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Polarization of Human CD4$^+$ T Cells to T-helper Type 1 and Type 2 Cells by Superantigen Stimulation

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To induce T-helper type 1 (Th1) and type 2 (Th2) cells in a short period, human adult peripheral blood T cells were cultured in combination with toxic shock syndrome toxin-1 (TSST-1) in the presence of IL-12 and anti-IL-4 antibody (Th1-polarizing condition), or in combination with IL-4, anti-IL-12 and anti-IFN-γ antibodies (Th2-polarizing condition) in the presence of IL-2 for a total of 7 days. CD4$^+$ T cell blasts that resulted from the Th1-polarizing condition of unprimed type CD4$^+$CD45RA$^+$ T cells exhibited massive IFN-γ production but quite low IL-4 production upon re-stimulation with TSST-1. Around 90% of the cells that exhibited the typical Th1 response were CXCR3-positive. These cells were divided into CCR4-positive and CCR4-negative fractions. The CD4$^+$ T cell blasts that resulted from the Th2-polarizing condition exhibited massive IL-4 production but quite low IFN-γ production. Around 70% of the cells that exhibited the typical Th2 response were CXCR3-negative but were CCR4-positive. Two preparations of CD4$^+$ T cell blasts resulting from the Th1 and the Th2 polarizing conditions of the memory-type CD4$^+$CD45RO$^+$ T cells produced both IFN-γ and IL-4 in substantial units and contained both CXCR3-positive and CXCR3-negative cells. The results indicated that the new system induced typical Th1 and Th2 cells from unprimed CD4$^+$ T cells within 7 days and that the expression of CXCR3 was the key factor to discriminate Th1 and Th2 cells.

Key words: bacterial superantigen, Th1, Th2, CD4$^+$CD45RA$^+$ T cells, CD4$^+$CD45RO$^+$ T cells

Introduction

Activated human CD4$^+$ T cells are known to be divided into two functional subsets, T-helper type 1 (Th1) and T-helper type 2 (Th2) cells, based upon the patterns of cytokines that they express in response to the stimulation by specific antigens. Th1 cells produce interferon-γ (IFN-γ) and interleukin-2 (IL-2) to mediate macrophage activation and delayed-type hypersensitivity reactions. On the other hand, Th2 cells produce IL-4 and IL-5 which promote the secretion of IgG1 and IgE and cause immediate-type hypersensitivity reactions[1)～4)]. It has been reported that Th1 and Th2 cells can be discriminated according to the expressions of chemokine receptors. Th1 cells express CCR5, CXCR6, and CXCR3, while Th2 cells express CCR3, CCR4, CCR8, and prostaglandin D2 chemoattractant receptor (CRTh2)[5)～8)]. Abnormal shift of the Th1/Th2 balance has been noticed to be deeply associated with the pathogenesis of allergies and inflammatory diseases[2)].

In vitro culture systems have been found to gen-

erate Th1 and Th2 cells by stimulating T cells with T cell stimulants such as the antibodies (Abs) to T cell receptor (TCR) or phytohemagglutinin (PHA) in the presence of appropriate cytokines and Abs. For example, T cells differentiate to Th1 cells when stimulated in combination with anti-CD3 Ab and anti-CD28 Ab in the presence of recombinant IL-12 (rIL-12) and anti-IL-4 Ab. And, T cells differentiate to Th2 cells when stimulated in the presence of rIL-4, anti-IL-12 Ab and anti-IFN-γ Ab[9)10)]. The above experimental system, however, has some disadvantages. T cell stimulants used above are not natural T cell ligands. In addition, it takes as long as 2 to 4 weeks to induce Th1/Th2 cells. Bacterial superantigens (SAGs) are produced by pathogenic bacteria and can work as natural ligands for human T cells. SAGs from *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Yersinia pseudotuberculosis* cause diseases such as toxic shock syndrome (TSS)[11)12)], neonatal TSS-like exanthematous disease (NTED)[13)] and *Yersinia pseudotuberculosis* infection[14)15)], through T cell stimulations. It is therefore possible to assume that SAGs are suitable T cell stimulants for inducing Th1 and Th2 cells within a short period of time.

In the present study we examined the effects of Th1- and Th2-polarizing conditions using the powerful TSS-causing superantigen, TSS toxin-1 (TSST-1) on *in vitro* induction of Th1 and Th2 cells obtained from human adult peripheral blood (APB) and cord blood T cells. Our data show that a CD45RA-positive fraction in APB $CD4^+$ T cells, and cord blood $CD4^+$ T cells which are almost CD45RA-positive, are highly vulnerable to the Th1/Th2 differentiation in response to our system, whereas APB $CD45RO^+$ T cells are not. We discuss the phenotypes of Th1 and Th2 cells based on the results obtained in human T cells cultured in the Th1- and Th2-polarizing conditions.

