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Immunological Functions of Human Cytotoxic T Cells at the Different Stages of Development

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Cytotoxic T lymphocytes (CTLs) play an important role in immune responses, and provide potent defenses against virus infection and intracellular pathogens. However, as compared with helper T lymphocytes, CTL development remains to be clarified. In this study we tried to find out the differences in maturity of human thymus, cord blood (CB), and adult peripheral blood (APB)-derived $CD8^+$ T cells, and examined whether these T cells are vulnerable or not to anergy induction. Using toxic shock syndrome toxin-1 (TSST-1), $CD8^+$ T cell blasts with different origins were prepared and re-stimulated to estimate their immunological responses. The results showed that the proliferative response and TNF-α production upon re-stimulation with TSST-1 in thymic and CB $CD8^+$ T cell blasts were much lower than those of APB, although the capacity for cytotoxic activity was comparable at all three different stages of T cell development. Therefore, thymic and CB $CD8^+$ T cells obviously had immature traits, but appeared to have the capacity to eliminate pathogenic factors in terms of cytotoxic activity.

Key words: CTL, CD8, T cell maturation, TSST-1

Introduction

T cell maturation is well-recognized as proceeding via distinct stages discriminated by CD4/CD8 surface phenotypes in the thymus[1)2)]. In terms of T cell function, they may maturate further post-thymically. In a previous report, we demonstrated that blasts obtained from cord blood (CB) $CD4^+$ T cells, which are immediate emigrants from the thymus, produced IL-2 and IL-4 marginally by re-stimulation with a superantigen, toxic shock syndrome toxin-1 (TSST-1) whereas adult peripheral blood (APB) T cell blasts showed high responses, indicating that CB $CD4^+$ T cells are rendered anergic[3)]. Consistently, human thymic $CD1a^-CD4$ single positive T cells, which are in the last stage of thymic maturation[4)], were even more susceptible to the anergy induction in proliferation and cytokine production in the same experimental system[5)]. These lines of evidence suggest that $CD4^+$ T cells undergo maturation as they reside in the peripheral lymphoid tissues. The molecular basis of anergy induction in human thymic CD4 single positive T cells was clarified. Fujimaki et al demonstrated that TSST-1-induced human thymic and peripheral $CD4^+$ T cell blasts are strongly phosphorylated to the same degree in Lck at the negative regulatory site of Tyr505, and subsequently dephosphorylated by re-stimulation with TSST-1 in peripheral $CD4^+$ T cell blasts but not in thymic $CD4^+$ T cell blasts[6)].

Susceptibility to the anergy induction is quite different between $CD4^+$ and $CD8^+$ T cells in mice. Kawabe et al[7)] reported that splenic $CD8^+$ T cells from the primed mice with a superantigen, staphylococcal enterotoxin B (SEB), proliferated vigorously upon *in vitro* re-stimulation. However, $CD4^+$ T cells from the same mice revealed lack of proliferative responses upon *in vitro* re-stimulation, indicating selective anergy in $CD4^+$ T cells. In contrast, we showed previously that *in vitro* SEE-induced splenic $CD4^+$ T cell blasts showed high responses in the

proliferation and lymphokine productions upon re-stimulation with SEE, whereas $CD8^+$ T cell blasts reacted poorly in these two responses[8]. Thus, the above-mentioned anergic properties of human thymic and CB $CD4^+$ T cells may not necessarily be applicable to corresponding $CD8^+$ T cells. Furthermore, in human CB T cells, Risdon et al found that they respond strongly against an alloantigen in mixed leukocyte cultures, but revealed unresponsiveness upon re-stimulation with the alloantigen without assessing their cytotoxic activities[9]. Therefore, to elucidate the immunological functions of human CB and thymic $CD8^+$ T cells, we examined their anergy induction and cytotoxic activity in the present study. Based upon the results obtained, we discuss the post-thymic maturation of human $CD8^+$ T cells.

Materials and Methods

Reagents

TSST-1 was purchased form Toxin technology Inc. (Sarasota, FL). The RPMI 1640 culture medium used contained 100 μg/ml of streptomycin and 100 U/ml of penicillin, 10% fetal calf serum, and 5×10^{-5}M 2-ME. Recombinant IL-2 (rIL-2) was kindly provided by Shionogi & Co (Osaka, Japan).

Monoclonal Abs

FITC-conjugated anti-CD38 (T16), FITC-conjugated anti-CD45RA (2H4LHDH11LDB9), FITC-conjugated anti-Vβ2 (MPB2D5), FITC-conjugated anti-CD4 (SFCI12T4D11), FITC-conjugated anti-CD25 (HT44H3), PE-conjugated anti-HLA-DR (immu 357), PE-conjugated anti-CD69 (TP1.55.3), PE-conjugated anti-CD56 (N901), PC5-conjugated anti-CD3 (UCHT1), PC5-conjugated anti-CD8 (B9.11), anti-CD4 (NU-TH/I), anti-glycophorin A (11E4B7.6), anti-CD56 (N901), and anti-γδ T cell receptor (TCR) (immu 510) were purchased from Immunotech-Beckman-Coulter (Villepinte, France). PE-conjugated anti-CD45RO (UCHL-1) and PE-conjugated anti-CD8 (SK1) were purchased from BD Biosciences (San Jose, CA).

