Early Human Thymocyte Proliferation Is Regulated by an Externally Controlled Autocrine Transforming Growth Factor-β1 Mechanism

By M. Djavad Mossalayi, Frank Mentz, Fateh Ouazaa, Ali H. Dalloul, Catherine Blanc, Patrice Debré, and Francis W. Ruscetti

Early thymocytes undergo extensive proliferation after their entry into the thymus, but cellular interactions and cytokines regulating this intrathymic step remain to be determined. We analyzed the effects of various T-cell growth factors and cellular interactions on in vitro proliferation of early CD2+CD3/TCR/CD4+CD8+ (triple negative [TN]) human thymocytes. Freshly isolated TN cells were then assayed for their growth capacity after incubation with CD2+MoAb+MoAb+IL-2. The addition of recombinant transforming growth factor β (TGFβ1) or autologous irradiated CD3+CD8+CD4+ cells to TN cell cultures dramatically decreased their growth responses to IL-2 and IL-7, whereas IL-4–induced proliferation was less sensitive to growth inhibition. We thus asked whether the CD8+ cell-derived inhibitory effect was due to TGFβ1. The addition of neutralizing anti-TGFβ1 or autologous CD8+CD4– cells to TN cell cultures completely abolished CD8+ cell-derived inhibition of TN cell growth. Analysis of CD8+ cell-derived supernatants indicated that these cells had low TGFβ1 production capacity, whereas TN cells secreted significantly higher levels of TGFβ1. Cell fixation studies showed that TN cells were the source of the TGFβ1. TGFβ1 released from TN cells was in the latent form that became the active inhibitory form through interaction of TN cells with CD8+ cells. Together, these data suggest a role for TGFβ1 as an externally controlled, autocrine inhibitory factor for human early thymocytes, with a regulatory role in thymic T-cell output.

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Materials and Methods

Antibodies. The following fluorescein isothiocyanate (FITC)-conjugated MoAbs were used: OKT6 (CD1), OKT11 (CD2), OKT3 (CD3), OKT4 (CD4), and OKT8 (CD8) (Ortho, Raritan, NJ); IOT14 (CD25), IOB4 (CD19), and IOT10 (CD57) (Immunotech, Marseille Lumigny, France); Leu11 (CD16) and MY4 (CD14) (Coulter Clone, Hialeah, FL); and D44 MoAb (kind gift of Dr. A. Bernard, Nice Medical School, Nice, France). CD8+D44+ and CD8+D44– cells were reported to comprise most functional cytotoxic and suppressor cells, respectively. For double marker analysis CD4–CD15–FITC and CD3–CD14–CD2–FITC (Coulter Clone) were also used. Cell analysis and sorting were performed using FACStar (Becton Dickinson, Mountain View, CA). To eliminate their fluorescent background for the reanalysis of their surface markers, sorted cells were incubated for 24 hours before staining. For cell activation, purified CD3+MoAb (OKT3; Ortho) and cotagentic CD2–MoAb (X11 and D66 clones, gifts of Dr. Alain Bernard) were used.

Cell preparations. Thymus fragments were obtained from children (<3 years old) undergoing corrective cardiac surgery. Various thymic cell subsets were obtained as described in detail elsewhere. Briefly, major thymic cell subsets were isolated from...
CD2+ (AET-sheep erythrocyte+) cells after their labeling with CD4-PE and CD8-FITC MoAb and subsequent cell sorting. Using this procedure, CD4+CD8+, CD4-CD8+, CD4+CD8+ (CD4+), and CD4+CD8+ (CD8+) subsets were obtained. CD2+ thymocytes were also treated with CD4, CD8, and CD3 MoAb and CD3-CD4-CD8- cells were sorted (termed TN, for triple-negative fraction). Each thymic cell subset was first analyzed for surface markers. Results in Table 1 indicate that the purity of these fractions was greater than 90%. By contrast to other thymic cells, a significant percentage (3% to 6%) of TN cells expressed IL-2 receptor, CD25. Labeling of TN cells with both CD7 and CD2 showed greater than 98% cell reactivity. No cell reactivity was observed with MoAbs for CD14, CD19, CD16, or CD57 antigens.

