主論文1.

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IL-7 induces proliferation, variable cytokine-producing ability and IL-2 responsiveness in naive CD4+ T cells from human cord blood [1]

Tetsuya Fukui*, Kenji Katamura*[2], Nobutaka Abe*, Takahiro Kiyomasu*, Jun Iio*, Hideki Ueno*, Mitsufumi Mayumi†, and Kenshi Furusho*

*Department of Pediatrics, Faculty of Medicine, Kyoto University, Kyoto, Japan,

†Department of Pediatrics, Fukui Medical school, Fukui, Japan.

[2]Address correspondence and reprint requests to Dr. Kenji Katamura, Department of Pediatrics, Faculty of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyoku, Kyoto, 606-01, Japan

FAX: 01181-75-752-2361 TEL: 01181-75-751-3294

E-mail: kkenji@kuhp.kyoto-u.ac.jp

1.Summary

We investigated the effects of IL-7 on the proliferation and acquisition of cytokine-producing ability of naive CD4+ T cells from human cord blood. Naive CD4+CD45RA+ T cells from human cord blood expressed CDw127 (IL-7R) at higher levels than adult CD4+ CD45RA+ T cells, and produced IL-2 and a small amount of IFN-γ upon stimulation with PMA and ionomycin. IL-7 induced IL-2-independent proliferation and both Th1-and Th2-type cytokine-producing abilities in cord blood CD4+CD45RA+ T cells without stimulation via TCR. These results suggest that this IL-7-induced antigen-independent activation mechanism could contribute to maintaining the clonal size of naive T cells with the potential to differentiate into either Th1 or Th2 cells at the sites of IL-7-expression.

2. Introduction

It is now generally agreed that naive CD4+ T cells produce mainly IL-2 in response to antigenic stimulation and that the differentiation of naive CD4+ T cells into Th1 or Th2 is regulated by factors present in the environment of naive T cells at primary stimulation [1-8]. However, recent studies have demonstrated that mature T cells and naive CD4+ T cells proliferate upon stimulation with cytokines in the absence of TCR stimulation *in vitro* [9-12]. Furthermore naive CD4+ T cells acquired the ability to produce cytokines other than IL-2 upon stimulation with these cytokines [10-13].

IL-7 was originally described as a cytokine supporting the growth of B cell precursors [14-15]. It was later found that IL-7 supports the growth of both murine and human T cell progenitors [16-21]. Furthermore, IL-7 affects not only immature, but also mature T cells as a costimulator [22-26]. Although bone marrow stroma cells were originally identified as a source of IL-7, recent studies demonstrated that IL-7 mRNA is also expressed in human intestinal epithelial cells and skin keratinocytes as well as spleen and thymic tissue [27-29]. Because the intestine and skin are continuously and directly exposed to the environment, they are important primary defensive organs that protect the host from ubiquitous environmental organisms or foreign antigens. Furthermore, they are

sites where naive T cells may encounter foreign antigens and get primary stimulation. These results suggested that IL-7 is involved in the differentiation of naive $CD4^+$ T cells.

Although much effort has been made to determine the factors that regulate the differentiation of naive $CD4^+$ T cells, little is known about the role of IL-7. We therefore investigated the effect of IL-7 on naive $CD4^+$ T cells.

3. Materials and Methods

Reagents

RPMI 1640 medium, FCS, human rIL-2, IFN-γ, IL-4, IL-5, PMA, ionomycin and Dynabeads M-450 Sheep anti-Mouse IgG were obtained as previously described [8]. Human rIL-7 were obtained from Research and Diagnostic Systems (Minneapolis, MN). Breferdin A and saponin were purchased from Epicentre Technologies (Madison, WI) and Sigma Chemical Co. (St. Louis, MO), respectively. Other reagents were obtained from Nacalai Tesque (Kyoto, Japan) unless otherwise specified.

