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Human thymic epithelial cells directly induce activation of autologous immature thymocytes

(thymus/T-cell ontogeny/interleukins)

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ABSTRACT **To study the role that epithelial cells of the thymic microenvironment play in promoting activation of immature CD7$^+$, CD2$^+$, CD4$^-$, CD8$^-$ (double-negative) human thymocytes, we have isolated thymocyte subsets from normal postnatal thymus and have cocultured autologous double-negative thymocytes with pure populations of thymic epithelial (TE) cells. We report that TE cells directly activate double-negative thymocytes to proliferate and that TE cells enhance the ability of double-negative thymocytes to proliferate in response to stimulation with exogenous interleukin 2. Activated double-negative thymocytes that proliferated *in vitro* in the presence of TE cells and interleukin 2 remained double-negative after 23 days in culture. Moreover, TE-cell culture supernatants in the absence of intact TE cells contain interleukin 1, interleukin 3, and granulocyte/macrophage-colony-stimulating factor activity for human bone marrow cells and can activate double-negative thymocytes to proliferate. Antibodies against interleukin 1 and against granulocyte/macrophage-colony-stimulating factor inhibited TE-cell-induced thymocyte activation. These data indicate that one role of TE cells *in vivo* may be to activate double-negative thymocytes to proliferate.**

The thymus is known to be the primary site of T-cell maturation during fetal and neonatal development (1–5). However, specific cellular interactions and activation signals necessary for growth and differentiation of T-cell precursors within the human thymus are largely unknown (4–6). Studies of T-cell ontogeny in mice have suggested that the earliest intrathymic T cells have the surface phenotype dLyt1$^+$ ("d" means that the immunofluorescence is faint, or "dull"), Lyt2$^-$, L3T4$^-$ and that a subpopulation of dLyt1$^+$, Lyt2$^-$, L3T4$^-$ thymocytes are able to reconstitute the T-cell repertoire (7–13).

Lobach and Haynes (14) showed that, during human fetal development, the earliest T-cell precursors are CD7$^+$ prior to entry into the thymus, and these cells acquire the CD2, CD4, CD8, CD3, and CD1 molecules in the thymus between 8½ and 14 weeks of fetal gestation. At 8½ weeks of fetal development, intrathymic T cells are CD7$^+$, CD2$^+$, CD4$^-$, CD8$^-$ (double-negative) (14–16).

To study the role that thymic epithelial (TE) cells might play in activating subsets of human double-negative thymocytes to proliferate and differentiate into mature T cells, we developed an *in vitro* coculture system of autologous TE cells and double-negative thymocytes (17). Here we show that TE cells up-regulate interleukin 2 (IL-2) receptor expression and IL-2 responsiveness of double-negative thymocytes and directly activate immature thymocytes to proliferate. Moreover, TE cells produce cytokines capable of providing activation signals to double-negative thymocytes in the absence of direct TE-cell–thymocyte contact.

MATERIALS AND METHODS

Antibodies. Antibodies 3A1 (CD7) (18), TS29.1 (anti-LFA-3) (19, 20), and TS122 (CD18) (19, 20) were used as previously described. Monoclonal antibodies 35.1 and 9.6 (21) were the gift of John Hansen. Monoclonal antibodies T3/RW2-4B6 (CD3), T4/19Thy5D7 (CD4), and T8/21Thy2D3 (CD8) were the gift of Ellis Reinherz (Dana–Farber Cancer Institute, Boston, MA). Antibodies Leu-M3 (22), WT31 (23), and anti-Tac (24) were gifts of Robert Winchester, Wil Tax, and Thomas Waldmann, respectively. Antibodies L243 (25) and 3F10 (26), which bind to nonpolymorphic determinants of major histocompatibility complex (MHC) class II and class I molecules, respectively, were obtained from the American Type Culture Collection. Rabbit polyclonal antibody against interleukin 1 (IL-1) (27) was the gift of Charles Dinarello. Rabbit antibodies against interleukin 3 (IL-3) and against granulocyte/macrophage-colony-stimulating factor (GM-CSF) were gifts of Steven Clark (Genetics Institute, Cambridge, MA).