**Cell cultures.** Total thymocytes or their various subsets were incubated (1 to 5 x 10^7 cells/100 µLwell) in 96-well microtiter in RPMI medium (Tebu, Paris, France) containing 20% human AB-sera, and various combinations of the following reagents were added to the cultures: comitogenic CD2+,CD3-MoAb (1/400 ascites from each), CD3-MoAb (OKT3 clone, 20 µg/mL), recombinant IL-2 (100 U/mL; Boehringer Mannheim, Meylan, France), IL-7 (10 ng/mL; Calbiochem, San Diego, CA), IL-4 (10 ng/mL; a gift from Dr J. Banchereau, Schering Plough, Dardilly, France), TGFβ1 (rTGFβ1; Bristol-Meyers-Squibb, Seattle, WA), neutralizing anti-TGFβ MoAb (clone 1-D11016, neutralizing both TGFβ1 and TGFβ2 forms), IOB4 MoAb as isotype-matched control (CD19, clone BC3; Immuno-techn), and polyclonal anti-TNFα antibody (Tebu). All human sera used were pretested to ensure that there were no inhibitory effects on thymocyte proliferation. Trinitiated thymidine (1 µCi/well; CEA, Les Ulis, France) was incorporated at day 4 and radioactive uptake was measured 18 hours later. Maximal growth responses were observed after this incubation period, as suggested by our previous study.33,35 TGFβ1 was quantified using a specific radioimmunoassay (NEN-Dupont, Paris, France) as recommended by the manufacturer. This assay detects more than 30 pg TGFβ1 in cell supernatants and measures active as well as latent TGFβ. For some experiments the cells were irradiated with 3,000 rad or fixed with paraformaldehyde before cultures.

**Statistics.** Various results were analyzed and compared using the Student's t-test for paired data.

**RESULTS**

**Definition of growth requirement of TN early thymocytes.** TN thymocytes were assayed for their proliferative response after their incubation with comitogenic CD2+,CD3-MoAb, CD3-MoAb, IL-2, IL-7, and/or IL-4 and compared with other major thymocyte subsets. Optimal concentrations of these reagents were previously determined.32,35 Results in Table 2 indicate that thymocytes varied in their response to the above physiologic signals. Double-positive (CD4+CD8+) and unfraccionated thymocytes displayed significantly lower growth ability than other thymic cell subsets (P < .001; Table 2). TN thymocytes, as predicted from their surface phenotype (Table 1), did not respond to CD3 ligation. Despite their heterogeneity,17-19,34 TN cells had significant and constant proliferative responses in the presence of IL-4, IL-7, or IL-2. Maximal proliferation of TN and CD4+CD8+ cells required CD2-MoAb and IL-2, whereas CD8+CD4+ cell growth was higher with IL-4 (compared with CD8+ cell response to CD2-MoAb + IL-2). Finally, optimal proliferation of TN and CD4+CD8+ cells required IL-2 and CD2-MoAb. In contrast, whereas CD2-MoAb increased the proliferation of CD8+CD4+ cells, optimal proliferation of these cells was seen with IL-4 stimulation, which was not affected by CD2-MoAb.

**Recombinant TGFβ1 inhibits the proliferation of TN thymocytes.** TGFβ is well known as a potent regulator of hematopoietic cell growth.35 Recombinant TGFβ1 was therefore added to early thymocytes cultured in the presence of CD2-MoAb+IL-2, IL-7, or IL-4. A dose-dependent inhibition of the proliferative responses was observed (Fig 1), which indicates that TGFβ1 displays a high inhibitory effect on IL-2– or IL-7–dependent thymocytes growth (P < .001), whereas IL-4–dependent TN cell proliferation was less sensitive to TGFβ1 addition (P < .05). This finding indicates that these cytokines induced distinct proliferation pathways in human early thymocytes. TGFβ effect can be reversed through the addition of neutralizing anti-TGFβ MoAb to the cultures but not through the addition of an isotype-matched control (CD19-MoAb; Fig 1).

**Mature CD8+ thymocytes downregulate the proliferation of TN thymocytes.** Cellular interactions regulating early thymocyte growth remain ill defined. However, we have previously shown that agar colony formation by prothymocytes was inhibited by CD8+4– thymic subset.30 We assayed here the effect of CD8+ as well as other major thymic cell subsets on the growth of TN cells stimulated with CD2-MoAb+IL-2. Data in Fig 2 show the ability of CD8+ cells to inhibit the proliferation of TN thymocytes (P < .007) in a dose-dependent manner, whereas TN, CD4+, and CD4+CD8- cell subsets displayed no such inhibitory effect. Higher amounts of exogenous IL-2 (500 to 1,000 U/mL) or CD2-MoAb (up to 100 µg/mL) did not significantly overcome this inhibitory effect (not shown). This finding indicated that the CD8+ cell-derived effect was not caused by IL-2 or CD2-MoAb consumption.