Antibodies

The antibodies 3G8 (anti-CD16), OKT8 (anti-CD8), 2.06 (anti-HLA-DR), TS1/22 (anti-CD11a) and rabbit anti-human IL-4 were obtained as described [8]. MHL-1 (anti-CD62L) and Tac (anti-CD25) were gifts from Dr. M. Miyasaka (Osaka University, Osaka, Japan) and Dr. T. Uchiyama (Institute for Virus Research, Kyoto University, Kyoto, Japan), respectively. The antibodies 3C10 (anti-CD14), 10F7MN (anti-glycophorin A), γ-2-11.1 (anti-human IFN-γ) were purchased from American Type Culture Collection (Rockville, MD) and purified with protein G. Leu4 (anti-CD3), Leu3a (anti-CD4) and X40 (RPE labeled mouse IgG_1) were purchased from Becton Dickinson Immunocytometry System (San Jose, CA). B-C15 (anti-CD45RA) was from Serotec (Oxford, England). FITC labeled 679.1Mc7 (mouse IgG_1), FITC labeled ALB11 (anti-CD45RA) and R34.34 (anti-human IL-7 receptor, anti-CDw127) were obtained from Immunotech (Marceille, France). DAKGO1 (mouse IgG_1), DAKGO5 (RPE labeled mouse IgG_{2a}), RPE labeled UCHL-1 (anti-CD45RO) and RPE or FITC labeled goat anti-mouse IgG were purchased from DAKO (Glostrup, Denmark). RPE labeled 8D4-8

(anti-human IL-4), FITC labeled 4S.B3 (anti-human IFN-γ) and RPE labeled TRAP1 (anti-human CD40 ligand) were obtained from Pharmingen (San Diego, CA). Polyclonal mouse IgG was obtained from Zymed Laboratories, Inc. (San Francisco, CA). Monoclonal mouse IgG_{2a} and rabbit neutralizing anti-IL-2 antibody were obtained from Chemicon International Inc. (Temecula, CA) and Becton Dickinson (Bedford, MA), respectively.

Purification of $CD4^+$ T cells

Naive $CD4^+$ T cells were purified from umbilical cord blood by the method described previously [8]. Briefly, mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation, washed twice with RPMI 1640 medium supplemented with 10% FCS, penicillin G (100 U/ml), streptomycin (100 μg/ml), 50 μM 2-ME and L-glutamine (2 mM) (complete culture medium: CM). Cells were applied to a nylon wool column preincubated with CM containing 5 mM *L*-leucine methyl ester. After incubation at 37 °C for 1 h, non-adherent cells were recovered and treated with saturating amounts of anti-CD8, anti-CD16, anti-CD14, anti-HLA-DR and anti-glycophorin A antibodies. For the purification of adult $CD4^+CD45RA^+$ T cells, recovered non-adherent cells were treated with both these antibodies described above and anti-CD45RO. $CD4^+$ T cells were negatively selected by the immunomagnetic method with Dynabeads M-450. These cells were shown by flow cytometry to be: 97-99% $CD3^+$, 96-98% $CD4^+$, <1% $CD8^+$, $CD16^+$, $CD14^+$ or $HLA\text{-}DR^+$.

Culture conditions

Naive $CD4^+$ T cells (10^6 /ml) were cultured in CM in 48-well plates (500 μl/well). Cytokines were added at the indicated concentrations from the beginning of culture. Seven days later, the cells were washed, then the concentration was readjusted to 10^6 /ml in fresh medium. The cells were then recultured with fresh cytokines as described above. After seven and 14 days of culture, the cells were washed, resuspended (10^6 /ml) in CM and stimulated with PMA (10 ng/ml) and ionomycin (1 μg/ml) for 24 h, then cytokines in the cell supernatant were assayed as described

Flow cytometric two-color cell analysis

Cells were stained with FITC- and RPE-labeled mAb for 30 min at 4 °C. When using unlabeled mAb, cells were first incubated with the mAb for 30 min, then stained with FITC- or RPE-labeled goat anti-mouse IgG for 20 min. After blocking free Ag-binding sites on the second Ab by incubation with a polyclonal mouse IgG for 20 min, the cells were counterstained with labeled mAb for 30 min. Stained cells were analyzed using an Ortho Cytron (Tokyo, Japan), designed to identify only live cells.