Materials and Methods

Antibodies and other reagents

Monoclonal antibodies (mAbs), Nu Ts/c (anti-CD8) and I2C3 (anti-HLA-DR/DP), and purified mAbs UCHL1 (anti-CD45RO) were described in previous studies[16)17)]. 2H4 (anti-CD45RA)[18)] was provided by Dr. C. Morimoto (Institute of Medical Science, University of Tokyo, Japan). The following Abs were obtained commercially: FITC-conjugated T4 (anti-CD4, Beckman Coulter, Hialeah, FL); PE-conjugated SK1 (anti-CD8), PE-conjugated UCHL-1, Cychrome-conjugated streptavidin, PE-conjugated HI100 (anti-CD45RA) and PE-conjugated SK3 (anti-CD4) (Becton Dickinson, Mountain View, CA); biotinylated-1G1 (anti-CCR4, BD PharMingen, San Diego, CA); PC-5-conjugated UCHT1 (anti-CD3), FITC-conjugated ALB 11 (anti-CD45 RA), and FITC-conjugated MPB2P5 (anti-Vβ2) (Immunotech, Marseille, France); FITC-conjugated 49801 (anti-CXCR3) (R & D Systems Inc., Minneapolis, MN); NIB42 (anti-IFN-γ), 8D4-8 (anti-IL-4), biotinylated-4S. B3 (anti-IFN-γ), and biotinylated-MP4-25D2 (anti-IL-4) (BD PharMingen, San Diego, CA); anti-IL-4, anti-IL-12, anti-IFN-γ Abs (Genzyme Techne, Minneapolis, MN), anti-mouse IgG coupled to magnetic beads (Dynabeads M-450, Dynal, Oslo, Norway).

RPMI medium 1640 (Invitrogen Corporation, Grand Island, NY) was used as the culture medium and was supplemented with 100 μg/ml streptomycin, 100 U/ml penicillin, 10% fetal calf serum, and 5×10^{-5} M 2-mercaptoethanol. TSST-1 were purchased from Toxin Technology (Sarasota, FL). Human rIL-2 was provided by Shionogi & Co (Osaka, Japan). rIL-12, and rIL-4 were purchased form Genzyme Techne.

Antigen-presenting cells and several T cell preparations

DR^+L cells (8124) used as antigen-presenting cells (APC)[19)] were treated with 50 μg/ml mitomycin C for 30 min at 37 ℃ and irradiated at 30 gray using an x-ray irradiator. The cells were cultured in 48-well plates at 1×10^5 cells/well or in 6-well plates at 1×10^6 cells/wells and were allowed to adhere overnight at 37 ℃. After non-adherent cells were removed, T cells were added. Mononuclear cells taken from APB of 3 healthy volunteers and cord blood of 4 normally full-term delivered babies, after obtaining written informed consent, were isolated by Ficoll-Conray density centrifugation. Whole APB T cells and cord blood T cells were obtained by the sheep red blood rosette method.

Methods of T cell fractionation were described in previous studies[17]. Briefly, to obtain $CD4^+$ T cells, whole APB or cord blood T cells were treated with Abs Nu Ts/c and I2C3. To obtain APB $CD4^+CD45RO^+$ T cells and $CD4^+CD45RA^+$ T cells, whole APB T cells were treated with a combination of Abs Nu Ts/c, I2C3 and 2H4, and a combination of Abs Nu Ts/c, I2C3 and UCHL1, respectively. After washing, the Abs-treated cells were mixed with anti-mouse IgG coupled to magnetic beads and the mixtures were kept on ice for 30 min. Antibodies-unbound cells were negatively selected with a magnet and were suspended in a culture medium. APB and cord blood $CD4^+$ T cells prepared contained $<7\%$ $CD8^+$ T cells. APB $CD4^+CD45RA^+$ T cells and cord blood $CD4^+$ T cells prepared contained $>97\%$ $CD4^+CD45RA^+$ T cells. Preparations of APB $CD45RO^+$ T cells contained $<30\%$ $CD45RA^+$ fraction.

Experimental protocol to induce Th1 and Th2 cells from human T cells

The experimental protocol to induce Th1 and Th2 cells *in vitro* was shown in Fig. 1. $CD4^+$ T cells were stimulated with 10 ng/ml TSST-1 in the presence of 50 ng/ml human rIL-12 and 1 μg/ml anti-IL-4 Ab (Th1-polarizing condition), or with 10 ng/ml TSST-1 in the presence of 50 ng/ml human rIL-4, 1 μg/ml anti-IL-12 Ab and 10 μg/ml anti-IFN-γ Ab (Th2-polarizing condition) on an APC monolayer in 6-well plates at 1×10^6 cells/ml for 3 days. Recovered cells were subjected to Percoll density centrifugation. Large lymphoblasts obtained at the interface of the culture medium and Percoll (density 1.068) were expanded in the presence of 100 U/ml of human rIL-2 for 2 days in 2 cycles. T cell blasts were obtained at the interface between densities 1.068 and 1.050 after being subjected to Percoll densities (1.068, 1.050) centrifugation. The percentage of TCR $V\beta2^+$ fractions, which were the major TSST-1-reactive T cell fraction were around 80% in all T cell preparations. Cells harvested were re-stimulated with varying doses of TSST-1 on APC monolayer for 6 h in 500 μl/well volumes using 48-well culture plates. The cultured supernatants were measured for amounts of IFN-γ and IL-4.