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**Table 1. Surface Marker Analysis of Various Thymocyte Subsets After Their Purification**

<table>
<thead>
<tr>
<th>Thymic Subsets</th>
<th>CD7</th>
<th>CD2</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD4.8*</th>
<th>Percentage of Thymocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total thymocytes</td>
<td>95</td>
<td>95</td>
<td>78</td>
<td>84</td>
<td>85</td>
<td>77</td>
<td>&gt;95</td>
</tr>
<tr>
<td>CD3+CD4−CD8+ (TN)</td>
<td>97</td>
<td>92</td>
<td>82</td>
<td>97</td>
<td>91</td>
<td>87</td>
<td>83</td>
</tr>
<tr>
<td>CD3+CD4+CD8− (CD4+)</td>
<td>94</td>
<td>93</td>
<td>85</td>
<td>96</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>CD3+CD4−CD8− (CD8+)</td>
<td>97</td>
<td>94</td>
<td>82</td>
<td>2</td>
<td>88</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

CD2+ thymocytes and their various subsets were analyzed by direct immunofluorescence and flow cytometry. Values are the percentage of positive cells (mean from 3 experiments).

* Double positive cells.
Table 2. Proliferation Capacity of Various Thymic Cell Subsets

<table>
<thead>
<tr>
<th>Thymic Cells</th>
<th>None</th>
<th>IL-2</th>
<th>IL-2 + CD2 MoAb</th>
<th>IL-2 + CD3 MoAb</th>
<th>IL-4</th>
<th>IL-4 + CD2 MoAb</th>
<th>IL-7</th>
<th>IL-7 + CD2 MoAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN(CD2')</td>
<td>860</td>
<td>5,327</td>
<td>27,149</td>
<td>5,535</td>
<td>15,059</td>
<td>16,245</td>
<td>6,632</td>
<td>7,212</td>
</tr>
<tr>
<td>CD2'CD4+CD8'</td>
<td>1,214</td>
<td>2,001</td>
<td>6,800</td>
<td>6,549</td>
<td>2,820</td>
<td>1,998</td>
<td>1,352</td>
<td>NT</td>
</tr>
<tr>
<td>CD2'CD4'</td>
<td>2,072</td>
<td>3,022</td>
<td>30,324</td>
<td>25,068</td>
<td>7,995</td>
<td>8,658</td>
<td>3,932</td>
<td>NT</td>
</tr>
<tr>
<td>CD2'CD8'</td>
<td>1,200</td>
<td>1,845</td>
<td>8,865</td>
<td>8,657</td>
<td>17,962</td>
<td>16,258</td>
<td>1,502</td>
<td>NT</td>
</tr>
<tr>
<td>E'(CD2')</td>
<td>1,196</td>
<td>2,134</td>
<td>8,453</td>
<td>7,932</td>
<td>5,232</td>
<td>6,525</td>
<td>2,397</td>
<td>3,022</td>
</tr>
</tbody>
</table>

Thymocyte subsets were incubated (10^4/100 µL/well) with rIL-2 (100 U/mL), CD3 MoAb (20 µg/mL), comitogenic CD2-MoAb (V400 ascites from each), rIL-7 (10 ng/mL), and rIL-4 (10 ng/mL). Tritiated thymidine was added at day 4 and radioactivity uptake was measured 18 hours later. Results represent the mean values from nine distinct thymocyte preparations, each performed in triplicate (SE <11%).

Abbreviation: NT, not tested.

