Selective support of a mouse thymus epithelial cell line (MTEC1) to the viability and proliferation of CD4$^+$ CD8$^-$, CD4$^-$ CD8$^-$, and CD4$^+$CD8$^+$ thymocytes in vitro

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ABSTRACT

The MTEC1 cell line, established in our laboratory, is a normal epithelial cell line derived from thymus medulla of Balb/c mice and these cells constitutively produce multiple cytokines. The selection of thymic microenvironment on developing T cells was investigated in an *in vitro* system. Unseparated fresh thymocytes from Balb/c mice were cocultured with MTEC1 cells or/and MTEC1- SN,then,the viability, proliferation and phenotypes of cultured thymocytes were assessed. Without any exogenous stimulus, both MTEC1 cells and MTEC1−SN were able to maintain the viability of thymocytes, while only the MTEC1 cells, not the MTEC1−SN, could directly activate thymocytes to exhibit moderate proliferation, indicating that the proliferative signal is delivered through cell surface interactions of MTEC1 cells and thymocytes. Phenotype analysis on FACS of viable thymocytes after coculture revealed that MTEC1 cells preferentially activate the subsets of CD4$^+$ CD8$^-$, CD4$^+$ CD8$^+$ and CD4$^-$ CD8$^-$ thymocytes; whereas MTEC1 - SN preferentially maintained the viability of CD4$^+$ CD8$^-$ and CD4$^-$ CD8$^+$ thymocyte subsets.

For the Con A-activated thymocytes, both MTEC1 cells and MTEC1-SN provided accessory signal (s) to significantly increase the number of viable cells and to markedly enhance the proliferation of thymocytes with virtually equal potency, phenotyped as CD4$^+$CD8$^-$, CD4$^-$ CD8$^+$, and CD4$^-$ CD8$^-$ subsets. In summary, MTEC1 cells displayed

Abbreviations used: MHC-major histocomptibility complex; N (M)TEC-(mouse) thymus epithelial cells; TSC −thymus stromal cells ; FACS - Fluorescence Activated Cell Sorter (flow cytometry) ; SN - supernatant.

selective support to the different thymocyte subsets, and the selectivity is dependent on the status of thymocytes.

Key words: *thymic selection, thymic epithelial cells, thymocyte subsets, cytokines, flom cytometry.*

INTRODUCTION

The advent of T cell receptor(TCR) encoding genes has provided a powerful tool to analyze the intrathymic T cell development. *In vivo* experiments have shown that the process of TCR gene rearrangement [1−3] and selections of developing T cells take place within thymus [4−6]. Thymic selection is mediated through the interactions of TCR$^+$ thymocytes with MHC (or MHC+self peptide) bearing thymic stromal cells (TSC); positive selection leads to maturation of the developing T cells, whereas negative selection results in the clonal deletion or clonal anergy[4−6]. The TCR repertoire is thus shaped as a consequence of thymic selection. The enigma, however, that the opposite selections are apparently mediated through the same interaction between TCR and MHC (or MHC + self peptide) remains unresolved. As the thymic microenvironment is extremely complex which contains many types of TEC and bone marrow (BM) derived TSC (macrophages, dendritic cells and fibroblasts)[7], it is hardly possible to define the types of TEC or TSC which induce thymic selections, and hardly to characterize the molecular basis underlying the selection events in the intact thymus. In this context, an in vitro system has been developed to analyze the outcomes of thymic selections with defined types of TSC and pro T cells as well as CD4$^-$ CD8$^-$ thymocytes. The preliminary results are promising[8−10].

To pursue and dissect thymic selections *in vitro*, we have established several lines of mouse TSC and characterized the cytokines they produced[11−13]. We presented here the interactions of MTEC1, a mouse nontransformed thymic epithelial cell line established from Balb/c strain, with fresh thymocytes from the same strain of mice in cocultures. The MTEC1 cells preferentially prolong the viability and support the proliferation of CD4$^+$CD8$^-$, CD4$^-$ CD8$^-$ and CD4$^+$CD8$^+$ thymocytes when cocultured *in vitro*. For the Con A activated thymocytes, both MTEC1 cells and MTEC1−SN provide accessory signals to enhance the activation of CD4$^+$CD8$^-$, CD4$^-$CD8$^-$ and CD4$^-$CD8$^+$ thymocytes.