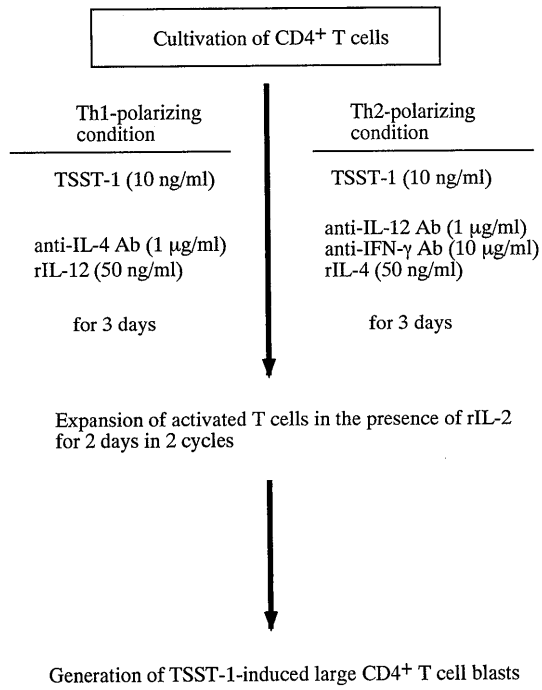

Fig. 1 Experimental conditions to polarize human T cells into Th1 or Th2 cells
APB or cord blood $CD4^+$ T cells were cultured in 5-ml volume in 6-well plates and stimulated on APC with 10 ng/ml TSST-1 for 3 days in the presence of 50 ng/ml rIL-12 and 1 μg/ml anti-IL-4 Ab (Th1-polarizing condition) or 50 ng/ml rIL-4, 1 μg/ml anti-IL-12 Ab, and 10 μg/ml anti-IFN-γ Ab (Th2-polarizing condition). T cell blasts collected were expanded in the presence of 100 U/ml rIL-2 for 2 days in 2 cycles.

We defined T cells which produced a large amount of IFN-γ but no IL-4 as Th1 cells, and cells which produced a large amount of IL-4 but no IFN-γ as Th2 cells. The dose of TSST-1 to induce Th1 and Th2 cells was determined to be 10 ng/ml because this dosage has induced sufficient activation of human T cells[19)~21)]. TSST-1-induced T cell blasts generated were cultured in the presence of rIL-2 for 2 days in 2 cycles because we found that this procedure induced substantial increase of the number of T cell blasts and a recovery of their reactivity to stimulation with TSST-1. Doses of cytokines and mAbs to another cytokines were chosen after our repeated experiments.

Cytokines assays

Amounts of IFN-γ and IL-4 in the culture supernatants were measured by sandwich ELISA, described in the previous study[17].