Cell Preparation. Thymic tissue was obtained from normal children (aged 1 day to 8 years) undergoing median sternotomy incision and corrective cardiovascular surgery. TE-cell cultures were established by an explant technique and were subcultured as described (28). Thymocytes were separated and purified as described (17). Double-negative (CD4$^-$, CD8$^-$) thymocytes were prepared by "panning" (29, 30) and preparative cell sorting.

Proliferation Assays. Thymocyte proliferation was assayed by measurement of [^{3}H]thymidine incorporation as described (17). Recombinant IL-2, kindly provided by Cetus Corporation (Emeryville, CA), was used at final concentrations of 0.5–10 units/ml. Recombinant human GM-CSF, a gift of Steven Clark, was used at final concentrations of 1–80 units/ml. In some experiments, anti-Tac, anti-IL-2 (Collaborative Research, Boston, MA), anti-IL-1, anti-IL-3, and anti-GM-CSF antibodies were added to cocultures of TE cells and double-negative thymocytes at saturating amounts (1:500 dilution of ascites, purified antibody at 50 μg/ml, 1:100 dilution of rabbit polyclonal antibody, 1:100 dilution of rabbit polyclonal antibody, and 1:500 dilution of rabbit polyclonal antibody, respectively).

RESULTS

Human TE Cells Directly Activate Double-Negative Thymocytes. Cocultures of autologous TE cells and double-

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Abbreviations: TE cell, thymic epithelial cell; Ti, T-cell antigen receptor; MHC, major histocompatibility complex; GM-CSF, granulocyte/macrophage-colony-stimulating factor; IL-1, interleukin 1; IL-2, interleukin 2; IL-3, interleukin 3.

negative thymocytes were established from seven subjects. Total thymocytes alone incorporated little [^{3}H]thymidine, and coculture with mitomycin C-treated TE cells (TE_M) resulted in a minimal increase in [^{3}H]thymidine incorporation (Fig. 1*A*). However, double-negative thymocytes cultured with 10% autologous TE cells were markedly activated after 5 days as determined by increased incorporation of [^{3}H]thymidine (Fig. 1*A*). $CD4^+$ and/or $CD8^+$ ($CD4^+$, $CD8^+$) thymocytes were not similarly activated when cocultured with TE cells. TE-cell-induced activation of double-negative thymocytes occurred as well in cocultures with allogeneic TE cells (data not shown). In separate experiments, purified populations of mature medullary thymocytes ($CD3^+$, $p80^+$) and immature cortical thymocytes ($CD1^+$) did not proliferate when cocultured with TE cells (17). As specificity controls, neither human erythrocytes, human epidermal cells, nor the human epithelial carcinoma cell line A-431 induced activation of double-negative thymocytes (data not shown).