MATERIALS AND METHODS

Animals

Balb/c mice, ♂ or ♀, 4−8 wk old, obtained from the Animal Research Center, Beijing Medical University, were used for the preperation of thymcytes.

Reagents

The following reagents were used in the experiments: concanavalin A (Con A, Pharmacia), mitomycin C (Sigma); 60% hypaque (Xin- Yi Laboratories, Shanghai, China), and 40% Ficoll (Shanghai Second Reagent Manu-

factory) to prepare Ficoll hypaque at density of 1. 090 g/ml; propidium iodide (PI, Sigma).

Radioisotope

[3]H TdR (23 Ci/mmol) was purchased from the Beijing Atomic Energy Research Institute.

Fluorescence-conjugated antibodies

Fluorescein isothiocyanate (FITC)-conjugated anti-mouse Ly 2 and phycoerythrin (PE)-conjugated anti-mouse L3T4 McAbs (Becton Dickinson, 250 μg/ml) were used at final dilution of 1 : 60 and 1 : 80 of stock solution respectively.

Media

Mouse tonicity, N 2 Hydroxyethylpiperazine N' 2 ethanesulfonic Acid (HEPES) buffered salt solution added with newborn calf serum to 5% (NCS BSS) was used for preparation of cell suspension and for cell washing. 7.5% FCS DMEM containing L valine (650 mg/L) was used for the culture of MTEC1 cells, and 7.5% FCS RP-M1 1640 used in cocultures of thymocytes and MTEC1 cells. Trypsin (0.25%, sigma) containing EDTA (0.01%) (TE) solution was used for digestion of adherent MTEC1 cells during cell passage.

Preparation of MTECI-cell culture, supernatants, and conditioned medium

The preparation of MTEC1 cell culture supernatants (MTECL-SN) has been described in detail previously[12]. The conditioned medium was Con A-activated rat spleen cell culture supernatants (CAS) which contains a mixture of natural cytokines[14].

Cocultures of thymocytes with MTEC1 cells

The fresh mouse thymocytes were cocultured with the monolayers of MTEC1 Cells, which wire placed in cell culture flasks or 96 well flat bottomed tissue culture plates for adhesion 12 14 h before the addition of thymocytes. The different cell numbers of thymocytes and MTEC1 cells in cultures were set up and cocultured for various days, so that the culture conditions were kept optimal for different assays. All cultures were incubated in humidified CO_2 incubator at 37℃, 5% CO_2 in air.

Viable thymocyte number in microcultures

For assessing the viability of thymocytes in cocultures, TE dispersed MTEC1 cells were added to 96 well flat bottomed plates at concentration of 1×10^4 cells/well and incubated overnight at 37℃ for cell adhesion. 5×10^5 fresh thymocytes were then added to each well. The cocultures were incubated at 37℃ for various days, and the viable thymocytes in each well after coculture were counted by eosin exclusion in hemocytometer under microscope at 200 fold magnification. Cell viability was calculated based on the average number of viable cells in triplicate microcultures.

lmmuno fluorescent staining

For phenotype analysis of thymocytes after cocultured with MTEC1 cells, 1 2×10^5 MTEC1 cells in 10 ml were seeded in 25 ml flasks and allowed to adhere for 12 14 h. Fresh thymocytes 5 10×10^6 were then added to the monolayers of MTECl cells and cocultured at 37℃ for 5 d. After cultivation, the cultured thymocytes were harvested and centrifuged through Ficoll Hypaque (1. 090 g/ml) to remove the dead cells. Viable cells 1×10^6 were stained with FITC anti mouse Ly 2 and PE anti mouse L3T4 McAbs for 30 min at 4℃, then washed twice with 5% NCS BSS containing 0. 1% sodium azide (NAN_3) by centrifugation. Freshly prepared mouse thymocytes were stained with the same fluorescent dye conjugated McAbs as control. Immediately before flow cytometry (FACS) analysis, PI was added at 0. 05 μg/ml.

Phenotype analysis of cultured thymocytes

The phenotypes of cultured fluorescent McAbs stained viable thymocytes were analyzed on FACS IV (Becton Dickinson). The dead cells displayed bright orange fluorescence after binding with PI and were gated out through setting window. The phenotypes of viable thymocytes were determined by 2 color FACS analysis according to the cell surface expression of CD4 and/or CD8 molecules. A Consort 30 statistical software was used for data analysis.