Flow cytometric analysis of intracellular cytokines

Intracellular cytokines were stained with mouse anti-hIL-4 or anti-hIFN-γ mAbs as described by Picker et al. [30]. Briefly, $1x10^6$ cells/ml in CM were stimulated with PMA (10 ng/ml) and ionomycin (1 μg/ml) for 8 h. Brefeldin A (10 μg/ml) was added for the last 4 h to inhibit protein transport. After fixation with 4% paraformaldehyde for 20 min at 4 °C, the cells were permeabilized with 0.1% saponin, stained with labeled anti-hIL-4 or anti-hIFN-γ mAb for 30 min and analyzed as described above.

Cytokine measurements

All cytokines were measured by two-site sandwich ELISA using a mouse mAb as the capture Ab and a rabbit polyclonal Ab for detection by essentially the same method as described except that the capture mAb to hIFN-γ (γ-2-11.1) and the ELISA plates (E.I.A./R.I.A. flat-bottomed plate, high binding) were obtained from American Type Culture Collection (Rockville, MD) and Corning Costar Japan, (Tokyo, Japan), respectively [8]. The sensitivity of the ELISA system was as described [8].

4. Results

Analysis of CDw127 on cord blood naive CD4+ T cells and adult CD4+ CD45RA+ T cells

We first analyzed the expression of CDw127 (IL-7R) on cord blood naive $CD4^+$ T cells and adult $CD4^+CD45RA^+$ T cells by two-color fluorescent cytometry. As shown in Figure 1, almost all human cord blood $CD4^+$ cells expressed CD45RA (96.8 ± 0.5 %, mean ± SEM, n=12) and CDw127 (97.2 ± 0.3 %, mean ± SEM,n=6). On the other

hand, 63.7 ± 3.0 % (mean ± SEM, n=4) of adult $CD4^+CD45RA^+$ T cells expressed CDw127.

Proliferative response of cord blood naive CD4+ T cells and adult CD4+CD45RA+ T cells to IL-7

To examine the effects of IL-7 on naive $CD4^+$ T cells, we cultured isolated naive $CD4^+$ T cells with IL-7. IL-7 induced vigorous proliferation of naive $CD4^+$ T cells from cord blood in a dose-dependent manner (Fig. 2a). In contrast to cord blood T cells, the proliferative response of adult $CD4^+CD45RA^+$ T cells to IL-7 was weaker than that of cord blood $CD4^+$ T cells (Fig. 2b). Their cell number decreased for the first 7 days, and then, proliferated to about 200% of the original number after 14 days. IL-2 neither induced the proliferation, nor maintained the viability of cord blood naive $CD4^+$ T cells (Fig. 2c). Furthermore, adding neutralizing anti-IL-2 Ab did not inhibit the proliferation of naive $CD4^+$ T cells by IL-7 (Fig. 2d). This concentration of anti-IL-2 Ab completely inhibited the proliferation of mouse IL-2-dependent HT-2 cells in the presence of 5 ng/ml of human IL-2 (data not shown). We did not detect spontaneous IL-2 production in the proliferating T cells by ELISA or reverse transcriptase-PCR (data not shown).

These results indicate that the proliferation of naive $CD4^+$ T cells was stimulated by IL-7 itself and not mediated by endogenous IL-2. Because the proliferative responses of these T cells reached a plateau when cultured with more than 30 ng/ml of IL-7 (data not shown), this concentration was used for the following study.

Cytokine production profile of cord blood naive CD4+ T cells proliferating in the presence of IL-7

To characterize the cytokine production profile of naive $CD4^+$ T cells in culture with IL-7 for 7 or 14 days without stimulation via TCR, we stimulated them with PMA and ionomycin. The levels of IL-2, IFN-γ, IL-4 and IL-5 in supernatants were measured by ELISA. Freshly isolated $CD4^+$ T cells produced mainly IL-2 and a little IFN-γ, but no IL-4 or IL-5 (Table I). After culture with 30 ng/ml of IL-7 for 7 days, we detected IL-2

and high levels of IFN-γ, but no IL-4 or IL-5. After 14 days of culture, we detected IL-2 and IFN-γ as well as small amounts of IL-4 and IL-5.