Phenotypical characterization of downregulating CD8+ cells. The data above led us to investigate the surface phenotype of the suppressor CD8+ cells. CD2'CD8+ thymocytes are a heterogeneous cell population and contain the precursors of functionally distinct peripheral T lymphocytes.14-16 Using negative selection procedure (by positive cell elimination), we attempted to further define the cell subset responsible for the inhibition of TN cell proliferation. CD8+ cells were therefore treated with CD3-, CD4-, CD57-, or D44-MoAb; negative cells were then sorted and assayed for their suppressive activity. After treatment with above MoAb, CD8+ cells contained less than 2% cell positivity with the MoAb used for cell elimination. D44 MoAb was used because it was previously described to recognize functionally distinct peripheral blood CD8+ lymphocytes.3'4' Although a minor subset of CD8+ cells (2% to 4%), CD44+ cells were also sorted and assayed in this study. Data in Fig 3A show the effect of various cell depletions on the ability of CD8+ cells to suppress the proliferation of TN cells. CD4+, CD57+, or D44+ cell depletion from CD8+ cells did not affect their inhibitory effect, whereas CD3+ cell elimination completely abolished CD8+ cell-derived effect. These findings indicate that the suppressor cells are likely to be CD3+CD8+CD4-CD57-D44+. Consequently, we have positively sorted these cells (2% to 4% total thymocytes) and assayed them for their ability to suppress TN cell growth. Data in Fig 3B confirm the high inhibitory effect of both irradiated and nonirradiated CD8+ cells on both IL-2- and IL-7-mediated proliferation of TN cells and indicate that suppression was not caused by CD8+ cell irradiation. Together, these data clearly show that CD3+CD8+ thymocytes comprise cells that downregulate the proliferation of TN thymocytes.

Anti-TGFβ1 MoAb reverses CD8+ cell-derived inhibitory effects. To clarify the mechanism of CD8+ cell-derived suppression, we first asked whether an inhibitory cytokine was involved in this phenomenon. In regard to the similarities between CD8+ cell-derived and TGFβ1-derived effects, we have analyzed the role of TGFβ1 in CD8+ cell-derived inhibition of TN cell proliferation. TN thymocytes were then cultured in the presence of CD2-MoAb+IL-2, irradiated CD8+ cells, neutralizing anti-TGFβ1 MoAb, anti-TNFα Ab, and/or an isotype-matched control (10B4). Interestingly, only anti-TGFβ1 addition to these cocultures significantly reversed the inhibitory effect of CD8+ cells (P < .001; Fig 4A). This effect was dose-dependent and was not observed with control isotype-matched MoAb (Fig 4B).

Human TN thymocytes produce high TGFβ1 levels. The effects of recombinant TGFβ1 on TN cell growth and the ability of anti-TGFβ1 MoAb to reverse the inhibitory effect of CD8+ cells led us to quantity the TGFβ1 levels produced by these cells as well as other thymic subsets. Unexpectedly,
CD8+ cells were poor producers of TGFβ1 (<0.8 ng/mL), even after their in vitro activation (Table 3). By contrast, TN thymocytes produced significantly high TGFβ1 levels, as did activated CD4+ cells. However, this TGFβ1 was not active because these cells proliferated in vitro in the presence of the same growth factors (Table 2). Finally, we have obtained similar data from three different TN cell preparations containing, respectively, 95%, 97%, and 99% CD2+ cells, which suggest that contaminating non-T cells may not account for TGFβ1 production. Figure 5 shows that TGFβ1 production is dependent on TN cell numbers added to the cultures. These data indicate that TN thymocytes differ from other thymic subsets in their high TGFβ1 releasing capacity in the absence of apparent in vitro activation.

**Mechanism of CD8+ cell-derived inhibition of early thymocyte proliferation: role for TGFβ1.** We here analyzed the mechanism by which CD8+ cells mediate the inhibitory effect on TN cells. In contrast to the cells themselves, the addition of CD8+ cell supernatants had no effect on IL-2- or IL-7-dependent early thymocyte growth (Table 4). This finding indicates that these cells did not produce biologically active TGFβ1. Because supernatants from cocultures of CD8+ and TN cells displayed important inhibitory effect (Table 4), we thus analyzed the role of each cell subset in inhibitory factor production. CD8+ TN thymocytes were then fixed by paraformaldehyde and cocultured with the other subset. As shown in Table 4, CD8+ cell fixation did not affect TN growth inhibition, whereas TN cell fixation completely abolished the release of the suppressor factor in these cocultures. CD8+ cell addition to TN cell supernatants also converted from inactive to active TGFβ1, which then inhibited TN cell growth. Incubation of TN-SN with CD8+ cells did not significantly increase TGFβ1 levels, as measured by radioimmunoassay (data not shown). Finally, the suppressor effect of CD8+ TN cell-derived supernatants was reversed through the addition of anti-TGFβ1 MoAb (Table 4). Together, these data indicate that TN cells are indeed the source of TGFβ1 that is converted to an active form after their interaction with CD8+ cells.