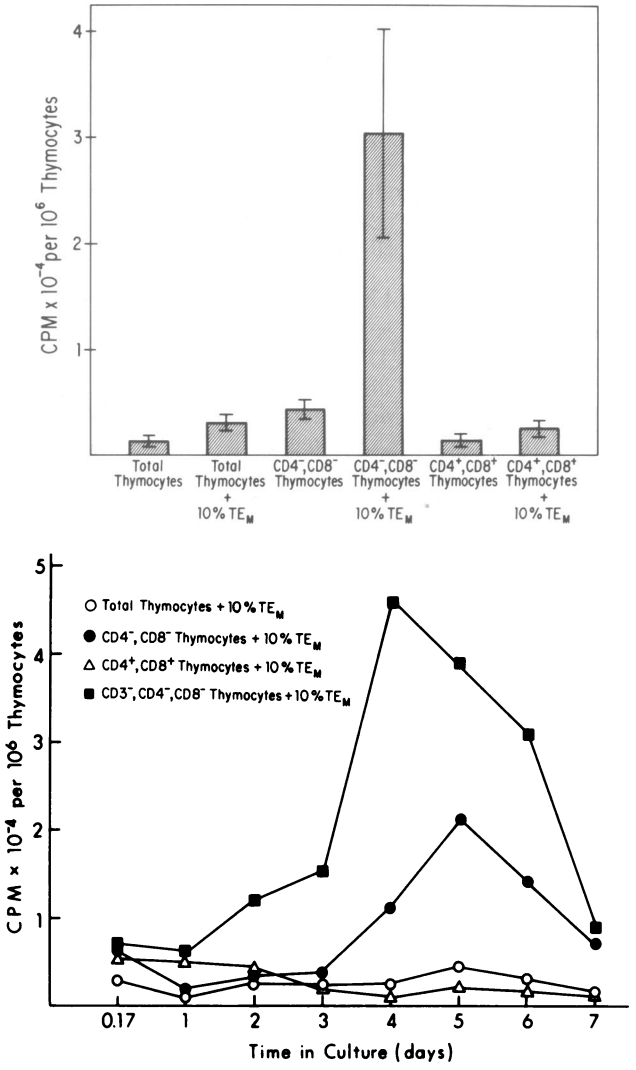

FIG. 1. Human TE cells directly induce proliferation of double-negative thymocytes. (*A*) Total thymocytes, $CD4^+$ and/or $CD8^+$ thymocytes, and purified $CD4^-$, $CD8^-$ (double-negative) thymocytes were cultured alone or in the presence (10%) of mitomycin C-treated TE cells (TE_M). After 5 days, [^{3}H]thymidine incorporation during a 4-hr period was measured. Bars show means ± SEM for six separate experiments. (*B*) Representative experiment showing the proliferative response of thymocyte populations at various times of coculture with TE_M. ○, Total thymocytes; △, $CD4^+$, $CD8^+$ thymocytes; ■, $CD3^-$ double-negative ($CD3^-$, $CD4^-$, $CD8^-$) thymocytes; ●, double-negative thymocyte suspensions that contain 20% $CD3^+$ cells ($CD4^-$, $CD8^-$ thymocytes). In every experiment, TE_M alone; $CD4^-$, $CD8^-$ thymocytes alone; and $CD3^-$, $CD4^-$, $CD8^-$ thymocytes alone incorporated <4000 cpm. The phenotype of double-negative ($CD4^-$, $CD8^-$) thymocytes, determined by indirect immunofluorescence (31), was 96% $CD7^+$, 95% $CD2^+$, 81% MHC class I-positive, 20% $CD3^+$, 90% $CD18^+$, <0.5% Leu-M3$^+$, and always <5% $CD4^+$ or $CD8^+$. Fewer than 5% of the double-negative thymocytes expressed surface or cytoplasmic α and β chains of the T-cell antigen receptor (Ti) as determined by reactivity with monoclonal antibody WT31. The phenotype of the $CD4^+$ and/or $CD8^+$ thymocyte population was 90% $CD4^+$ and/or $CD8^+$, 92% $CD7^+$, and 93% $CD2^+$.

To determine the number of double-negative thymocytes responding to TE-cell activation signals in 5-day cocultures, we counted blasts and mitotic figures in Wright-stained cytocentrifuge preparations (17). In three separate experiments, whereas double-negative thymocyte suspensions cultured alone for 5 days contained 5 ± 3% lymphocyte blasts and no mitotic figures, double-negative thymocyte suspensions cultured for 5 days with TE cells contained 30 ± 6% lymphocyte blasts and 2 ± 1% mitotic figures.