Changes in surface molecule expression on CD4$^+$ T cells after culture with IL-7

Previous studies showed that naive CD4$^+$ T cells mainly produce IL-2 and express CD45RA molecules [1, 31-33]. In our study, cord blood naive CD4$^+$ T cells which were stimulated with IL-7 but not through TCR produced IFN-γ, IL-4 and IL-5 as well as IL-2. Therefore, we examined the surface molecules expressed on these cells by flow cytometry. As shown in Figure 3, CD4$^+$ T cells cultured with IL-7 had a higher mean fluorescent intensity (MFI) of CD45RA and CD11a than freshly isolated cord blood naive CD4$^+$ T cells. The expression of CD62L was stable. There was a small proportion of CD45RA$^-$ and CD45RO$^+$ cells. Furthermore, IL-7 induced CD25 and CD40 ligand expression on about 75 and 20% of these T cells, respectively. These results indicated that the phenotype of naive CD4$^+$ T cells cultured with IL-7 for 14 days was a mixture of naive, memory and activated T cells.

Production of IFN-γ and IL-4 by CD4$^+$CD45RA$^+$ T cells

The T cells proliferating in the presence of IL-7 contained a small amount of CD45RA$^-$ T cells and CD45RO$^+$ T cells. This suggests that IFN-γ and IL-4 were produced by the CD45RA$^-$ T, and not by the CD45RA$^+$ T cells. To rule out this possibility, we investigated IL-4- and IFN-γ-production in the CD45RA$^+$ and CD45RA$^-$ cell population by intracellular cytokine staining. After 14 days of culture with IL-7, T cells were cultured in CM only or with PMA and ionomycin for 8 h, and brefeldin A was added for the last 4 h to inhibit cytokine secretion as described in Materials and Methods. Expression of intracellular IL-4, IFN-γ and surface CD45RA determined by two color fluorescence flow cytometry showed that CD45RA$^+$ T cells produced IL-4 and IFN-γ (Fig. 4).

Induction of IL-2Rα (CD25) and IL-2 responsiveness by stimulation with IL-7

As shown in Figure 3, we demonstrated that IL-7 induced CD25 expression on naive CD4$^+$ T cells from cord blood. We then investigated the IL-2 responsiveness of these T cells. As shown in Figure 5, IL-2 dose-dependently induced the proliferation of CD4$^+$ T cells that had been cultured with IL-7 for 14 days.

5. Discussion

We investigated the effects of IL-7 on naive CD4$^+$ T cells. We first analyzed the presence of CDw127 on cord blood naive CD4$^+$ T cells and adult CD4$^+$CD45RA$^+$ T cells. Virtually all cord blood naive CD4$^+$ T cells expressed CDw127. We also detected CDw127 expression on about 65% of adult peripheral blood CD4$^+$CD45RA$^+$ T cells, indicating that adult peripheral CD4$^+$CD45RA$^+$ T cells were heterogeneous in regard to the expression of CDw127. Although Watanabe *et al.* reported that adult peripheral blood T cells did not express CDw127, a new mAb against CDw127 has detected CDw127 on all these cells [27, 34]. These differences may be due to the affinity of the mAb used to detect CDw127 Ag and to the different levels of CDw127 expression on various T cell subsets.

We demonstrated that in the absence of stimulation via TCR, these cord blood CDw127$^+$ naive CD4$^+$ T cells vigorously proliferated in response to IL-7 and the cell number increased 5 to 6-fold within two weeks. Whereas, adult CD4$^+$CD45RA$^+$ T cells showed a weaker response to IL-7. The difference in the proliferative response to IL-7 might have been due to the difference in expression of CDw127. There would be no essential difference in the IL-7 responsiveness between cord blood and adult peripheral CD4$^+$CD45RA$^+$CDw127$^+$ T cells. We used cord blood CD4$^+$ T cells because they are homogeneous in regard to the expression of CD45RA and CDw127. Although CD45RA molecules were originally thought to be putative naive markers [31-33], CD45RA expression is fully regained after stimulation [35-36]. These observation might explain the difference in the expression of CDw127 between cord blood and adult peripheral CD4$^+$CD45RA$^+$ T cells. A difference in proliferative response to IL-4 between cord blood naive CD4$^+$ T cells and adult CD4$^+$CD45RA$^+$ T cells has also been reported [37].