Preparation of thymic, CB, and APB $CD8^+$ T cells

This study was approved by the institutional review board of Tokyo Women's Medical University as for obtaining human peripheral blood, CB and thymus fragments. The informed consent to examine CB samples was obtained from mothers before uncomplicated births. CB was obtained from neonates with a gestational age of 28-37 weeks (CB preterm) or >37 weeks (CB term). Informed consent to examine thymic fragments also was obtained from patients who had undergone corrective cardiac surgery. APB was obtained from healthy adult donors. Mononuclear cells from CB and APB were isolated by Ficoll-Conray density gradient centrifugation, and whole T cells were purified using the sheep red blood cell rosette formation method. To enrich the suspension for mature thymic cells, agglutination with peanut agglutinin was used. Non-aggregated thymic cells were isolated. Next, whole T cells from CB, APB, and thymus were incubated with a mixture of anti-CD4, anti-γδ, and anti-CD56 for 30 min on ice to deplete $CD4^+$ T cells, γδ T cells, and NK cells, then washed, added to a 5-fold number of anti-mouse IgG-coupled magnetic beads (Dynal, Biotech, Oslo, Norway), and incubated on ice for another 30 min, whereafter unbound cells ($CD8^+$ T) were collected. The purity of $CD8^+$ T cell preparations was 62.1% (APB), 39.7% (CB), and 21.2% (thymus) respectively, and that of $CD4^+$ T cells was ≤ 6.2%.

Preparation of TSST-1-induced $CD8^+$ T cell blasts

Thymic, CB and APB $CD8^+$ T cells (2×10^6 cells/well) were stimulated with 10 ng/ml of TSST-1 and DR^+L cells (8124) (4×10^5 cell/well) using a 24 well-plate with 1 ml culture medium per well for 3 days. DR^+L cells were treated with 50 μg/ml of mitomycin C prior to use. The activated cells were subjected to Percoll (density=1.068) centrifugation, and the large lymphoblasts obtained at the interface of the culture medium and Percoll were expanded by incubation with 100 U/ml of rIL-2 for 2 days in 2 cycles.

Assay for cell-mediated cytotoxic activity

Thymic, CB, and APB $CD8^+$ T cell blasts were tested for cytotoxic activity against ^{51}Cr-labeled Daudi cells as target cells when re-stimulated with 10 ng/ml of TSST-1. First, Daudi cells (1×10^6) were incubated with 0.1 μCi of ^{51}CrNa for 1 hour at 37℃,

and then washed 3 times with complete RPMI 1640 medium. Next, $CD8^+$ Tell blasts (effectors) were mixed with Daudi cells (1×10^4) at various ratios from 0.625 : 1 to 20 : 1 in 200 μl per well in triplicate using round bottom 96-well plates. After 4 hours incubation at 37℃, 100 μl culture supernatant from each well was transferred into filter tubes to determine ^{51}Cr release with an ARC 370 γ-counter (Aloka, Tokyo, Japan). The percentage of total ^{51}Cr release was calculated as follows:

$$\frac{(\text{Sample release cpm} - \text{spontaneous release cpm})}{(\text{Maximal cpm} - \text{spontaneous release cpm})} \times 100$$

Sample release cpm indicated the ^{51}Cr release in the presence of $CD8^+$ effector T cells, Daudi target cells, and TSST-1, while spontaneous release indicated the ^{51}Cr release from Daudi target cells and TSST-1. Maximal cpm referred to the total amount of ^{51}Cr incorporated into the target cells, which was obtained by pipetting the wells. Data of specific killing was calculated by subtracting the percentage of total ^{51}Cr release of samples (with TSST-1) with that of controls (without TSST-1). Data are presented as the mean ± SD of average values of triplicate cultures in separate experiments.

Assay for proliferative responses

To determine the proliferative response of $CD8^+$ T cell blasts to TSST-1, these cells (5×10^4 cells/well) were re-stimulated with various doses of TSST-1 (range from 0.0001 to 10 ng/ml) plus mitomycin C-treated DR^+L cells (5×10^3 cells/well) in 0.2 ml volumes in triplicate in a round bottom 96-well plate for 2 days. Cultures were pulsed with 0.5 μCi (18.5 kBq) of [^{3}H]-thymidine for the last 16 hours of the culture period. Each specific proliferation was calculated by subtracting the value of $CD8^+$ T cells, DR^+ L cells plus TSST-1 in each culture with the background value of $CD8^+$ T cells alone. Data were expressed as the mean cpm ± SD of triplicate cultures.