Selection of thymic epithelial cells on thymocyte subsets

Thymocyte proliferation assay

The monolayers of MTEC1 cells at confluent growth were treated with mitomycin C(50 μg/ml) at 37℃ for 1 h to inhibit the cell growth yet maintain the cell viability. The mitomycin C were then washed out and the cell layer digested with TE to make MTEC1 in a single cell suspension. 1×10^4 mitomycin C -treated MTEC1 cells were added to each well of 96 well flat bottomed tissue culture plates and incubated at 37℃ overnight for cell adhesion. 1×10^5 fresh thymocytes were added to the MTEC1 monolayers in each well the next morning. The cocultures were incubated at 37℃ for 3 d in the presence or absence of Con A (2.5 μg/ml). For control and/or for comparison, the same number of fresh thymocytes were also cultured with either 25% MTEC1 -SN or 10% CAS in the presence or absence of Con A. ^{3}H−TdR was pulse added at 0. 5 ci/well 12 h before harvest. The incorporation of ^{3}H−TdR into cellular DNA was determined by scintillation counting. Data were calculated based on the average of triplicates.

RESULTS

1. Characteristics of MTEC1 cells

MTEC1 cell line was established from cultures of Balb/c mouse thymus cells and has been maintained in our laboratory for 3 yr. The MTEC1 cells are homogeneous in morphology and are epithelial in nature as identified by cytokeratin positive, and possessing desmosomes between neighbouringcells [11]. The MTEC1 cells exhibit normal cell characteristics, growing as monolayer, and 75% of the cells contain 40 chromosomes. Within the cytoplasm, no virus−like particles could be identified under electron microscope. All the MTEC1 cells were MTS 33$^+$, a McAb specific against the molecules espressed on mouse thymus medullary epithelial cells. MTEC1 cells constitutively produce many types of cytokines including IL−1, IL−6, IL−7, GM−CSF, and chemotactic factor (s), of which, IL−6, GM−CSF and chemotactic factor are quite abundant[12,13].

2. MTEC1 cells maintained the viability of fresh thymocytes

MTEC1 cells effectively maintained the viability of thymocytes. After being cultured for 5 days, 13.2 % of input thymocytes were viable in cocultures, whereas all thymocytes died when cultured alone (Tab i). Even cocultured for 2 wk, a few but significant number of viable thymocytes were still visible. MTEC1−SN also prolonged the viability of thymocytes as efficient as that of MTEC1 cells (Tab 1). Combination of MTEC1 cells and MTEC1−SN could not further increase the number of viable thymo-

Tab 1. MTEC1 cell prolonged the viability of fresh thymocytes *in vitro*.

Thymocyte plus	Viable thymocyte number ($\times 10^{-4}\pm$SD)			
	Cultivation period (d)			
	0	3	5	7
−	50	8.3±1.7	0	0
MTEC1 cells	50	17.3±3.4*	6.6±0.9	2.2±0.4
MTEC1−SN	50	12.0±1.5*	5.0±0.7	1.8±0.8
MTEC1 cells + MTEC1−SN	50	17.5±2.7*	6.7±1.4	2.8±1.2

The number of viable thymocytes was the mean of 4 seperated experiments. *P<0.05.

cytes than in cocultures with MTEC1 cells alone.

3. MTEC1 cells maintained the viability of Con A-activated thymocytes

In *in vitro* cultures, the viable cell recovery rate of Con A−activated thymocytes was increased and the survival time prolonged compared with those of inactivated thymocytes cultured alone (Tab 2 vs Tab 1). Coculture with MTEC1 cells and/or MTEC1 −SN, the viability of Con A−activated thymocytes was further improved. On d 5 of culture, the viable cell recovery was 33−37% of initial input in cocultures with MTEC1 cells or MTEC1−SN, that is, the viable cells were increased 2.2 folds compared with those of Con A−activated thymocytes cultured alone (Tab 2). Similar to the cocultures in the absence of Con A, there appeared no significant difference between MTEC1 cells and MTEC1−SN in the ability to improve cell viability of Con A−activated thymocytes. In positive control cultures, where thymocytes were incubated under optimal conditions (added Con A and CAS, a mixture of natural cytokines), the viable cell recovery rate was 92% of initial input on d 3 of culture, which was 1.7−2.0 folds higher than that in cocultures with MTEC1 cells or MTEC1−SN. It implied that CAS provides more appropriate factors required to maintain the cell viability of Con A−activated thymocytes than does the MTEC1 cells or MTEC1−SN.