Because the $CD3^+$, $CD4^-$, $CD8^-$ subset of thymocytes may constitute a separate lineage from thymocytes that become mature Ti α/β^+ cells (i.e., cells expressing the α/β heterodimeric receptor for antigen) (32–35), we determined whether TE cells could activate $CD3^-$ as well as $CD3^+$ double-negative thymocytes. For this purpose, we performed kinetic studies to compare TE-cell-induced proliferation of double-negative ($CD4^-$, $CD8^-$) thymocytes (containing 20–50% $CD3^+$ cells) with TE-cell-induced proliferation of $CD3^-$, $CD4^-$, $CD8^-$ cells (containing <1% $CD3^+$ cells) (Fig. 1*B*). TE-cell induction of proliferation of double-negative thymocytes containing $CD3^+$ cells was maximal at day 5 of culture, whereas TE-cell-induced activation of $CD3^-$, $CD4^-$, $CD8^-$ thymocytes was maximal at day 4. Moreover, in four separate experiments, $CD3^-$, $CD4^-$, $CD8^-$ thymocytes in the presence of TE cells incorporated more [^{3}H]thymidine than did the double-negative thymocyte subset containing $CD3^+$ cells ($P < 0.05$).

Response of Immature Thymocytes to TE Cells and IL-2. As shown in Table 1, addition of as little as 0.5 unit of recombinant IL-2 per ml of culture medium induced double-negative thymocytes to proliferate. Furthermore, addition of 10% TE cells substantially enhanced the proliferative response of double-negative thymocytes to IL-2 ($P < 0.005$).

In indirect immunofluorescence assays of 20 different samples, 11 ± 3% of double-negative thymocytes prior to *in vitro* culture expressed 55-kDa (p55) IL-2 receptor subunits as determined by reactivity with anti-Tac antibody. After 5 days of culture, there was no difference in the number of cells expressing p55 IL-2 receptors when double-negative thymocytes cultured alone (30 ± 8%) were compared to double-negative thymocytes cocultured with TE cells (32 ± 8%). However, addition of TE cells to double-negative thymocytes resulted in a marked increase in p55 IL-2 receptor

Table 1. Response of double-negative ($CD4^-$, $CD8^-$) thymocytes to IL-2 is augmented by TE cells

Addition(s) to thymocyte suspension	[^{3}H]Thymidine incorporation, cpm per 10^6 $CD4^-$, $CD8^-$ thymocytes
Medium (control)	3,000 ± 1,300
rIL-2	28,100 ± 8,000
10% TE_M	20,600 ± 3,800
10% TE_M + rIL-2	103,000 ± 16,100

Thymocytes were prepared and cultures initiated as described in *Materials and Methods* and the legend to Fig. 1. Results shown are means ± SEM for four separate experiments. Proliferation in the presence of recombinant IL-2 (rIL-2, 0.5 unit/ml) was significantly greater than control ($P < 0.02$). Proliferation in the presence of 10% TE_M (mitomycin C-treated TE cells) plus rIL-2 was significantly greater than that in the presence of TE_M without rIL-2 ($P < 0.005$).

density compared to double-negative thymocytes cultured alone. This was observed as an increase in anti-Tac binding [as measured by increase in mean fluorescence channel (MFC) on cytofluorographic analysis] from 49 ± 14 MFC for double-negative thymocytes cultured alone to 84 ± 24 MFC for double-negative thymocytes cultured with TE cells ($P < 0.05$). Addition of saturating amounts of anti-Tac or anti-IL-2 antibody to TE cell/double-negative thymocyte cocultures stimulated with IL-2 (0.5 unit/ml) inhibited the TE-cell augmentation of the proliferative response of double-negative thymocytes to added IL-2 (data not shown).

TE-Cell-Induced Thymocyte Activation Leads to Expansion of Both the CD3$^+$ and the CD3$^-$ Double-Negative Thymocyte Populations. First, the phenotype of double-negative thymocytes after 5 days in coculture with TE cells was determined (Table 2). We found that 91% of thymocytes after 5 days in culture with TE cells remained CD4$^-$ and CD8$^-$.