Assay for cytokine production

To assess possible cytokine secretion, $CD8^+$ T cell blasts (2×10^5 cells/well) were re-stimulated by TSST-1 (10 ng/ml) in the presence of mitomycin C-treated DR^+L cells (1×10^4 cells/well) in 0.2 ml in duplicate to quadruplicate (depending on the cell number we obtained) in a round-bottom 96-well plate for 1 day at 37℃. Equal volumes of culture supernatant from these wells were then taken and added together. TNF-α and IFN-γ production in these supernatants was quantitated with a sandwich ELISA using Ab pairs purchased from BD Biosciences (San Diego, CA), avidin-conjugated peroxidase (Sigma, St Louis, MO), and substrate solution TMB (Dako, Glostrup, Denmark). Standard curves were obtained by using recombinant human TNF-α or IFN-γ (BD Biosciences). The data were calculated by subtracting control values without TSST-1 from the culture values in the presence of TSST-1.

Flow cytometry analysis

The purity of the $CD8^+$ T cells was checked by staining T cells with PC5-anti-CD3, FITC-anti-CD4, and PE-anti-CD8. Regarding the expression level of other surface markers, $CD8^+$ T cells were stained with PC5-anti-CD8 plus combinations of the appropriate PE- or FITC-conjugated Abs such as CD25 versus CD69, CD38 versus HLA-DR, CD45RA versus CD45RO, and Vβ2 versus CD45RO, and then analyzed with 3-color flow cytometry using the EPICS XL flow cytometer (Beckman Coulter, Fullerton, CA). The entire procedures for cells staining were conducted on ice.

Statistics

Data were analyzed with the Student's t-test. $P<0.05$ was taken as significant.

Results

Preparation and purity of $CD8^+$ T cell blasts as responders

First of all, T cells were purified from three different tissues such as thymus, CB from neonates with a normal delivery, and APB by the rosette formation method using sheep red blood cells (Fig. 1). Thymus, CB, and APB had different percentages in whole $CD3^+$ T cells (72.4, 18.8, and 79.6% respectively) as well as in $CD8^+$ T cells (39.8, 7.3, and 27.5% respectively). After removal of $CD4^+$ T cells, NK cells, and γδ T cells (the second step), the enriched-$CD8^+$ T cells were stimulated for 3 days with the bacterial superantigen TSST-1 in combination with L cells transfected with HLA-DR. Through their expansion and resting in the presence of human rIL-2, and further purification with Percoll-gradient cen-