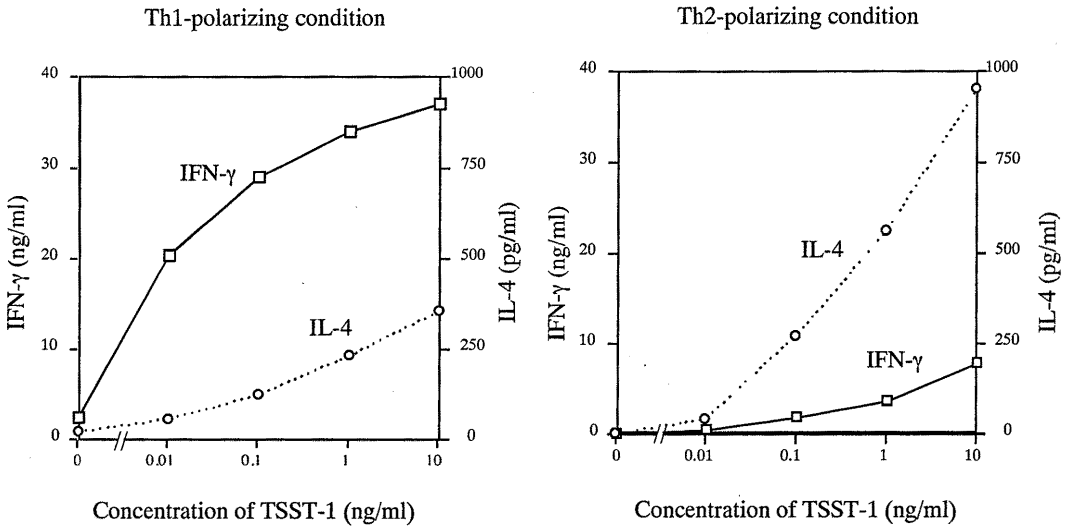

Fig. 2 Patterns of cytokine production of APB $CD4^+$ T cells cultured in Th1- or Th2-polarizing condition
Whole APB $CD4^+$ T cells were stimulated with 10 ng/ml TSST-1 for 3 days in the presence of rIL-12, anti-IL-4 Ab, or rIL-4, anti-IL-12 Ab, anti-IFN-γ Ab. T cell blasts collected expanded with 100 U/ml rIL-2 for 4 days and re-stimulated with varying concentrations of TSST-1 for 6 h. Culture supernatants were measured for amounts of IFN-γ and IL-4.

Flowcytometric analysis

T cell samples were analyzed for expression of CD4 vs CD8 gated in CD3, Vβ2 vs CD4 gated in CD3, CXCR3 vs CCR4 gated in CD45RA or CD45 RO, CD45RA vs CD45RO gated in CD3. T cells were stained with several combinations of the appropriate PE-, PC5- and FITC-conjugated Abs and examined by three-color flowcytometric analysis using as EPICS CS flow cytometer (Coulter Electronics, Hialeah, FL). All procedures for cell staining were conducted on ice.

Results

Induction of Th1 and Th2 cells from APB or cord blood $CD4^+$ T cells, APB $CD4^+CD45RA^+$ T cells, APB $CD4^+CD45RO^+$ T cells

Results from one of the several experiments are presented in Fig. 2. $CD4^+$ T cell blasts from the culture of the Th1-polarizing condition produced high amounts of IFN-γ at 0.01 ng/ml or more of TSST-1, and low but substantial amounts of IL-4 at 0.1 ng/ml or more of TSST-1. $CD4^+$ T cells blasts from the culture of the Th2-polarizing condition produced high amounts of IL-4 at 0.1 ng/ml or more of TSST-1, and low but substantial amounts of IFN-γ at the same TSST-1 doses. The results indicate that whole $CD4^+$ T cells are resistant to polarization with the present experimental system.

Two possibilities could be thought to explain the above findings. First, the Th1- and Th2-polarizing conditions used here were not enough to induce Th1 and Th2 cells. Second, since it is well known that APB T cells mainly consist of naive type $CD45RA^+$ T cells and memory type $CD45RO^+$ T cells, it seems likely that the latter T cell fraction is responsible for the resistance of the whole $CD4^+$ T cells to the Th1 /Th2-polarizing conditions.

Then, fractionated APB $CD4^+CD45RA^+$and $CD4^+$ $CD45RO^+$ T cells from several healthy donors were cultured in the Th1- or Th2-polarizing conditions and the resulting $CD4^+$ T cell blasts were examined for the cytokine production patterns to re-stimulation with TSST-1. Cord blood $CD4^+$ T cells which consisted of mostly $CD45RA^+$ cells were included in parallel experiments (Table). The data in Fig. 3 are representative of all the 6 experiments (Exp. 2 and Exp. 3 in the Table). The patterns of cytokine production in T cell blasts derived from $CD4^+CD45RO^+$ T cells cultured in the Th1- and Th 2-polarizing conditions were almost the same as those seen in the blasts from whole $CD4^+$ T cells

Table Cytokine production of APB and cord blood $CD4^+$ T cells cultured in the Th1- and Th2-polarizing conditions

Exp	Source of $CD4^+$ T cell blasts	Polarizing condition			
		cytokine production after re-stimulation with TSST-1 (10 ng/ml)			
		Th1		Th2	
		IFN-γ (ng/ml)	IL-4 (pg/ml)	IFN-γ (ng/ml)	IL-4 (pg/ml)
1	APB CD45RA-1	57.0	0	1.9	90
2	cord blood 1	61.5	0	0.9	270
3	APB CD45RA-2	27.5	7	0.9	170
	APB CD45RO-2	23.0	86	6.8	145
4	APB CD45RA-3	87.0	0	3.6	375
	APB CD45RO-3	64.5	330	30.0	345
5	cord blood 2	27.5	0	0.8	190
6	cord blood 3	40.0	0	1.2	260
	cord blood 4	40.0	0	0.4	210

APB $CD4^+$ T cells, APB $CD4^+CD45RA^+$ T cells, APB $CD4^+CD45RO^+$ T cells, and cord blood $CD4^+$ T cells (5×10^6/well) were cultured on APC monolayer in 5 ml-volume in 6-well plates, and stimulated with 10 ng/ml TSST-1 in Th1- or Th2-polarizing condition. The T cell blasts (5×10^5/well) collected were re-stimulated with varying doses of TSST-1 in 0.5 ml-volume in 48-well plates for 6 h. Amounts of IFN-γ and IL-4 in the culture supernatants were measured by ELISA method. Data obtained at 10 ng/ml of TSST-1 are presented.

