas those MoAbs enhanced the cytotoxicity against Daudi and HL-60, probably through anti-CD3/TCR redirected cytotoxic mechanism. The cytotoxicity was not restricted by MHC since the cytotoxicity was observed against various cell lines lacking surface MHC antigens. Further studies showed that isolated γδTCR+ cells exhibited strong cytotoxicity to K562, while αβTCR+ cells exhibited little cytotoxicity. Furthermore, anti-CD3 and anti-γδTCR MoAbs strongly blocked cytotoxicity of the

![Diagram](image)

Fig 4. Rearrangement patterns of TCRγ gene (A) and TCRδ gene (B) in the patient. (A) EcoRI digests identified by the Jγ probe yielded 3.3 and 1.8 kb bands in germline DNA (C) and two faint rearranged bands (arrows) and germline bands in the patient’s DNA (P). (B) HindIII fragments identified by the MH6 probe demonstrated 5.3 and 2.7 kb bands in germline DNA (C) and two additional rearranged bands (arrows) in the patient’s DNA (P).

<table>
<thead>
<tr>
<th>Table 3. Functional Analysis of Patient’s PBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulants</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Medium</td>
</tr>
<tr>
<td>PHA</td>
</tr>
<tr>
<td>ConA</td>
</tr>
<tr>
<td>Anti-CD3 (lgG2a) MoAb</td>
</tr>
<tr>
<td>PPD</td>
</tr>
<tr>
<td>Candida</td>
</tr>
<tr>
<td>Allogeneic cells</td>
</tr>
<tr>
<td>Autologous non-T cells</td>
</tr>
</tbody>
</table>

Results are expressed as the mean counts of [3H] thymidine incorporated per minute of three experiments. Control values are expressed as the mean of experiments with six healthy subjects.
patient's PBMC and isolated \( \gamma \delta TCR^+ \) cells, but not of isolated \( \alpha \beta TCR^+ \) cells. Anti-\( \alpha \beta TCR \) MoAb (WT31) was unable to block the cytotoxicity of the patient's whole PBMC, isolated \( \alpha \beta TCR^+ \) cells and \( \gamma \delta TCR^+ \) cells, as shown in Fig 6.

The effects of \( \gamma \delta TCR^+ \) cells on hematopoietic progenitors were investigated to determine a role of \( \gamma \delta TCR^+ \) cells in the pathogenesis of PRCA. Few CFU-E or BFU-E colonies were formed with the patient's bone marrow cells, even if the patient's marrow cells were depleted of T cells with sheep RBC or anti-CD3 (IgM subclass) and complement. Therefore, normal donor's marrow cells were used in the subsequent studies. As shown in Fig 7, the patient's PBMC obtained at the time of relapse inhibited BFU-E but not CFU-GM in a dose-dependent manner. Control PBMC showed little inhibition to either BFU-E or CFU-GM. Furthermore, isolated \( \gamma \delta TCR^+ \) cells strongly inhibited BFU-E but not CFU-GM; and the inhibition by \( \gamma \delta TCR^+ \) cells was almost completely reversed by anti-CD3 MoAb (\( P < .01 \)) and partially reversed by anti-\( \gamma \delta TCR \) (\( \delta TCS1 \)) MoAb (\( P < .05 \)), but not by anti-\( \alpha \beta TCR \) (WT31) MoAb (\( P > .1 \)), as shown in Fig 8. Supernatants derived from the patient's T cells similarly inhibited BFU-E but not CFU-GM in a concentration-dependent manner (Fig 9). The patient's serum did not suppress either BFU-E or CFU-GM (data not shown).

**DISCUSSION**

We have described that human \( \gamma \delta TCR^+ \) cells, freshly isolated from a patient with a combination of type 1 autoimmune polyglandular syndrome and PRCA, exhibited non-MHC-restricted cytotoxicity and suppression of erythropoiesis.

PRCA of acquired form is mostly considered to be of an autoimmune etiology due to antibody-mediated or T cell-mediated inhibition of erythropoiesis. Involved immunologic mechanisms in the pathogenesis of PRCA is further supported by its frequent association with other autoimmune diseases and by its responses to immunomodulation therapies. With respect to antibody-mediated immunologic abnormalities, we were unable to find serum erythropoietic inhibitor for PRCA or anti-adrenal antibody for autoimmune polyglandular syndrome. However, absence of...
antigadrenal antibody at this time cannot rule out a possibility that organ-specific autoantibody had been initially present because autoantibodies could disappear if target organs were completely destructed.

In support of a cell-mediated autoimmune etiology for PRCA and polyglandular syndrome in this patient, the T cells bearing γδ TCR were predominantly expanded to 68% of whole lymphocytes in the peripheral blood. There have been only a few reports on a peripheral expansion of γδ TCR + cells, in an immunodeficient state after bone marrow transplantation and in primary immunodeficiency. The γδ TCR + cells in our patient mostly exhibited granular lymphocytes of small to medium size, as reported in human γδ TCR + lymphocytes. Our patient had underlying autoimmune disease without hepatosplenomegaly or lymphadenopathy. The lymphocytes of the patient showed neither cellular atypia, clonal cytogenetic abnormality,
nor monoclonal rearrangement with DNA analysis, which were suggestive of non-neoplastic lymphocytosis. The $\gamma$T$\delta$TCR-bearing lymphocytes were CD4- and 16$\%$ dim-CD8+. In addition, the $\gamma$T$\delta$TCR + cells also expressed an in vivo activation marker, HLA-DR. It is also noteworthy that the $\gamma$T$\delta$TCR + cells exclusively expressed a V$\delta$/J$\delta$1-encoded determinant (J$\delta$CS1 +) and no V$\delta$2-encoded determinant (BB3 -). In the previously reported two cases with type I autoimmune polyglandular syndrome and PRCA, one had normal T cell phenotypes and the other had no data available.