It is now generally agreed that naive $CD4^+$ T cells bearing CD45RA essentially produce only IL-2 and that once they are primed through their TCR, they produce multiple cytokines [1, 32]. However, we demonstrated that IL-7-primed $CD4^+$ T cells, which were "naive" in the sense that they had not been stimulated through TCR and had originally produced mainly IL-2, also produced IFN-γ after 7 days upon stimulation with PMA and ionomycin. These findings indicated that IL-7 induced a typical Th1-type cytokine production profile in naive $CD4^+$ T cells without stimulation via TCR. Similar results have been obtained in cord blood naive $CD4^+$ T cells cultured with IL-4, and in peripheral blood $CD45RA^+$ T cells stimulated with a mixture of IL-2, TNF-α and IL-6 [11, 12].

Furthermore, after 14 days of culture with IL-7, $CD4^+$ T cells acquired the ability to produce Th2-type cytokines, although at low levels. The combination of IL-2 and costimulation through CD28 induces IL-4-producing ability in naive $CD4^+$ T cells [13]. Long term culture with IL-4 and either IL-12 or IL-2 also induced IL-4-producing ability in cord blood naive $CD4^+$ T cells [11]. However, the effects of these cytokines and CD28 were accompanied by conversion from CD45RA to CD45RO. In our studies, $CD4^+CD45RA^+$ cells cultured with IL-7 produced IL-4. These IL-7-primed $CD4^+$ T cells, like murine $CD4^+NK1.1^+$ T cells [38], produced various cytokines without requiring IL-4 or stimulation via TCR to induce IL-4 production.

These IL-7-primed $CD4^+$ T cells had both naive and memory surface phenotypes. They had increased levels of CD45RA and stable expression of CD62L, indicating the naive phenotype. However, they also had a higher mean fluorescent intensity of CD11a than that of freshly isolated $CD4^+$ T cells, also with the memory phenotype. This surface phenotype of $CD4^+$ T cells that proliferated in the absence of stimulation via TCR was discussed by Unutmaz *et al.* as a split phenotype of $CD4^+$ T cells [12]. In addition, we also found that IL-7 induced CD40 ligands, which play a critical role in delivering helper signals to B cells [39], on about 25 % of these proliferating cells.

Although fresh naive $CD4^+$ T cells neither proliferated nor maintained their viability upon stimulation with IL-2, IL-7 induced the surface expression of CD25 as described

[9, 24]. And these $CD4^+$ T cells proliferated in response to IL-2 without IL-7 or stimulation via TCR. Recently Fujihashi *et al.* reported that IL-7 by itself induces CD25 in murine intraepithelial $\gamma\delta$ TCR-positive lymphocytes and that combination of IL-2 and IL-7 induces synergistic proliferation in these cells [40]. These results, including ours, demonstrate that there is a TCR-independent proliferation and differentiation pathway in both $\alpha\beta$ and $\gamma\delta$ T cells that involves IL-7.

IL-7 mRNA is also expressed in human intestinal epithelial cells and skin keratinocytes [27, 28]. Human intestinal epithelial cells not only produce IL-7 but also express MHC class II molecules and can present food and microbial antigens as well as intestinal APC [27, 41-42]. Because the intestine and skin are continuously exposed to the environments, they are the sites where naive T cells may encounter foreign antigens and receive primary stimulation. The intestinal epithelial layer contains many intra-epithelial lymphocytes, including $\alpha\beta$ and $\gamma\delta$ receptor T cells and they constitute the mucosal immune network where both IL-7 and CDw127 are thought to play critical roles [27, 40, 43, 44]. Our results suggest that IL-7-primed naive intestinal intra-epithelial $CD4^+$ T lymphocytes produce mainly Th1-type cytokines at the primary stimulation and play a critical role in host defense. Further investigation of TCR independent differentiation pathways of naive $CD4^+$ T cells by cytokines may help to elucidate the precise mechanism of $CD4^+$ T cell differentiation and immune response.