(Fig. 3A, 3B and Fig. 2). Fig. 3A showed that there was production of massive amounts of IFN-γ, and low but substantial amounts of IL-4 in the Th1-polarized T cells. Fig. 3B shows massive amounts of IL-4 and low but substantial amounts of IFN-γ in the Th2-polarized T cells. $CD4^+$ T cell blasts derived from $CD4^+CD45RA^+$ T cells cultured in the Th1-polarizing condition exhibited a massive IFN-γ production at 0.01 ng/ml or more of TSST-1 and a quite low IL-4 production at 10 ng/ml of TSST-1 (Fig. 3C). The corresponding $CD4^+$ T cell blasts derived from the Th2-polarizing condition exhibited a massive IL-4 production at 0.1 ng/ml or more of TSST-1 and a quite low IFN-γ production at 1 ng/ml or more of TSST-1 (Fig. 3D). $CD4^+$ T cell blasts derived from cord blood $CD4^+$ T cells also exhibited similar Th1- and Th2-patterns of cytokine production according to the Th1- and Th2-polarizing conditions (Fig. 3E, 3F).

The results indicated that $CD4^+CD45RA^+$ T cells, irrespective of sources, could differentiate Th1 or Th2 cells almost completely according to the different polarizing condition while $CD4^+CD45RO^+$ T cells were not vulnerable to the Th1- and Th2-polarizing conditions.

Expression of chemokine receptors in Th1 and Th2 cells

We examined how APB, cord blood $CD4^+CD45RA^+$ and APB $CD4^+CD45RO^+$ T cells cultured in the present Th1/Th2-polarizing conditions expressed the chemokine receptors, CXCR3 and CCR4 by flow cytometry. The analysis may clarify that $CD4^+CD45RA^+$ T cells were highly vulnerable to Th1/Th2-polarizing conditions, while $CD4^+CD45RO^+$ T cells were not.

More than 90% of the unstimulated APB $CD4^+CD45RA^+$ T cells were negative for CXCR3 and CCR4 expressions (Fig. 4A). After culturing them in the Th1-polarizing condition, around 90% of the resulting activated $CD4^+$ T cells were positive for CXCR3 expression (Fig. 4B). As for the CCR4 expression, the activated cells could be divided into CCR4-positive and CCR4-negative fractions (around 80% and 20%, respectively). After culturing APB $CD4^+CD45RA^+$ T cells in the Th2-polarizing condition, around 70% of the activated cells were negative for CXCR3 expression (Fig. 4C). More than 95% of the activated cells were positive for CCR4 expression (Fig. 4C). $CD4^+$ T cell blasts derived from cord blood $CD4^+$ T cells cultured in Th1- and Th2-polarizing conditions exhibited similar patterns of chemokine receptor expression as the $CD4^+$ T