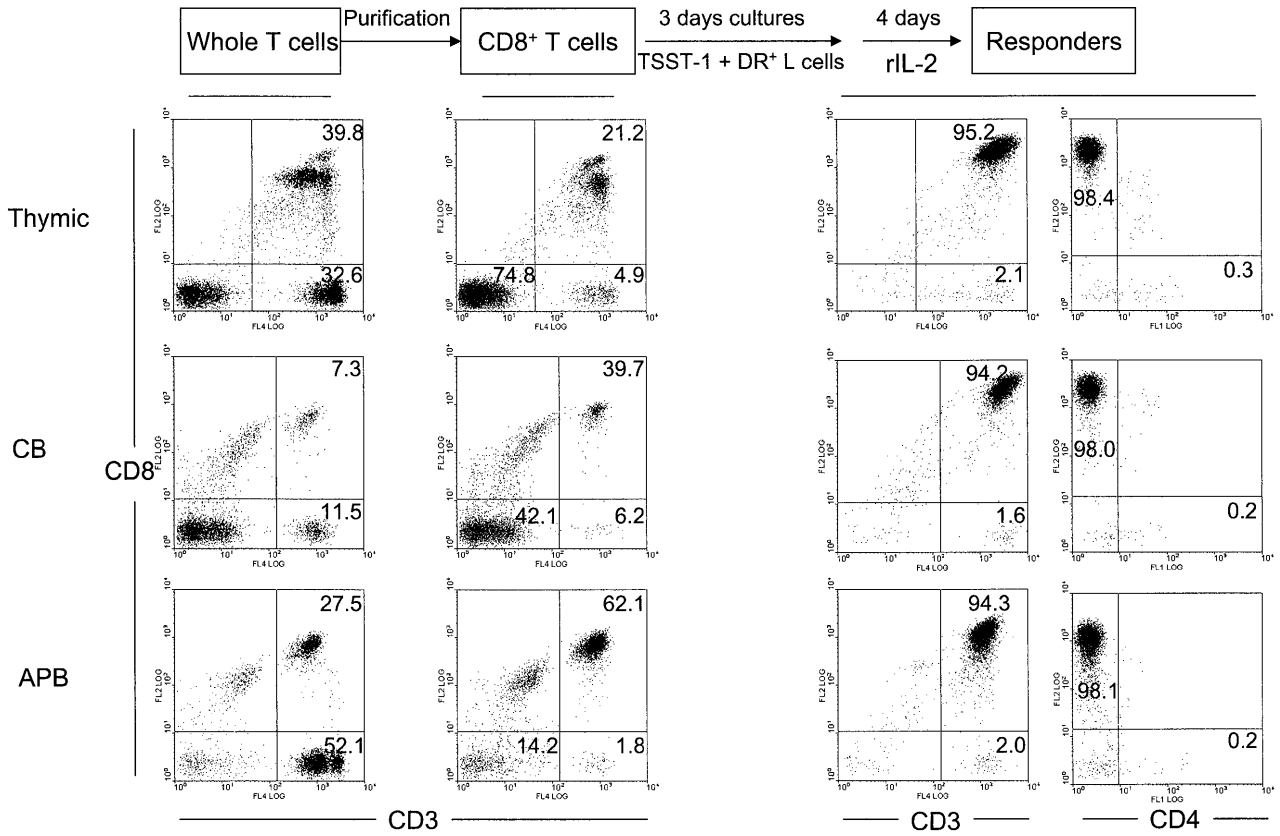

Fig. 1 Preparation and purity of CD8+ T cell blasts as responders
At the first step whole T cells were prepared from human thymus, CB and APB by the sheep RBC rosette method. At the second step CD8+ T cells were negatively purified by adding an Abs cocktail to remove CD4+ T cells, NK cells, and γδ T cells. Next, the purified CD8+ T cells were stimulated with 10 ng/ml TSST-1 presented on DR+ L cells for 3 days, and expanded, rested in rIL-2 for a total of 4 days. These CD8+ T cell blasts (responders) were finally stained with Abs as indicated, and the purity of each preparation is shown. Numbers indicate the percentage of positive cells in each quadrant.

trifugation, activated effector CD8+ T cell blasts were obtained to see if these T cell blasts had any differences in maturity and function. The purity of the final CD8+ T cell blast preparations (responder) was very clearly comparable: thymic, 95.2%; CB, 94.2%; and APB, 94.3% respectively. Consistently, among the CD3+ T cell-gate, CD8+CD4- cells were 98.4, 98.0, and 98.1% in the same order, respectively.

Immunologic phenotypes of TSST-1-induced CD8+ T cell blasts derived from thymus, CB preterm, CB term, and APB

Next we tried to determine in detail the surface phenotypes of responder CD8+ T cell blasts of four different origins (Table). CB preterm was also included to explore more immature cells. TSST-1 is known to activate a particular TCR beta chain repertoire Vβ2. The percentages of TCR Vβ2 markedly increased from 5-6% in unstimulated cells (data not shown) to 47.6% in thymic, 60.2% in CB from a preterm delivery (premature birth), 52.0% in CB from a normal delivery, and 82.4% in APB CD8+ T cells, indicating that a sufficient number of TSST-1-reactive cells was included in all the preparations, although there was a relatively higher percentage of APB CD8+Vβ2+ T cells than other cell types. No consistent difference was detected between preterm and normal delivery CB. As to the relatively late activation marker, CD25 and the activation/diffferentiation marker, CD38 expression was high in all the samples (95-99%), while the early activa-

Table Immunologic phenotype of Thymic, CB preterm, CB term and APB $CD8^+$ T responders[a]

Surface molecules	Cell type of responders			
	Thymic (n = 5)	CB preterm (n = 5)	CB term (n = 6)	APB (n = 6)
Vβ2	47.6 ± 14.1 **	60.2 ± 18.7 *	52.0 ± 20.0 *	82.4 ± 4.3
CD25	96.2 ± 1.3	97.9 ± 1.3	97.6 ± 4.0	97.0 ± 1.3
CD69	13.6 ± 5.1 ***	3.2 ± 1.9	5.6 ± 3.0	4.9 ± 2.6
CD38	95.8 ± 3.6	98.1 ± 4	99.0 ± 1.2	94.7 ± 5.1
HLADR	21.0 ± 13.2	3.3 ± 1.0 **	8.0 ± 5.0 **	24.2 ± 9.8
$CD45RA^+$ RO^-	1.6 ± 0.9	1.1 ± 1.3	2.2 ± 1.7	1.3 ± 1.5
$CD45RO^+$ RA^-	71.8 ± 25.3	48.9 ± 39.7	65.9 ± 27.5	56.8 ± 19.5

[a] T cells (2×10^6 cells/well) were stimulated with TSST-1 (10 ng/ml) presented on DR^+ L cells (4×10^5 cells/well) for 3 days in 24-well plates and expanded with rIL-2 (100 U/ml) for 2 days in 2 cycles. The blast cells were stained with Abs conjugated with fluorescence (PC5, PE or FITC) and then analyzed by flow cytometry. The data show the mean percentage of positive cells in $CD8^+$ T cells ± SD of all experiments, * $p < 0.05$, ** $p < 0.01$, *** $p < 000.1$ as compare with APB.

tion marker, CD69 was low (3-14%). Another T cell activation marker, HLA-DR was 21-24% in thymus and APB, but 3-8% in CB, which may suggest a lower induction of HLA-DR in CB $CD8^+$ T cells. The T cell memory/activation marker, CD45RO expression ranged from 49 to 72%. Consequently, various surface markers showed similar levels of their expression on these activated T cell blasts.