Tab 2. MTEC1 cells prolonged the viability of Con A-activated thymocytes *in vitro*

Thymocyte plus	Viable thymocyte number ($\times10^{-4}\pm$SD)			
	Cultivation period (d)			
	0	3	5	7
Con A	50	16.0±2.4	7.5±0.5	3.3±0.3
MTEC1 cells + Con A	50	22.5±3.1*	16.7±1.9	5.5±1.7
MTEC1−SN + Con A	50	27.5±4.0*	18.7±2.1	2.5±0.2
MTEC1 cells + MTEC1−SN				
+ Con A	50	28.7±3.5*	23.3±3.2	5.0±0.7
CAS + Con A	50	45.8±6.1*	28.3±2.7	11.7±1.4

The number of viable thymocytes was the mean of 4 seperated experiments. *P<0.05.

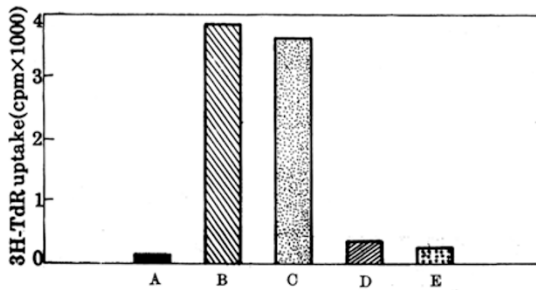### 4. MTEC1 cells directly promoted the proliferation of fresh thymocytes

MTEC1 cells were treated with mitomycin C to block their DNA synthesis. Fresh thymocytes were cocultured with mitomycin C-treated MTECl cells and the proliferation of thymocytes was assessed by DNA synthesis. As shown in Fig 1, MTEC1 cells moderately supported the proliferation of thymocytes, whereas MTEC1-SN was essentially unable to induce the thymocytes to proliferate. Addition of MTEC1-SN to the coculture of thymocytes and MTEC1 cells could no more enhance the thymocyte proliferation. Thus, the activation signal to induce thymocyte proliferation is delivered via the interaction of MTEC1 cells and thymocytes. This activation signal is not attributed to the cytokines present in MTEC1−SN.

5. MTEC1 cells and MTEC1 SN augmented the proliferation of Con A-activated thymocytes

Con A could activate the thymocytes and induce their DNA synthesis at the cell concentration assayed (Fig 2). Cocultures with either MTEC1 cells or MTEC1 SN in the presence of Con A, the thymocyte proliferation was significantly enhanced, which was 2.1 2.4 folds higher than that of Con A-activated thymocytes cultured alone. Under Con A-activation, there was no significant difference in the capability of augmenting the thymocyte proliferation between MTEC1 cells and MTEC1 SN. Combination of MTEC1 cells and MTEC1 SN only marginally enhanced the proliferation of Con A activated thymocytes. In comparison with DNA synthesis in positive control cultures, the DNA synthesis of Con A activated thymocytes induced by MTEC1 cells or MTEC1-SN was 2.2 fold less than that induced by CAS. It is suggested that the cytokines present in CAS (IL 2 mainly) is more efficient than the MTEC1 cell produced cytokines for supporting Con A activated thymocyte to proliferate.