To determine whether TE-cell-induced thymocyte activation as measured by [^{3}H]thymidine incorporation indeed reflected proliferation of the double-negative thymocytes, three experiments were performed in which 500,000 purified double-negative thymocytes (>99% CD4$^-$, CD8$^-$) were cocultured with 10% TE cells and recombinant IL-2 (80 units/ml) for 23 days. Phenotypic analysis was performed weekly. During this time double-negative thymocytes underwent 4–5 doublings and grew to 10–18 × 10^6 cells. Phenotypic analysis at 23 days (Fig. 2) demonstrated that 92% of the cells were CD4$^-$, CD8$^-$ (double-negative) with varying proportions expressing CD2 (60%) and CD3 (27%). Importantly, double-negative thymocytes at day 23 were nonreactive with antibodies against Ti α and β chains (WT31) and the CD1 (T6) antigen (Fig. 2). Myeloid marker expression on these cells was minimal, with 6% of the cells Mo-1$^+$.

In contrast to day 5, when 32 ± 8% of double-negative thymocytes expressed IL-2 receptors (as measured by anti-Tac), by day 23 only 2% were Tac$^+$ (Fig. 2).

TE-Cell Culture Supernatant Contains GM-CSF and IL-3 Activity and Can Activate Double-Negative Thymocytes. TE-cell–thymocyte binding is inhibited by anti-CD2 and anti-LFA-3 antibodies (37). To investigate whether TE-cell activation of double-negative thymocytes could be similarly inhibited, we assayed the effect of a panel of monoclonal antibodies on TE-cell/double-negative thymocyte cocultures. Neither antibodies to MHC class I or class II antigens nor antibodies to CD2, LFA-3, or CD7 antigens significantly inhibited double-negative thymocyte activation as measured by [^{3}H]thymidine incorporation ($P > 0.5$; data not shown). Since anti-CD2 and anti-LFA-3 antibodies blocked binding of TE cells to thymocytes but did not block TE-cell-induced activation of double-negative thymocytes, we reasoned that direct cell contact could not be solely responsible for the activation. Thus, we tested whether TE-cell supernatant contained cytokine activities other than IL-1 (38) and whether this supernatant could activate double-negative thymocytes in the absence of intact TE cells. As shown in Table 3, TE-cell supernatant indeed contained both GM-CSF- and IL-3-like activity as determined by its effect on bone marrow mononuclear cells. In addition, in three separate experiments, both TE-cell supernatant [at a concentration of 1:5 (vol/vol) in culture] and IL-3 were capable of activating double-negative thymocytes to proliferate (Table 4). When recombinant GM-CSF was added to cultures of double-negative thymocytes, proliferative responses were observed with GM-CSF concentrations as low as 4 units/ml, with peak responses at 16 units/ml (Table 4).

Table 2. Phenotype of purified CD7$^+$, CD4$^-$, CD8$^-$ thymocytes after 5 days of coculture with human TE cells

Monoclonal antibody-defined leukocyte marker	% positive cells
CD7	60.6 ± 3.5
CD2	46.5 ± 13.9
CD4	3.0 ± 2.3
CD8	8.4 ± 7.8
CD3	7.7 ± 5.0
WT31	3.3 ± 3.3

Purified CD7$^+$, CD4$^-$, CD8$^-$ thymocytes (<1% CD4$^+$, CD8$^+$) were cocultured for 5 days with allogeneic TE cells. Thymocyte phenotype was determined by indirect immunofluorescence assay and cytofluorographic analysis. Data represent the means ± SEM of three separate experiments. Analysis performed with a combination of CD4 and CD8 antibodies showed that all CD4$^+$ thymocytes at day 5 were CD8$^+$ as well.