Comparison of immunologic functions between CB and APB $CD8^+$ T cell blasts

To confirm an immature property of a particular T cell stage, we have shown that a secondary response of activated T cell blasts was very useful[3)5)]. Thus, in figure 2A, a cell-mediated cytotoxicity assay of $CD8^+$ T cell blasts was conducted by re-stimulation, and compared between CB from a normal delivery and APB using Daudi cells, a human B cell line as a target, but showed no significant difference at various effector vs. target ratios from 0.625 : 1 to 20 : 1. In contrast, CB $CD8^+$ T cell blasts failed to proliferate in the secondary response to TSST-1 presented on DR^+L cells, while APB $CD8^+$ T cell blasts proliferated in a dose-dependent manner (Fig. 2B). Cytokine production such as TNF-α and IFN-γ was also assessed and compared between these two $CD8^+$ T cell blast populations (Fig. 2C). In two independent experiments, TNF-α was robustly produced by APB T cells, while CB T cells were secreted only marginally. On the other hand, the amount of IFN-γ production by CB was smaller in experiment 1, but the same as that of APB in experiment 2. Collectively these data revealed selective immaturity in CB $CD8^+$ T cells, such as proliferation and TNF-α production.

Cytotoxic activity of preterm CB $CD8^+$ T cells

Furthermore, specific killing activity by preterm delivery CB-derived $CD8^+$ T cell blasts was also estimated (Fig. 3). As a result, similar to normal CB, preterm CB T cells also killed target cells in the same degree as did APB $CD8^+$ blast cells, showing no difference in cytotoxicity. However, we were unable to attempt to estimate cytokine production capacity of preterm CB because of the limited cell number obtained.

Comparison of immunologic functions between thymic and APB $CD8^+$ T cell blasts

Finally we compared thymic $CD8^+$ T cell blasts with the corresponding APB cells in terms of killing activity and proliferation (Fig. 4). Thymic $CD8^+$ T cell blasts showed no difference and efficiently killed target cells as APB cells did in all the ratios between effector and target. On the contrary, thymic $CD8^+$ T cells did not proliferate at all in response to TSST-1, which indicated an immature trait with selected unresponsiveness.

Discussion

In the present study, human cord and thymic $CD8^+$ T cell blasts responded marginally in the proliferation and weakly in the production of cytokines upon re-stimulation with TSST-1 *in vitro*, as com-