6. Phenotype analysis of thymocytes after coculture with MTEC1 cells

Phenotypes of viable thymocytes after cocultured with MTEC1 cells or MTEC1 SN for 5 d were determined by 2-color FACS analysis. A typical FACS profile is shown in Fig 3, and the average data of 4 experiments are shown in Tab 3. The proportion of four subsets defined by expression of CD4 and/or CD8 molecules of uncultured fresh thymocytes was listed in Tab 3(Row 1) for comparison, in which the subsets of $CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^+CD8^-$ and $CD4^-CD8^+$ thymocytes constitutes 3.8%, 81.2%, 10. 2% and 4.8% respectively. When cocultured with MTEC1 cells in the presence or absence of Con A, the frequencies of the 4 subsets were different. Coculture in the absence of Con A, the frequency of $CD4^+CD8$ (single positive, SP) and CD4 CD8 (double negative, DN) thymocytes was increased by 3.6 and 5.8 folds respectively,whereas the percentage of $CD4^+CD8^+$ (double positive, DP) was decreased by 50%, though still constituting 40% of the total viable cells. These results indicated that the MTEC1 cells preferentially maintained the viability and growth of the subsets of $CD4^+$ SP, DN and, in a minor degree, of DP thymocytes. In contrast, MTEC1 SN maintained the viability of $CD4^+$ SP thymocytes, and, to a lesser extent, of $CD8^+$ SP thymocytes, constituting 56% and 23% of the total viable cells respectively. The MTEC1 SN apparently did not provide efficient support to maintain the viability of DP and DN thymocytes. Coculture in the presence of Con A, the frequencies of the 4 subsets after cultivation were distinct from that in the absence of Con A. Both MTEC1 cells and MTEC1 SN preferentially maintained the viability and promoted the proliferation of the subset of Con A activated $CD4^+$ SP thymocytes, and, to a lesser extent, of the subsets of activated DN as well as $CD8^+$ SP thymocytes, constituting $30-42\%$, $27-33\%$ and $16-19\%$ of the total viable cells respectively. Notably, the ratio of viable $CD4^+$ SP to $CD8^+$ SP thymocytes was essentially the same in cocultures of Con A activated thymocytes with MTECl cells or with MTEC1 SN (1.8 vs 2.2), implying that the support from MTEC1 cells for activated thymocytes is probably attributable to the cytokines they produced . In the cultures of thymocytes stimulated with Con A or Con A plus CAS for 5 d , the highest rate of viable cells was CD 8^+ SP thymocytes , constituting

Fig 1. MTEC1 cells promoted the proliferation of fresh thymocytes

Thymocytes $(1 \times 10^5$/well) cocultured with mitomycin C treated MTEC1 cells $(1 \times 10^4$/well) and/or MTEC1 -SN for 3 days.
A. Thymocytes alone, B. Thymocytes + MTEC1 cells, C. Thymocytes + MTEC1 cells + MTEC1−SN, D. Thymocyte + MTEC1−SN,
E. MTEC1 cells treated by mitomycin C.
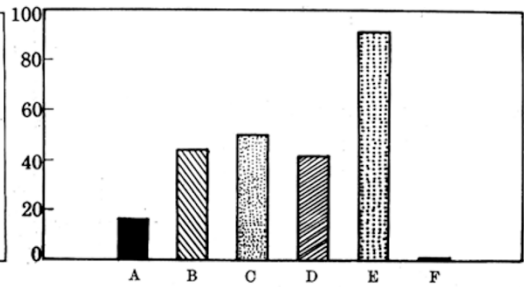
Fig 2. MTEC1 cells promoted the proliferation of Con A−activated thymocytes

Thymocytes $(1 \times 10^5$/well) cocultured with mitomycin C treated MTEC1 cells $(1 \times 10^4$/well) and/or MTEC1−SN for 3 days.
A. Thymocytes + Con A, B. Thymocytes + MTEC1 cells + Con A, C. Thymocytes + MTEC1 cells + MTEC1 -SN + Con A, D. Thymocytes + MTEC1 -SN + Con A, E. Thymocytes + CAS + Con A, F. MTEC1 cells treated by mitomycin C.