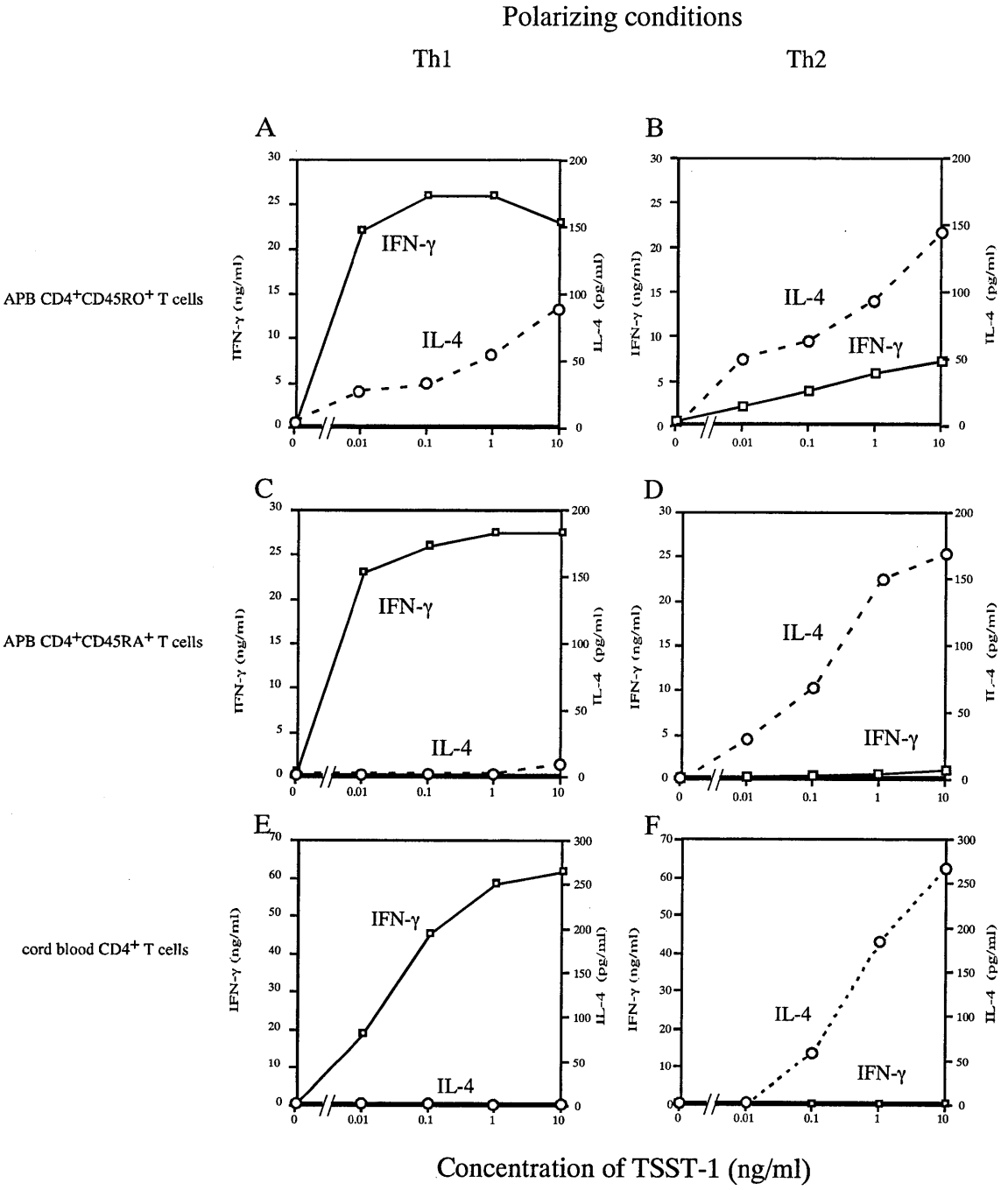

Fig. 3 Patterns of cytokine production of APB and cord blood $CD4^+CD45RA^+$ T cells, APB $CD4^+CD45RO^+$ T cells cultured in Th1- or Th2-polarizing condition
APB $CD4^+CD45RA^+$ T cells, APB $CD4^+CD45RO^+$ T cells and cord blood $CD4^+$ T cells were stimulated with 10 ng/ml TSST-1 for 3 days in the Th1- or Th2-polarizing condition. The percentage of $CD45RA^+$ fraction of cord blood $CD4^+$ T cells was about 97%. T cell blasts collected were expanded with 100 U/ml rIL-2 for 4 days, and re-stimulated with varying concentrations of TSST-1 for 6 h. Culture supernatants were measured for amounts of IFN-γ and IL-4.

cell blasts derived from APB $CD4^+CD45RA^+$ T cells cultured in the same conditions (Fig. 4E, 4F).

In unsensitized APB $CD4^+CD45RO^+$ T cells, cells positive for expression of CXCR3 alone, or CCR4 alone, or negative for both CXCR3 and CCR4 expressions were seen as major fractions (around 30% in each fraction) (Fig. 4G). After culturing these cells in the Th1- and Th2-polarizing conditions, gen-