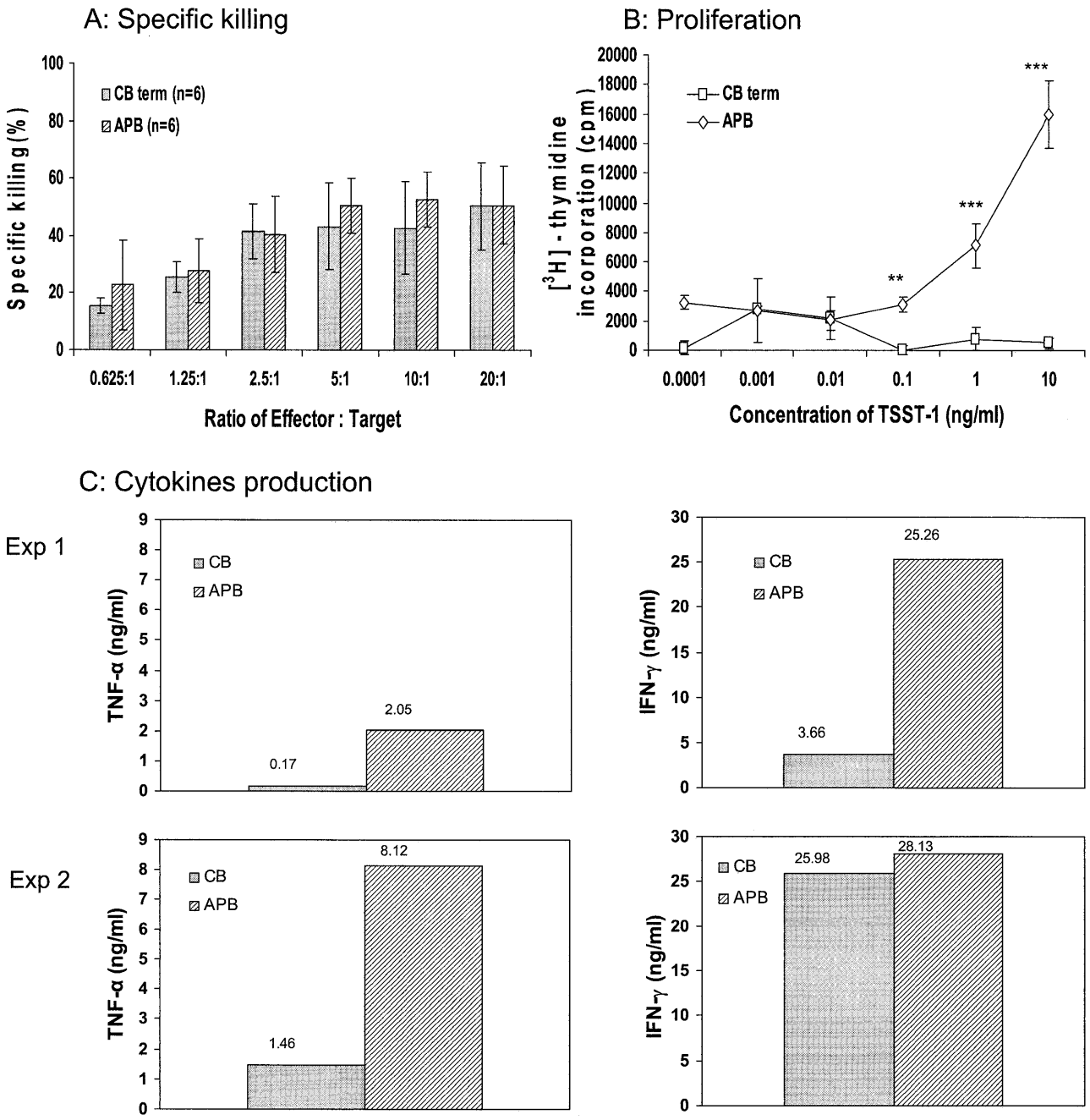

Fig. 2 Comparison of immunologic functions between CB and APB $CD8^+$ T cell blasts

A: Cytotoxic activity was measured in a standard 4 hours ^{51}Cr release assay at various effectors to targets (E : T) ratios. $CD8^+$ T cell blasts were re-stimulated with TSST-1 (10 ng/ml) in the presence of ^{51}Cr-labeled Daudi cells (1×10^4 cells/well) in triplicate in flat bottom 96-well plates. The data of specific killing activity are presented as the mean ± SD for 6 independent experiments. $p > 0.05$.

B: Proliferative responses of CB and APB $CD8^+$ T blast cells. The cells (5×10^4 cells/well) were re-stimulated with various doses of TSST-1 in the presence of DR^+ L cells (5×10^3 cells/well), that had been treated with 50 μg/ml of mitomycin C for 30 min at 37℃, for 2 days in a round bottom 96 well-plate and pulsed with 0.5 μCi (18.5 kBq) of $[^3H]$-thymidine for the last 16 h of culture period. The data are representative of 2 independent experiments. ** $p < 0.01$, *** $p < 0.001$.

C: TNF-α and IFN-γ production in secondary stimulation. CB and APB $CD8^+$ T cell blasts (2×10^5 cells/well) were re-stimulated with TSST-1 (10 ng/ml) in the presence of mitomycin C-treated DR^+ L cells (1×10^4 cells/well) for 24 h at 37℃. Subsequently, the culture supernatants were measured for cytokine production with a sandwich ELISA.

pared with APB CD8$^+$ T cell blasts, albeit the equivalent IFN-γ production in one experiment between cord and APB cells. Thus, human cord and thymic CD8$^+$ T cells appear to be immunologically immature, and undergo post-thymic maturation as we have previously indicated in the corresponding CD4$^+$ T cells[3)5)]. The mechanism of post-thymic maturation has not been elucidated yet. Environmental factors such as hormones may influence the T cell maturation, or T cells may inherently undergo maturation. In contrast, in the cytotoxic activity upon re-stimulation with TSST-1, human cord, thymic and APB CD8$^+$ T cell blasts revealed comparable levels. Therefore, cord and thymic CD8$^+$ T cells themselves may be functionally intact in mediating cell-mediated immunity. Furthermore, preterm CB CD8$^+$ T cells exhibited an equal cytotoxic activity to corresponding term cells, indicating that fetal CD8$^+$ T cells possess competence as immune cells against infectious agents. Clinical data have shown that in CB stem cell transplantation, donor T cells exhibit a relative low incidence of graft-versus-host disease[10)11)]. Supporting this, Risdon G. et al. reported that human CB T cells were rendered unresponsive in proliferation by stimulation with an alloantigen[9)]. However, our present data suggest that the cytotoxic potential of CB CD8$^+$ T cells in CB stem cell transplantation should exist in spite of the low incidence of graft-versus-host disease.