It has been reported that $\gamma$T$\delta$TCR + cells acquire nonspecific cytolytic activity after activation in vitro, but freshly isolated $\gamma$T$\delta$TCR + cells display little if any cytotoxic activity on the resting state. The present case has shown that freshly isolated $\gamma$T$\delta$TCR + cells, if already activated in vivo, were capable of exhibiting cytotoxic function without further activation in vitro. The cytotoxic activity of the patient's PBMC was strongly blocked by the MoAb against CD3 and $\gamma$T$\delta$TCR, but not by the MoAb against $\alpha$$\beta$TCR (WT31), suggesting that $\gamma$T$\delta$TCR + cells exhibited most of cytotoxicity observed through CD3/$\gamma$T$\delta$TCR complex, as reported by others. It was further confirmed by the cytotoxicity study using purified $\gamma$T$\delta$TCR+ cells by panning method. At the moment, the ligand of the $\gamma$T$\delta$TCR + cells are not known in this patient, although $\gamma$T$\delta$TCR + cells have recently been shown to recognize MHC class I or its associated molecules in murine and human systems in certain experimental conditions. Isolated $\gamma$T$\delta$TCR + cells inhibited BFU-E but not CFU-GM by precoculture experiments. The inhibition was observed at relatively high effector:target ratios, probably because the patient's $\gamma$T$\delta$TCR + cells showed the non-monoclonal nature of the proliferation. In fact, inhibition of erythroid progenitors was observed at high effector:target ratios in patients with benign lymphoproliferative disorders and PRCA. Selective inhibition of BFU-E by the patient's $\gamma$T$\delta$TCR + cells was reversed by the addition of anti-CD3 or anti-$\gamma$T$\delta$TCR($\delta$TCS1) MoAb, but not by the addition of anti-$\alpha$$\beta$TCR (WT31) MoAb. It is not likely that reversion by MoAb was mediated by complement killing of T cells since the serum used was heat-inactivated at 56°C for 30 minutes and $\delta$TCS1 MoAb of IgG1 subclass does not bind complement. Therefore, $\gamma$T$\delta$TCR/CD3 complex on the patient's $\gamma$T$\delta$TCR + cells appears to work as a regulatory molecule to secrete a factor capable of inhibiting erythroid progenitors rather than working as a specific receptor for target cell recognition in this situation. Selective suppression of erythropoiesis by both T cells and their supernatants has recently been shown in PRCA and $\gamma$T$\delta$TCR + granulocyte leukemia or chronic Tγ-lymphoproliferative disease. Further studies are necessary to determine what factor $\gamma$T$\delta$TCR + cells secrete.

As it has been suggested that activated T cells may have a role in the pathogenesis of aplastic anemia and PRCA, this patient's improvement of PRCA correlated with decreases of total numbers of lymphocytes and of $\gamma$T$\delta$TCR + cells, especially HLA-DR + $\gamma$T$\delta$TCR + cells, which suggests a possible link between in vivo-activated $\gamma$T$\delta$TCR + cells and PRCA in our patient.

It remains to be determined whether or not $\gamma$T$\delta$TCR + cells might be involved in the pathogenesis of autoimmune polyglandular syndrome. The observation that amelioration of hypocalcemia was associated with improvement of PRCA suggests that a common immune mechanism might be involved. However, no clinical correlation was observed between mucocutaneous candidiasis and PRCA.

The present study has suggested a possible relationship between $\gamma$T$\delta$TCR + cells and PRCA, one of the representative diseases in which involvement of T cells has been well demonstrated by in vivo and in vitro studies. Thus, the $\gamma$T$\delta$TCR + cells may play a role in the pathogenesis of a certain type of PRCA.

ACKNOWLEDGMENT

We thank Dr J. Borst (The Netherlands Cancer Institute, Amsterdam) for kindly providing the MoAb TCR$\gamma$/81, Dr L. Moretta (Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy) for BB3, and Dr M. Tsudo (The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan) for Mik-B1. We also thank Dr T.H. Rabbitts (Laboratory of Molecular Biology, Cambridge, UK) for $\beta$ probe.

REFERENCES

34. Comans-Bitter WM, van Dongen JMJ, Neijens HJ, Friedrich W, Belohradsky BH, Kohn Th, Borst J: Expression of the classical T cell receptor (TcR-αβ) and the alternative T cell receptor (TcR-γδ) in athymic children and normal children. Seventh International Congress of Immunology, Berlin, July 30-August 5, 1989, 36-9 (abstr)
42. Matis LA, Ceron R, Bluestone JA: Major histocompatibility