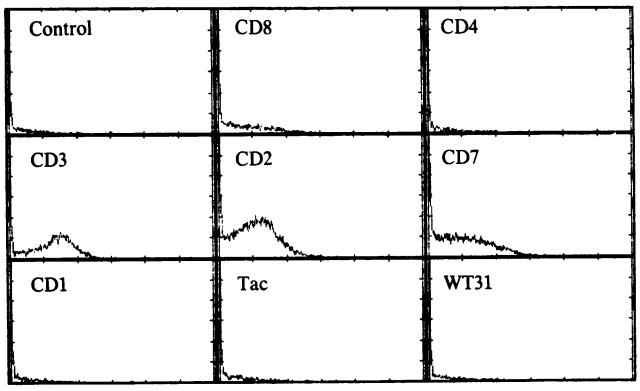

FIG. 2. Purified human double-negative thymocytes cultured for 23 days with TE cells plus IL-2 proliferate and express CD2 and CD7 antigens but remain double-negative. Percentages for the panels shown above [% above control using P3X63Ag8 ascites (5%)] are as follows: CD8, 8%; CD4, 0%; CD3, 27%; CD2, 63%; CD7, 40%; CD1, 0%; Tac, 2%; WT31, 0%. Long-term TE-cell/thymocyte cocultures were established as described (36). Medium conditioned by human peripheral blood T cells for 72 hr in the presence of 1% phytohemagglutinin was added to long-term double-negative thymocyte cultures at a final concentration of 10% (vol/vol), and recombinant IL-2 was added at 80 units/ml. Thymocytes in culture were characterized by indirect immunofluorescence (31) with monoclonal antibodies 3A1 (CD7), 35.1 (CD2), anti-Leu-2a (CD8; Becton Dickinson), anti-Leu-3a (CD4; Becton Dickinson), anti-Leu-4 (CD3; Becton Dickinson), anti-Leu 6 (CD1; Becton Dickinson), anti-Tac (CD25), WT31 (anti-Ti), and P3X63Ag8 ascites. Cells were analyzed (10,000 cells counted) with an EPICS 753 flow cytometer (Coulter Electronics, Hialeah, FL).

Table 3. TE-cell culture supernatant contains IL-3 and GM-CSF activity

Addition(s) to bone marrow mononuclear cells	Colonies per 10^6 bone marrow mononuclear cells		
	CFU-GM	CFU-GEMM	BFU-E
Medium (control)	0	0	0
IL-3 + Epo	ND	70	220
GM-CSF	1380	ND	ND
TE-cell supernatant	2420	1320	1300

Bone marrow mononuclear cells were prepared and the frequency of nonlymphoid hematopoietic progenitor colonies was assayed in 0.9% methylcellulose as described (36, 39–42). Placental conditioned medium (10%, vol/vol) or conditioned medium from the human bladder carcinoma line 5637 (10%, vol/vol) was added as a source of GM-CSF and IL-3, respectively. Recombinant erythropoietin (Epo) was added to give a final concentration of 2 units/ml. TE-cell conditioned medium (culture supernatant) was added at a final concentration of 20% (vol/vol). Colonies were inspected microscopically at 14 days of culture and assessed as granulocyte/macrophage colonies (CFU-GM; CFU, colony-forming unit), mixed granulocyte/erythrocyte/macrophage/megakaryocyte colonies (CFU-GEMM), or erythrocyte colonies (BFU-E, burst-forming unit, erythroid). Data shown are representative of five experiments. ND, not done.

Table 4. TE-cell culture supernatant induces proliferation of double-negative thymocytes

Addition to double-negative thymocytes	[^{3}H]Thymidine incorporation, cpm per 10^6 CD4$^-$, CD8$^-$ thymocytes				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
Medium (control)	1,990	400	750	2,750	2,800
TE-cell supernatant	5,300	8,100	6900	ND	ND
IL-3	12,700	10,500	7700	ND	ND
GM-CSF	ND	ND	ND	25,300	15,200

Conditioned medium from the human bladder carcinoma line 5637 (10%, vol/vol) was added as a source of IL-3. Conditioned medium from TE-cell cultures was used in a 20% (vol/vol) concentration. Recombinant GM-CSF was added at a concentration of 16 units/ml. ND, not done.