Questions arise as to the molecular mechanism underlying the clear difference between proliferative and cytotoxic responses in cord and thymic

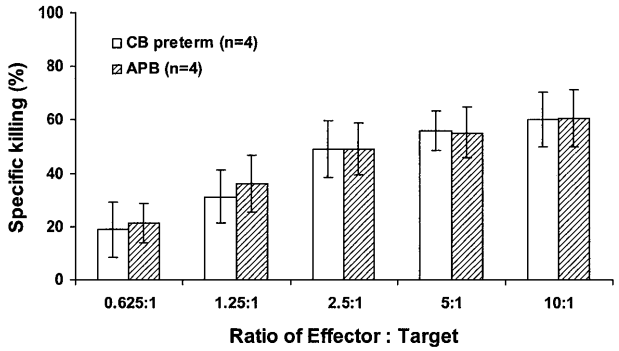

Fig. 3 Cytotoxic activity of preterm CB CD8$^+$ T cells
In a similar method to Fig. 2A, CD8$^+$ T cell blasts were re-stimulated with TSST-1 (10 ng/ml) in the presence of ^{51}Cr-labeled Daudi cells (1×10^4 cells/well) in triplicate in a flat bottom 96-well plate. Specific killing activity was compared between CB from neonates with premature birth (preterm) and APB CD8$^+$ T cell blasts. The data show the mean ± SD of all experiments (n = 4). $p > 0.05$.

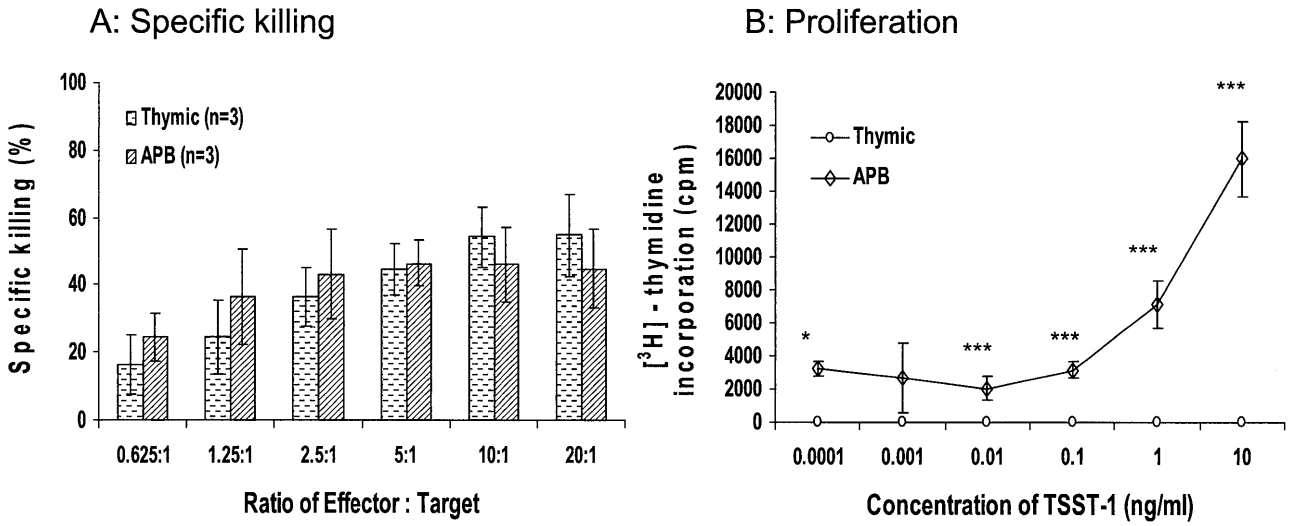

Fig. 4 Comparison of immunologic functions between thymic and APB CD8$^+$ T cell blasts
A: Specific killing activity of CD8$^+$ T cell blasts was measured as in Fig. 2A. The data show the mean ± SD from all the experiments done (n = 3). $p > 0.05$.
B: Proliferative responses of thymic and APB CD8$^+$ T cell blasts were examined as conducted in Fig. 2B. The result is a representative of 2 independent experiments with a similar tendency. * $p < 0.05$, ** $p < 0.01$, *** $p < 000.1$.