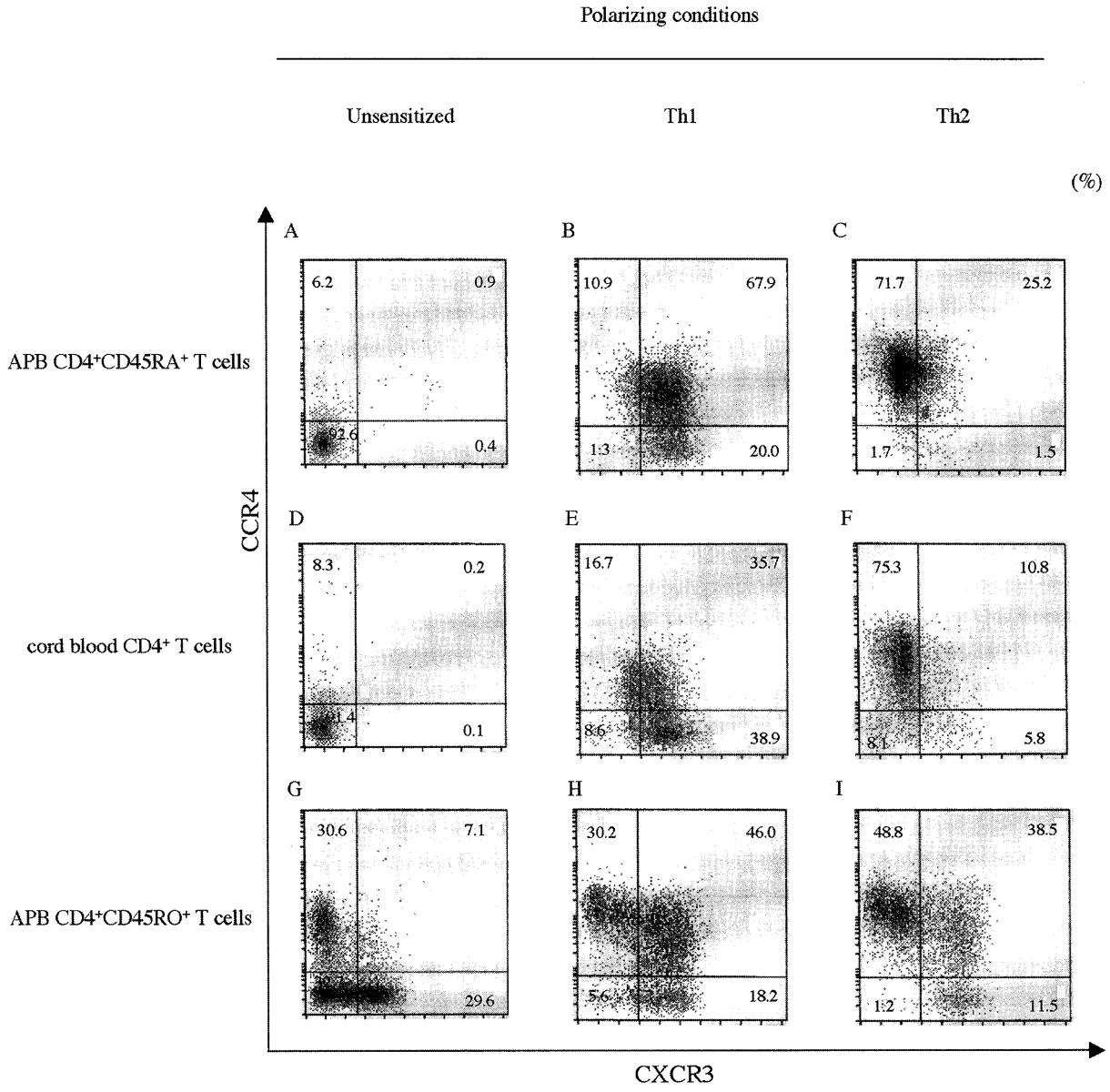

Fig. 4 Expression of chemokine receptors in human $CD4^+$ T cells cultured in Th1- and Th2- polarizing conditions

APB $CD4^+CD45RA^+$ T cells, $CD4^+CD45RO^+$ T cells and cord blood $CD4^+$ T cells (>97%CD45RA-positive) were stimulated with 10 ng/ml TSST-1 for 3 days in the Th1- or Th2-polarizing condition. T cell blasts expanded with 100 U/ml rIL-2 for 4 days were stained by appropriate mAbs, and analyzed by flowcytometry for the expression of chemokine receptors (CXCR3, CCR4). The quadrant lines shown were set according to the staining pattern of control mAbs.

erated $CD4^+$ T cell blasts contained both CXCR3-positive and CXCR3-negative cells. The ratio of the CXCR3-positive and CXCR3-negative cells in each T cell blasts changed according to the polarizing conditions (around 65% and 50% in the Th1- and Th2-polarizing conditions, respectively) (Fig. 4H, 4 I).

Discussion

In the present study, we conducted *in vitro* experiments to establish a system to effectively induce Th1 and Th2 cells from human T cells in a short period of time. In most experimental systems reported so far, to obtain Th1 and Th2 cells from human $CD4^+$ T cells took 2 to 4 weeks, including $CD45RA^+$fraction which were highly susceptible

for the Th1/Th2 polarization[9)10)22)]. The superantigen TSST-1 is a powerful T cell stimulant, which induces massive T cell blasts by quite a small dose in *in vitro* culture systems. Massive expansion of TSST-1-reactive Vβ2$^+$ T cells is seen in patients with TSST-1-associated diseases, TSS and NTED[11)~13)]. We could polarize APB and cord blood CD4$^+$CD45RA$^+$ T cells into Th1 cells that produced IFN-γ without IL-4, and into Th2 cells that produced IL-4 without IFN-γ within 7 days with the new experimental system using the superantigen TSST-1. We believe that our present study would be useful for the analysis on the differentiation mechanisms of Th1 and Th2 cells and the pathogenic mechanisms of allergies.