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Footnotes

Abbreviations

CM: complete culture medium

RPE: R-phycoerythrin

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Table I

Cytokine production by freshly isolated CD4+ T cells and CD4+ T cells cultured with IL-7 [a]

Treatment	IL-2 (ng/ml)	IL-4 (pg/ml)	IL-5 (pg/ml)	IFN-γ (ng/ml)
Freshly isolated CD4+ T cells	80 ± 13 [b]	<16	<16	0.87 ± 0.13
IL-7 (30 ng/ml) for 7 days	79 ± 10	<16	<16	76 ± 12
IL-7 (30 ng/ml) for 14 days	92 ± 10	480 ± 130	85 ± 6	81 ± 6

[a] Freshly isolated CD4+ T or CD4+ T cells cultured with IL-7 for indicated days were stimulated with PMA (10 ng/ml) and ionomycin (1 μg/ml) at a density of 10^6/ml for 24 h. Cytokines in the supernatant were assayed by ELISA as described in Materials and Methods.

[b] Data were the mean ± SEM of 5 independent experiments.

Figure legends

Figure 1

Analysis of CD127w (IL-7R) expression on freshly isolated cord blood CD4+ T cells and adult CD4+CD45RA+ T cells. Both CD4+ T cells were stained as described Materials and Methods. Results are representative from several independent experiments. Vertical and horizontal axes represent logarithmic red or green fluorescent intensity and the percentage of cells in each region is indicated.

Figure 2

(a) Proliferation of cord blood naive CD4+ T cells cultured with IL-7.

Freshly isolated cord blood CD4+ T cells (10^6 /ml) were cultured with the indicated concentrations of IL-7. After a 7 day incubation, cells were washed and readjusted to a density of $1x10^6$ /ml and cultured with each concentration of fresh IL-7. Viable cell numbers were determined by Trypan blue dye exclusion. Data are the mean ± SEM of 3 independent experiments.

(b) Proliferation of adult CD4+CD45RA+ T cells cultured with IL-7.

Purified adult CD4+CD45RA+ T cells were cultured with IL-7 and viable cell numbers were determined as described above. Data are the mean of 3 independent experiments. SEM was below 30% of the number.

(c) Effect of IL-2 on the proliferation of cord blood CD4+ T cells.

Freshly isolated cord blood CD4+ T cells were cultured with the indicated concentrations of IL-2 and viable cells were counted as described above. Data are the mean ± SEM of 3 independent experiments. SEM of the cell number cultured with IL-2 or medium was always below 14 %.

(d) Effect of anti-IL-2 Ab on the proliferation of cord blood CD4+ T cells by IL-7.

Freshly isolated CD4+ T cells were also cultured with 30 ng/ml of IL-7 and rabbit polyclonal anti-human IL-2 Ab (10 μg/ml). Viable cells were counted as described above. Results are representative of 3 independent experiments. SEM was always below 5 %.

Figure 3

IL-7-primed cord blood naive $CD4^+$ T cells display a mixed phenotype of naive and memory T cells. Surface molecules of cord blood naive $CD4^+$ T cells were analyzed by flow cytometry before and after culture with IL-7 for 14 days. The surface expression of CD25 and CD40 ligand on $CD4^+$ T cells cultured with IL-7 for 7 days was similar to that shown in figure. Data are representative results from 3 or 4 experiments.

Figure 4

Flow cytometry of intracellular IL-4, IFN-γ and surface CD45RA of IL-7-primed cord blood $CD4^+$ T cells. Freshly isolated cord blood $CD4^+$ T cells were incubated with 30 ng/ml of IL-7 for 14 days. These proliferating cells were stimulated with PMA and ionomycin for 8 h, then with brefeldin A during the last 4 h of culture to inhibit cytokine secretion. Intracellular cytokines and surface molecules were stained as described in Materials and Methods. Vertical and horizontal axes represent logarithmic red or green fluorescent intensity, respectively. Cultured with medium (left lane) and stimulated with PMA and ionomycin (right lane). The percentage of cells in each region is shown. Data are representative of 4 independent experiments.