$CD8^+$ T cell blasts. Cell cycle progression is well-recognized as being regulated by the balance between cyclin-dependent kinases (Cdks) and Cdk inhibitors[12)13)]. A recent report indicated that TCR signaling activates the Cdk/E2F pathway, leading to cell cycle progression and apoptosis, the latter of which is blocked by NFκB activation induced by the co-stimulatory signal via CD28 engagement, to allow for proliferation[14)]. Our previous report showed that a key molecule for TCR signaling, Lck, was defective in anergic human thymic $CD4^+$ T cells in the dephosphorylation upon re-stimulation. In the present study, human cord and thymic $CD8^+$ T cell blasts responded weakly in proliferation and cytokine production as shown in the corresponding $CD4^+$ T cell blasts, as was the case in the same *in vitro* TSST-1 re-stimulation in our previous report[3)5)]. The flow cytometric analysis showed that the expressions of perforin and granzyme B in cord and thymic $CD8^+$ T cell blasts were similar to those in APB $CD8^+$ T cells blasts (data not shown). Collectively, it is most likely that such a defect in TCR signaling would also be present in human cord and thymic $CD8^+$ T cell blasts, but would not affect the cytotoxic activity induced by the effector molecules such as perforin and granzyme[15)]. Since the signaling pathway for the release of the above molecules in cytotoxicity has not been fully understood, it should be important and intriguing to examine this possibility in future studies. On the other hand, Fas-FasL interaction[16)] may be functioning mainly in our experiments and remain intact to induce cytotoxicity in human cord and thymic $CD8^+$ T cell blasts. This alternative possibility also should be examined.

We have previously shown in *in vitro* experiments that SEE-induced $CD8^+$ T cell blasts revealed weak responsiveness in proliferation and cytokine production upon re-stimulation with SEE, whereas the corresponding $CD4^+$ T cell blasts reacted strongly in these responses[8)]. However, in the present study, human $CD8^+$ T cells were not rendered anergic by *in vitro* TSST-1 stimulation. The difference in the immunological functions in species has been noticed. For instance, the expression of a co-stimulatory molecule, ICOS, on T cells is up-regulated by IL-4 produced by T cells in mice[17)], whereas ICOS expression is induced by IL-12 and IL-23, but not IL-4, in human T cells[18)]. Thus, the apparent different immune regulation in mouse and human T cells is striking and urges caution when extrapolating mouse data to human responses.

Since the neonatal T cells show weakness in the responses in the production of cytokines following various stimuli *in vitro*, compared with APB T cells[19)~23)], the immaturity of T cells as shown in the present and our previous studies may continue until some period after birth. In support of this concept, adult TSS is a fatal disease caused by TSST-1, whereas neonatal TSS-like exanthematous disease (NTED), which is also induced by TSST-1, does not cause shock and is associated with milder symptoms than adult TSS[24)25)]. Thus, our results obtained in the present study and further elucidation of the mechanism underlying the immaturity of $CD8^+$ T cells would provide insight into, and an understanding of the defense against infectious agents in newborn infants.

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ヒト細胞傷害性T細胞の異なる発達段階での免疫機能

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CD8陽性の細胞傷害性T細胞（CTL）は，ウイルス感染細胞や腫瘍細胞の排除などの免疫応答において重要な役割を演じる．しかしその機能的な成熟に関しては，不明である．我々は，以前in vitro培養系において，細菌性スーパー抗原，毒素性ショック症候群毒素-1（TSST-1）の一次刺激で得られた成人末梢血由来CD4陽性T細胞芽球が，TSST-1の再刺激に対し強い増殖とサイトカイン産生を示すのに対し，臍帯血および胸腺由来のCD4陽性T細胞芽球は，両反応において無反応性（アナジー）を示し機能的に未熟であることを見出した．本研究は，同様の実験系を用いてヒトCD8陽性T細胞の機能的成熟について明らかにすることを目的とした．

TSST-1刺激で得られた成人末梢血CD8陽性T細胞芽球は，TSST-1再刺激に対する増殖およびサイトカイン（TNF-α，IFN-γ）産生，TSST-1特異的なヒトB細胞（Daudi細胞）傷害において強い反応を示した．一方，臍帯血および胸腺CD8陽性T細胞芽球は，増殖でアナジーを認め，臍帯血CD8陽性T細胞芽球は，低サイトカイン産生を示した．細胞傷害活性は両者とも，成人末梢血CD8陽性T細胞芽球と同レベルの誘導を認めた．また，早産の臍帯血CD8陽性T細胞芽球も成人末梢血CD8陽性T細胞芽球と同レベルの細胞傷害活性を認めた．

以上の結果から，ヒト臍帯血および胸腺CD8陽性T細胞は，CD4陽性T細胞と同様な機能的未熟性を有することが明らかになった．しかし，異物の排除に係る細胞傷害活性は，成人末梢血CD8陽性T細胞と同レベルに誘導されることを見出した．一般に，末梢リンパ組織においてT細胞が機能的に成熟するためには出生後ある程度の時間を要することが示唆されている．したがって，本研究の結果は，新生児CD8陽性T細胞の免疫応答を理解するための有用な情報になると考えられる．