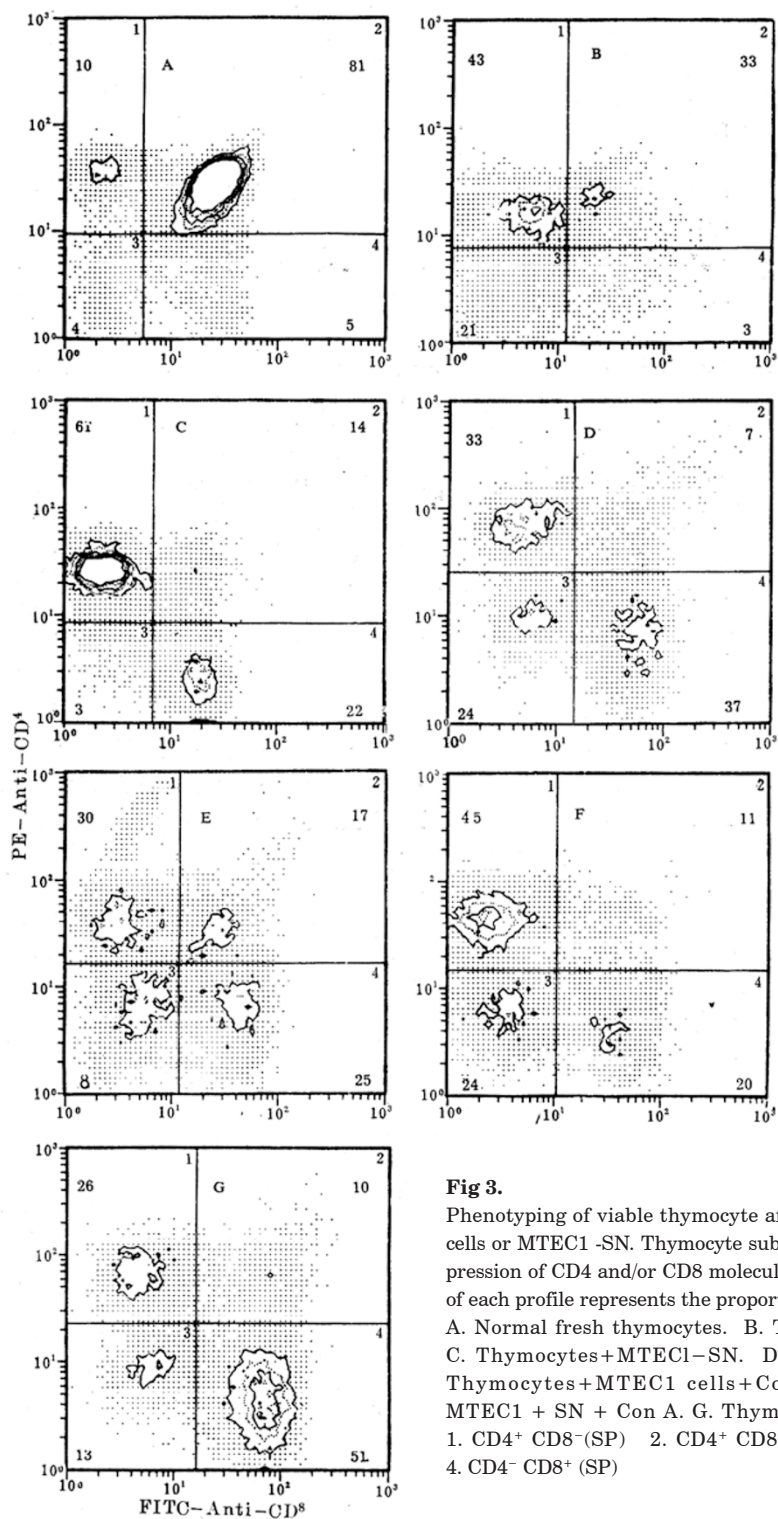**Tab 3** Phenotyping of thymocyte subsets after coculturing with MTEC1 cells or MTEC1 -SN

Thymocyte plus	Percentage of thymocyte subsets (Mean±s)				
	CD4$^+$CD8$^-$	CD4$^+$CD8$^+$	CD4$^-$CD8$^-$	CD4$^-$CD8$^+$	CD4$^+$/CD8$^+$
Fresh thymocytes	10.2±1.1	81.2±1.8	3.8±0.4	4.8±0.3	2.1
MTEC1 cell	35.7±8.9	39.9±9.2	22.5±6.8	3.9±1.0	9.2
MTEC1−SN	56.0±7.1	15.1±4.5	5.8±1.6	22.7±3.2	2.5
Con A	31.0±2.8	7.1±0.5	20.5±3.2	42.4±5.7	0.7
MTECl cell + Con A	29.7±3.6	23.0±4.7	32.7±9.4	16.2±4.4	1.8
MTECl −SN+ Con A	42.1±4.5	12.2±2.2	26.5±2.5	19.3±2.7	2.2
CAS + Con A	27.6±1.8	9.8±1.2	16.7±4.5	45.9±8.1	0.6