Inhibition of TE-Cell Activation of Double-Negative Thymocytes. To investigate the mechanisms of TE-cell-induced thymocyte proliferation, anti-Tac (anti-p55 IL-2 receptor), anti-IL-2, anti-IL-1, anti-IL-3, and anti-GM-CSF antibodies were added singly or in combination to 5-day cocultures of TE cells and double-negative thymocytes. Neither anti-Tac, anti-IL-2, nor anti-IL-3 antibodies inhibited the activation of double-negative thymocytes by TE cells (data not shown). However, antibodies to IL-1 and to GM-CSF each partially inhibited the activation. Moreover, anti-IL-1 and anti-GM-CSF antibodies together inhibited the activation by 70% (Fig. 3).

DISCUSSION

In this study, we have shown that human TE cells can activate autologous immature CD4$^-$, CD8$^-$ (double-negative) thymocytes and augment immature thymocyte responses to IL-2. TE-cell-induced activation of double-negative thymocytes led to expansion of both the CD3$^+$ and the CD3$^-$ subpopulations of double-negative thymocytes.

A major consequence of TE cell/double-negative thymocyte coculture was an increase in surface expression of p55 IL-2 receptors on the double-negative thymocytes. This increase was accompanied by an enhancement of the proliferative response of these thymocytes to IL-2. Howe *et al.* (43) have shown that IL-1 enhances the IL-2-dependent mitogen response of murine double-negative (Lyt2$^-$, L3-T4$^-$) thymocytes and increases thymocyte production of IL-2 in response to phorbol 12-myristate 13-acetate and the calcium ionophore ionomycin. Additionally, murine double-negative thymocytes express receptors for IL-1 and rapidly internalize bound IL-1, suggesting that the receptors are functional. Ceredig (44) reported that phorbol myristate acetate and ionomycin stimulation can similarly increase the response of murine double-negative thymocytes to IL-2 and cause proliferation without differentiation of these double-negative cells. The observations that human TE cells constitutively produce an IL-1-like activity (38) and that thymocyte binding or anti-LFA-3 antibody binding increases TE-cell production of IL-1 (P.T.L., B.F.H., and K.H.S., unpublished data) suggest that TE-cell IL-1 may partially mediate these effects of TE cells on double-negative thymocytes. Evidence directly supporting the importance of IL-1 is the recent isolation of a TE-cell line (SK-13) that does not produce IL-1 and does not activate double-negative thymocytes (K.H.S. and B.F.H., unpublished observations). That anti-IL-1 antibodies can partially inhibit the activation of double-negative thymocytes by TE cells lends support to the concept that IL-1 has a role in that activation.

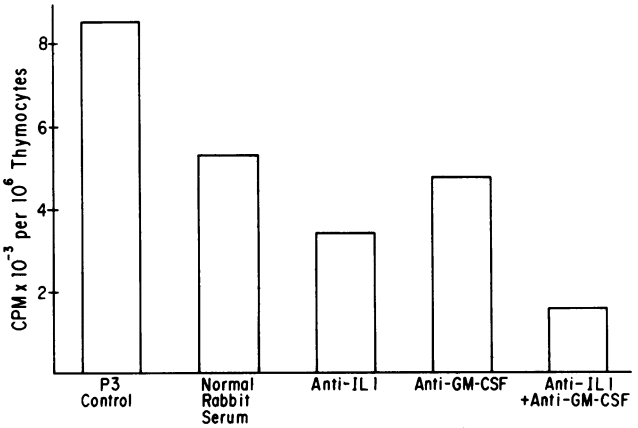

FIG. 3. Anti-IL-1 and anti-GM-CSF antibodies inhibit TE-cell-induced proliferation of double-negative thymocytes. Double-negative thymocyte/TE-cell cocultures were initiated as described in *Materials and Methods*. Both anti-IL-1 and anti-GM-CSF partially inhibited the activation of double-negative thymocytes; the combination of anti-IL-1 and anti-GM-CSF inhibited the activation by 70%. Neither anti-Tac nor anti-IL-3 inhibited the activation (data not shown). P3X63Ag8 ascites (P3) and normal rabbit serum were tested as controls.