**DISCUSSION**

The present study attempted to dissect the growth and the activation capacities of human early thymocytes, their interactions with other thymic cells, and the effect of TGFβ1 on their in vitro growth response. Several novel findings...
emerged from these data. (1) Human thymocytes differ in their proliferation potential and sensitivity to IL-2, IL-7, and IL-4, which may be related to their developmental stages. (2) The growth of human TN thymocytes is downregulated by a subset of autologous CD8+ cells, an effect requiring cell-cell interactions and reversed by MoAb to TGFβ1. (3) Recombinant TGFβ1 dramatically inhibited the proliferation of human TN thymocytes. (4) TN cells produce high TGFβ1 levels that, after contact with CD8+ cells, converted into biologically active form.

TGFβ is a member of a highly conserved family of polypeptide factors regulating cell growth and differentiation. It is secreted by most human cells and selectively inhibits CSF-induced growth of both murine and human immature hematopoietic cells.1 TGFβ1 also acts as an important immunomodulatory protein2 for cells of the immune system as it regulates the proliferation of T6,9,12,36 and B lymphocytes,37,38 inhibits Ig release by B cells,39,40 and modulates cytokine production by T lymphocytes.41,42 Some of these effects seems to be mediated through counteracting IL-1 functions43,36 by inhibiting IL-1R expression.43

The role of TGFβ on human early T-cell development remains to be defined. The intrathymic sojourn of T lymphocytes constitutes an essential step in the generation of immunocompetent cells. Bone marrow prothymocytes migrate through the thymus, where they undergo extensive expansion together with the acquisition of various surface antigens including CD3/TCR.15,16 These events allow subsequent positive and negative selection of appropriate clones. Present work shows that, although heterogeneous,16,19 early CD3-CD4-CD8- (TN) thymocytes display a significantly higher proliferation potential than most thymic cells (Table 2). The absence of their response through CD3 cross-linking further confirmed the CD3- phenotype of these cells (Tables 1 and 2). High growth potential of TN thymocytes correlates with the fact that they belong to the proliferative compartment of thymic outer cortex.15 In vivo, early thymocyte activation may be initiated through CD2-triggering by LFA-3 antigen on epithelial cells.16,44,45 Early thymocytes also proliferated in response to IL-4 and IL-7, corroborating with previous reports on the role of these cytokines in early T-cell development.18,23-27

Our data show the presence of a limited population of

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**Table 3. TGFβ1 Production by Various Thymocyte Subsets**

<table>
<thead>
<tr>
<th>Thymocytes</th>
<th>TGFβ1 (ng/mL/10^6 cells)</th>
<th>Supernatants From Cells Incubated With</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1.4</td>
<td>CD2 MoAb</td>
</tr>
<tr>
<td>TN</td>
<td>4.1</td>
<td>CD2 MoAb</td>
</tr>
<tr>
<td>CD4+</td>
<td>0.6</td>
<td>CD3 MoAb</td>
</tr>
<tr>
<td>CD8+</td>
<td>0.5</td>
<td>CD2 MoAb + IL-2</td>
</tr>
</tbody>
</table>

Values are TGFβ1 levels quantified by radioimmunoassay in 48-hour supernatants from various thymocyte subsets. Values reflect representative data from one experiment (SD <15%) of three.
thymic CD8+ cells that inhibited the proliferation of early TN precursors. Phenotypical characterization of these suppressor cells indicated that they are CD3+CD4−CD8+CD57−D44+. Such a phenotype was previously reported as a characteristic of PBL-derived T lymphocytes suppressing Ig production by B lymphocytes and to be distinct from NK cells (CD3+CD57+CD16+) and cytolytic (CD8+D44+) cells. Interestingly, anti-TGFβ MoAb completely reversed the inhibitory effect of CD8+ cells. The failure of supernatants from CD8+ cells to suppress thymocyte proliferation suggested that the interaction between these thymocytes and TN cells is required for the activation of TGFβ1. Further analysis showed the presence of high TGFβ1 levels in supernatants from TN cells, whereas CD8+ cells produced comparatively low TGFβ1 amounts (Table 3). CD8+ cells induced the conversion of TN cell-derived TGFβ1 from latent to active form, leading us to postulate that the mature cell pool in the thymus may have a feedback control on the proliferation of early precursor (Fig 5). Even though we feel most of the TGFβ is made by CD2+ TN cells, we cannot rule out a contribution by other cell types such as thymic epithelial cells.