Thymocytes were cocultured with MTEC1 cells or MTEC1−SN for 5 d. Viable thymocytes were stained by PE−Anti−CD$_4$ and FITC−Anti−CD$_8$ McAb, then analyzed by FACS 440.
Mean ± s̲were the average of 4 seperated experiments−

49−50% of the total viable cells, which is accounted for by the activity of IL−2 present in CAS. Thus, regarding to the capability of maintaining the viability and of enhancing the growth of thymocytes, MTEC1 cells and the cytokines they produced are relatively favorable to the subset of CD4$^+$ SP thymocytes, whilst Con A−activated thymocyte produced lymphokines (IL−2 mainly) are relatively favorable to the subset of CD8$^+$ SP thymocytes.

Fig 3.
Phenotyping of viable thymocyte after coculturd with MTEC1 cells or MTEC1 -SN. Thymocyte subsets were defined by the expression of CD4 and/or CD8 molecules. The figure on the corner of each profile represents the proportion of each subsets.
A. Normal fresh thymocytes. B. Thymocytes+MTEC1 cells. C. Thymocytes+MTECl−SN. D. Thymocytes+Con A. E. Thymocytes+MTEC1 cells+Con A. F. Thymocytes + MTEC1 + SN + Con A. G. Thymocytes + CAS + Con A.
1. $CD4^+$ $CD8^-$(SP)   2. $CD4^+$ $CD8^+$(DP)   3. $CD4^-CD8^-$(DN)
4. $CD4^-$ $CD8^+$ (SP)

DISCUSSION

The fact that *in vivo* thymic positive selection is able to induce the immature T cells to differentiate into mature T cells has been confirmed by *in vitro* experiments. Cocultivation of defined thymic epithelial cells (TEC) with various stages of developing T cells has demonstrated that Pro-T cells could give rise to $CD4^+CD8^-$ or to $CD4^- CD8^+$ thymocytes[8, 9], and $CD4^+ CD8^+$ thymocytes could develop into $CD4^- CD8^+$ thymocytes [15]. It has yet been only few reports indicating that TEC could directly induce immature T cells to proliferate without addition of stimulus and/or exogenous eytokines[16]. In this report, we have provided evidence that MTEC1 cells, a thymic medullary type epithelial cell line, can directly maintain the viability and promote the proliferation of MHC matched fresh thymocytes. To maintain cell viability and to promote cell proliferation are likely to be 2 distinct bioactivities, and are apparently mediated through different triggering events. Both MTEC1 cells and MTEC1-SN, which contains cytokines including IL-1, IL-6, GM-CSF and others, could maintain cell viability with equal potency. By contrast, the thymocyte proliferative response takes place only in cocultures with MTEC1 cells, which can not be replaced by MTEC1 SN. The resulis implied that the MTEC1 cell-produced cytokines could maintain cell viability at G1 phase, but can not activate the thymocytes to enter S phase (DNA synthesis); a prerequisite signal delivered from MTEC1 cell thymocyte interaction is needed and appears to be sufficient to activate a minor population of thymocytes initiating DNA synthesis. The possibility, however, can hardly be neglected, that the mitomycin C treated MTEC1 cells may produce cytokines which function as accessory signal for thymocyte proliferation, m as much as the mitomycin C treated thymic stromal cells retain the capacity of synthesizing cytokines[17].

The phenotypes of viable thymocytes were analyzed on FACS on d 3 to 5 of cocultures in the absence of Con A. MTEC1 cells preferentially supported the activation of mature $CD4^+CD8^-$ SP thymocytes and immature $CD4^-CD8^-$ DN as well as $CD4^+CD8^+$ DP thymocytes. By contrast, the MTEC1 SN chiefly maintained the viability of $CD4^+$ SP and, to a lesser extent, DN, as well as $CD8^+$ SP thymocytes. These results suggest that: [1] a population of thymocytes capable of responding to the signals generated from direct interaction of thymocytes with MTEC1 cells to enter DNA synthesis is resided in the subsets of $CD4^+SP$, DN and DP thymocytes. [2]$CD4^+ CD8^-$ helper thymocytes are favorably responsive to the signals derived from the direct contact with MTEC1 cells and to the MTEC1 cell-produced cytokines.