Figure 5

Induction of IL-2 responsiveness in cord blood naive $CD4^+$ T cells with IL-7. Naive $CD4^+$ T cells from cord blood were cultured for 14 days with 30 ng/ml of IL-7. These cells were washed and resuspended in CM containing the indicated concentrations of IL-2 or IL-7. After 7 days of culture with each concentrations of cytokine, viable cells were determined by Trypan blue dye exclusion. IL-2 (150 ng/ml) had a similar effect to 30 ng/ml of IL-2 on the proliferation of IL-7-primed $CD4^+$ T cells. Data are the mean $\pm$ SEM of 3 independent experiments.

Fig. 1.

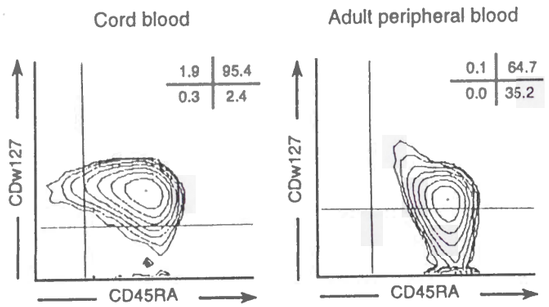

Cord blood
Adult peripheral blood
1.9
95.4
0.3
2.4
0.1
64.7
0.0
35.2
CDw127
CD45RA
CDw127
CD45RA

Fig. 2.

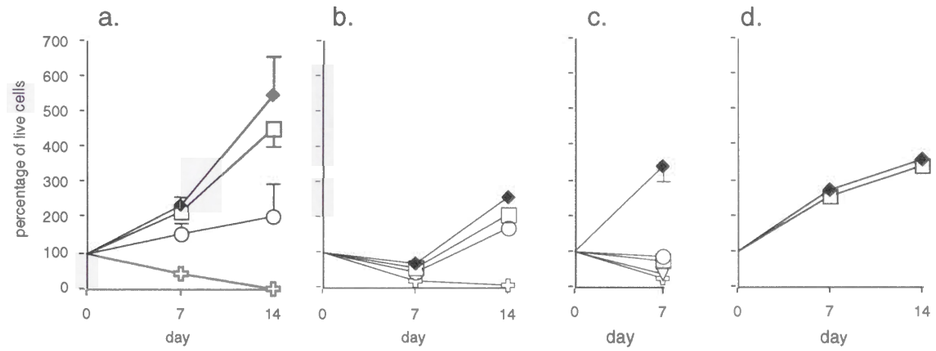

a.
b.
c.
d.
percentage of live cells
700
600
500
400
300
200
100
0
0
7
14
day
IL-7 30 ng/ml
IL-7 10 ng/ml
IL-7 3 ng/ml
Medium
IL-2 30 ng/ml
IL-2 6 ng/ml
IL-2 1.2 ng/ml
IL-7
IL-7 + anti-IL-2

Fig. 3.