Recently, Takahama et al.13 reported the role of TGFβ in murine CD4+CD8+ cell differentiation into CD4+CD8+ thymocytes through a paracrine mechanism. This study did not address the effect of TGFβ on human TN cell differentiation; however, it differs from the work of Takahama et al.13 in that it shows an autocrine source for TGFβ. Our data are supported by previous reports that point to the autocrine inhibitory effect of TGFβ on human lymphoblastoid cells in culture and murine hematopoietic stem cells. In addition, by contrast to Takahama et al.,13 we have reversed TN inhibition by antibody to TGFβ, further supporting the direct involvement of this cytokine. However, our data do not exclude a role for paracrine TGFβ on human TN cell proliferation.

TGFβ is generally secreted in a latent form composed of a homodimer of 105 kD of which the c-terminal 112 amino acids of each chain form the mature active 25-kD cytokines. In vitro, the release of active TGFβ from latent complex can be facilitated by enzymatic or physicochemical treatment.

Table 4. Cell-Cell Interaction Requirement for the Generation of Inhibitory Factor From TN Cells

<table>
<thead>
<tr>
<th>TN Cells Cultured With</th>
<th>CD2 MoAb + IL-2</th>
<th>Inhibition</th>
<th>IL-7</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. None</td>
<td>22,652 ± 5,2411</td>
<td>—</td>
<td>8,965 ± 1,242</td>
<td>—</td>
</tr>
<tr>
<td>2. CD8+ cells (10^4/well)</td>
<td>5,253 ± 2,147</td>
<td>Yes (77%)†</td>
<td>1,284 ± 254</td>
<td>Yes (86%)</td>
</tr>
<tr>
<td>3. (CD8+ + TN)-SN</td>
<td>10,267 ± 1,502</td>
<td>Yes (55%)</td>
<td>4,239 ± 323</td>
<td>Yes (53%)</td>
</tr>
<tr>
<td>4. (CD8+ + TN)-SN + anti-TGFβ</td>
<td>20,839 ± 2,125</td>
<td>No (8%)</td>
<td>8,856 ± 1,245</td>
<td>No (1%)</td>
</tr>
<tr>
<td>5. CD8+ -SN</td>
<td>21,142 ± 1,247</td>
<td>No (7%)</td>
<td>9,623 ± 2,027</td>
<td>No (0%)</td>
</tr>
<tr>
<td>6. TN-SN</td>
<td>21,425 ± 2,555</td>
<td>No (5%)</td>
<td>8,858 ± 862</td>
<td>No (1%)</td>
</tr>
<tr>
<td>7. (CD8+-fixed + TN)-SN</td>
<td>12,124 ± 1,856</td>
<td>Yes (46%)</td>
<td>4,989 ± 1,005</td>
<td>Yes (45%)</td>
</tr>
<tr>
<td>8. (CD8+ + TN-fixed)-SN</td>
<td>22,119 ± 1,186</td>
<td>No (2%)</td>
<td>7,967 ± 212</td>
<td>No (11%)</td>
</tr>
<tr>
<td>9. (TN-SN + CD8+)-SN</td>
<td>13,429 ± 2,452</td>
<td>Yes (41%)</td>
<td>5,436 ± 259</td>
<td>Yes (39%)</td>
</tr>
<tr>
<td>10. (TN-SN + CD8+)-SN + anti-TGFβ</td>
<td>20,259 ± 3,214</td>
<td>No (11%)</td>
<td>8,002 ± 2,351</td>
<td>No (11%)</td>
</tr>
</tbody>
</table>

* TN cells were cultured (10^4/100 μL) in the presence of CD2 MoAb + IL-2 and (1) none; (2) CD8+ cells; (3) 24-hour supernatants (SN) from CD8+ + TN cells; (4) CD8+ + TN cell SN and anti-TGFβ MoAb; (5) CD8+ cell-derived SN; (6) TN cell-derived SN; (7) SN from CD8+ cells fixed with paraformaldehyde + TN cell cocultures; (8) SN from CD8+ + fixed TN cell cocultures; (9) 24-hour SN from TN cells incubated with CD8+ cells for an additional 24 hours; (10) same as 9 + anti-TGFβ MoAb. SN were added at 20% final dilution.
† Thymidine uptake per 10^6 TN thymocytes cultured as in Table 2.
‡ Rate of inhibition compared with that of TN cells cultured with medium alone (none). Yes indicates significant inhibition with P < .01; no indicates that no significant inhibition was observed.

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Early human thymocyte proliferation is regulated by an externally controlled autocrine transforming growth factor-beta 1 mechanism

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