The observation that the CD3$^-$ subset of double-negative thymocytes proliferated in response to both IL-2 and TE cells suggests a thymocyte-activation pathway independent of the CD3/Ti complex (52) early on in T-cell ontogeny. Additionally, anti-CD3 antibodies, either alone or in combination with IL-1 or IL-2, did not activate this CD3$^-$, CD4$^-$, CD8$^-$, thymocyte subset (S.M.D. and B.F.H., unpublished observations).

Also of interest was the observation that whereas 30% of double-negative thymocytes after 5 days in coculture with TE cells were Tac$^+$ (i.e., expressed the p55 chain of the IL-2 receptor), only 3% of double-negative thymocytes were Tac$^+$ after 23 days in culture with TE cells and recombinant IL-2 (80 units/ml) (Fig. 2). Shimonkevitz *et al.* (45) showed that transient expression of IL-2 receptors by a subset of murine double-negative thymocytes precedes differentiation to mature thymocytes. Our data suggest that the loss of IL-2 receptors represents either an early stage of thymocyte maturation or, alternatively, a selection *in vitro* of Tac$^-$ double-negative thymocytes during long-term culture. That the Tac$^-$ double-negative thymocytes in the 23-day cultures proliferated in response to IL-2 at 80 units/ml may indicate that these cells use the p70 (intermediate-affinity) chain of the IL-2 receptor (46–48).

A surprising observation in this study was that anti-CD2 and anti-LFA-3 antibodies that block TE-cell–thymocyte binding (37) did not inhibit TE-cell-induced activation of double-negative thymocytes. Rather, TE-cell supernatant activated double-negative thymocytes in the absence of intact TE cells, and the combination of anti-IL-1 and anti-GM-CSF antibodies inhibited TE-cell-induced thymocyte activation (Fig. 3). Production of cytokines including macrophage-colony-stimulating factor (CSF-1) has also been found in cloned human TE cells transformed by simian virus 40 (49). A potential role for GM-CSF or similar cytokines in early T-cell proliferation is suggested by the recent establishment of a human T-cell leukemia line that was GM-CSF-dependent and did not proliferate in response to IL-2 (50). At present, we cannot rule out that TE cells may induce a subset of double-negative thymocytes to produce GM-CSF, leading to thymocyte proliferation in this system.

We have shown that TE cells produce cytokines with GM-CSF- and IL-3-like activities (Table 3) in addition to IL-1 (38). A clue to the significance of these findings may come from the observation that the T-cell acute lymphoblastic leukemia syndrome in which the malignant cells are CD7$^+$, CD4$^-$, CD8$^-$ is a malignancy of pluripotent cells that are capable of responding to GM-CSF, IL-3, granulocyte-

colony-stimulating factor, macrophage-colony-stimulating factor, and erythropoietin, resulting in terminal myeloid and erythroid differentiation (51). In the presence of intact TE cells (with binding to thymocytes via CD2 molecules), double-negative thymocytes proliferate, remain lymphoid, and stay double-negative ($CD4^-$, $CD8^-$) (Fig. 2). Moreover, in the absence of intact TE cells, TE-cell supernatant induces subsets of normal double-negative thymocytes to differentiate along myeloid lineage pathways (J.K., P.T.L., S.M.D., and B.F.H., unpublished data). Therefore, TE cells may function *in vivo* by providing multiple activation signals to immature thymocytes and, by as-yet-uncharacterized mechanisms, participate in lineage determination of T-cell precursors at the time of their arrival in the thymus.

Note Added in Proof. Wiranowska *et al.* (53) recently reported syngenetic triggering of immature T cells from nude mouse spleen by IL-2 and human TE cells.

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