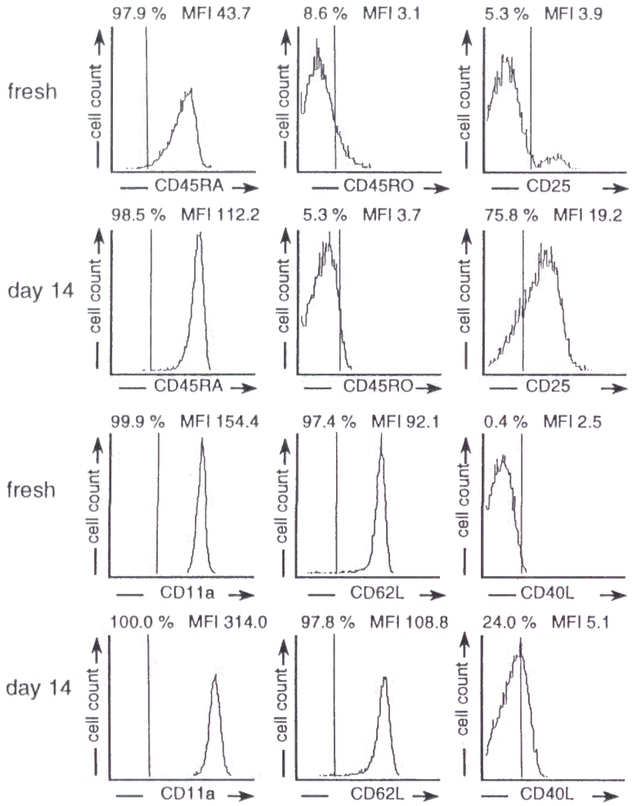

fresh
day 14
fresh
day 14
cell count
CD45RA
CD45RO
CD25
CD11a
CD62L
CD40L
97.9 % MFI 43.7
8.6 % MFI 3.1
5.3 % MFI 3.9
98.5 % MFI 112.2
5.3 % MFI 3.7
75.8 % MFI 19.2
99.9 % MFI 154.4
97.4 % MFI 92.1
0.4 % MFI 2.5
100.0 % MFI 314.0
97.8 % MFI 108.8
24.0 % MFI 5.1

Fig. 4.

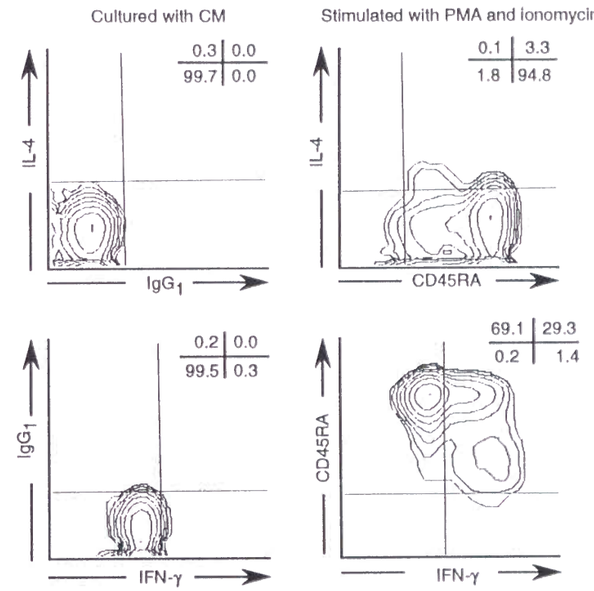
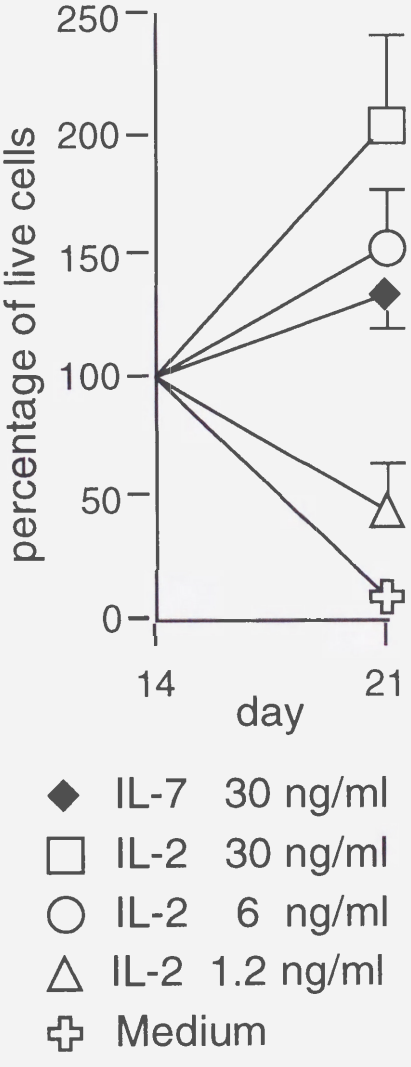

Cultured with CM
Stimulated with PMA and Ionomycin
IL-4
IgG1
CD45RA
IFN-γ
0.3 0.0
99.7 0.0
0.1 3.3
1.8 94.8
0.2 0.0
99.5 0.3
69.1 29.3
0.2 1.4

Fig. 5.


250
200
150
100
50
0
percentage of live cells
14
21
day
IL-7 30 ng/ml
IL-2 30 ng/ml
IL-2 6 ng/ml
IL-2 1.2 ng/ml
Medium