We then analyzed the expressions of CXCR3 and CCR4 in unsensitized and TSST-1 stimulated T cells from APB and cord blood. Most of the unsensitized APB and cord blood CD4$^+$CD45RA$^+$ T cells which were highly vulnerable to the Th1- or Th2-polarizing conditions as shown in the Table and Fig. 3, expressed neither CXCR3 nor CCR4. Recently Kim et al[5)] reported that unsensitized CXCR3-positive fraction in whole APB CD4$^+$ T cells expressed high IFN-γ and negligible IL-4 after *in vitro* stimulation with a combination of PMA and ionomycin, indicating that CXCR3-positive cells have been triggered into differentiation to Th1 cells. Unsensitized CCR4-positive fraction in whole APB CD4$^+$ T cells expressed both IFN-γ and IL-4 after stimulation with PMA and ionomycin, indicating that CCR4-positive T cells consisted of either Th1 or Th2 cells[5)]. In accordance to the report, CD4$^+$ T cell blasts generated after culturing APB CD4$^+$CD45RA$^+$ T cells in the Th1-polarizing condition that exhibited the Th1 phenotype could be recognized as CXCR3-positive cells, regardless of the CCR4. CD4$^+$ T cell blasts generated after culturing them with the Th2-polarizing condition which exhibited the Th2 phenotype could be recognized as CXCR3-negative and CCR4-positive cells. As CCR4 was expressed in both Th1 and Th2 cells, it could be deduced that expression of CXCR3 was a key factor to discriminate *in vitro* induced Th1 and Th2 cells. It seemed likely that CXCR3-negative cells in the Th1-phenotype CD4$^+$ T cell blasts and CXCR3-positive cells in the Th2-phenotype CD4$^+$ T cell blasts were responsible for the slightly detected IL-4 and IFN-γ production.

The present study was able to explain why CD4$^+$CD45RO$^+$ T cells were not vulnerable to the Th1- and Th2-polarizing conditions. Unsensitized APB CD4$^+$CD45RO$^+$ T cells were considered to contain both cells expressing CXCR3 alone, which exhibited Th1 phenotype and cells expressing CCR4 alone, which exhibited Th1 and Th2 phenotype according to the report by Kim et al cited before. Actually CD4$^+$ T cell blasts resulted from *in vitro* culture of CD4$^+$CD45RO$^+$ T cells in the Th1- and Th2-polarizing conditions are composed of two fractions, CXCR3-positive and CXCR3-negative, the ratio of which changed according to the Th1- and Th2-polarizing conditions.

A report showed that human Th1 and Th2 cells that were obtained around 20 days after the initial *in vitro* stimulation acquired the opposite phenotypes when re-cultured under the polarizing conditions opposite to the initial conditions[23)]. Another report showed that the reversibility of phenotypes of murine Th1 and Th2 cells was lost after a long-term stimulation[24)]. On the other hand, a report showed that the reversibility of phenotypes was different between Th1 and Th2 cells: murine Th1 cells could be converted into Th2 cells when re-cultured in second Th2-polarizing stimulation, but Th2 cells could not be converted into Th1 cells when re-cultured in second Th1-polarizing stimulation[25)]. It will be examined in our future studies whether Th1 and Th2 cells induced in the present experiments would change their phenotype when cultured in the opposite polarizing conditions.

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ヒト CD4$^+$ T 細胞のスーパー抗原による Th1, Th2 type 細胞への偏向

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免疫応答に重要な役割をもつ CD4$^+$ヘルパー T 細胞に 2 つのサブセット（Th1, Th2）があり，Th1/Th2 バランスの変調が，様々な疾患の病因に大きく関与していることが明らかになってきた．これまで，人工的な刺激物質である抗 CD3 抗体などを用いて *in vitro* 実験系で偏向させたヒト Th1 細胞と Th2 細胞サンプルを得るまでに 4 週間ほど時間が必要であった．本研究では自然環境においてヒト T 細胞の強力な活性化抗原である細菌性スーパー抗原を用い，短期間に Th1 細胞と Th2 細胞を誘導できる実験システムを確立した．ヒト T 細胞の未感作分画である CD4$^+$CD45RA$^+$T 細胞は 1 週間以内に Th1 細胞と Th2 細胞に分化を遂げた．一方，末梢血感作 T 細胞分画である CD4$^+$CD45RO$^+$T 細胞は Th1/Th2 偏向刺激に対して抵抗性を示した．ケモカインレセプター表現の解析では，従来の報告と同様に CD4$^+$CD45RA$^+$T 細胞から誘導された Th1 細胞は CXCR3 陽性であり，Th2 細胞は CXCR3 陰性を示す成績が得られた．また，Th1/Th2 偏向刺激を受けた CD4$^+$CD45RO$^+$T 細胞は，いずれにおいても CXCR3 陽性細胞と CXCR3 陰性細胞が混在していた．