The significance of these results is that the DP and DN immature thymocytes can be activated by a particular type of thymic epithelial cells (MTEC1) via direct cell-cell interaction. The existence of direct cell-cell contact is evidenced by our another experiment, in which the dominant subsets of thymocytes binding to MTEC1 cells areof CD4 CD8 and $CD4^+ CD8^+$ phenotypes. It is imperative that the interaction is mediated through the receptor ligand molecules expressed on MTEC1 cells and immature thymocytes. There are several systems of receptor-ligand capable of mediating the thymic stromal cell thymocyte interaction, including TCR−CD3-syngenic MHC + endogenous

peptides; TCR β chain endogenous superantigen; adhesion molecules, such as CD4 or CD8 to non-polymorphic moieties of MHC molecules; CD2 to LFA-3; LFA-1 to ICAM-1; and fibronectin to their respective receptors [18]. Because of lack of appropriate McAbs to identify these molecules, we are at present unable to unravel the exact molecular basis underlying the interactions between MTEC1 cells and immature thymocytes.

In this report, we have also described the accessory function of MTEC1 cells and MTEC1 SN in the augmentation of Con A-activated thymocyte proliferation. The dominant subsets supported by MTEC1 cells and MTEC1 SN were CD4$^+$ and, to a lesser extent, CD8$^+$ SP thymocytes as well as DN thymocytes. There was no significant difference between MTECl cells and MTEC1 SN in their capacity of enhancing the activated thymocytes to grow, and in the supported proportion of SP thymocyte subsets (CD4$^+$/CD8$^+$: l.8 vs 2.2). It is conceivable that the augmentation of SP thymocyte response to Con A activation by proliferation is attributed to the activities of MTEC1 cell produced cytokines, and the effect of signal derived from thymocyte MTEC1 cell interaction seems to be vague in the presence of a strong activator Con A. In principle, our results are consistent with that in the cocultures of human thymic epithelial cells (TEC) and human thymocytes[16], in which human TEC cells directly supported growth of immature (T6$^+$) and mature (T6$^-$) thymocytes; whereas in the cultures of PHA-activated thymocytes, TEC cells only supported the growth of mature thymocytes. An explanation for augmentation of MTEC1 cell produced cytokines in Con A-activated SP thymocyte proliferation is due to the synergistic effect of multiple cytokines. Both CD4$^+$ and CD8$^+$ SP thymocyte subsets can be activated by Con A to produce a limited amount of IL 2, IL 4 (mainly from CD4$^+$ SP), and IFNγ (mainly from CD8$^+$ SP), as well as receptors for these cytokines, inducing the proliferation of both SP thymocyte subsets, especially of CD8$^+$ SP subset (Tab 3, Row 4 and 7). When the IL-1, IL-6 (produced by MTEC1 cells), as well as IL-2 (produced by Con A-activated CD4$^+$ SP thymocytes) are available, the synergy among them would be more preferential to induce the proliferation of CD4$^+$ SP thymocytes than that of CD8$^+$ SP thymocytes [19]. This probably underlies the mechanism that CD4$^+$ SP thymocytes came out as dominant population in our cocultures of Con A activated thymocytes with MTEC1 cells or with MTEC1-SN.

To compare the growth and viable cell recovery of thymocytes cocultured with MTEC1 cells in the presence or absence of Con A, DNA synthesis of thymocytes was 11 fold higher, while the viable cell recovery was only 20% higher in the presence of Con A than in its absence. These observations implied that the Con A-activated CD4$^+$ SP thymocyte produced lymphokines (IL 2, IL 4, etc) are capable of inducing DNA synthesis in the thymocytes whereas MTEC1 cell-produced cytokines (IL 1, IL 6, IL 7, etc) have the function to maintain cell viability. The fact that much less DNA synthesis of thymocytes in the cocultures with MTEC1 cells in the absence of Con A than in its presence implied that MTEC1 cells can only trigger a minor population of thymocytes to synthesize DNA, or alternatively, MTEC1 cells act only as accessory signal to CD4$^+$ thymocytes and are not able to fully activate them to produce lymphokines to further gear up DNA synthesis. Further experiments coculturing MTEC1 cells with separated thymocyte subsets supplemented with IL 2 and/or IL 4 are now proceeding to verify these assumptions.

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