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THYMOCYTE BINDING TO HUMAN THYMIC EPITHELIAL CELLS IS INHIBITED BY MONOCLONAL ANTIBODIES TO CD-2 AND LFA-3 ANTIGENS[1]

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With the use of cultured human thymic epithelial (TE) cells, we have previously shown that thymocytes bind to TE cells in suspension in a rosette-forming assay. To identify cell surface molecules involved in human TE-thymocyte rosette formation, we assayed a large panel of monoclonal antibodies for their ability to inhibit rosette formation. We found anti-CD-2 (LFA-2, T11), and anti-LFA-3 antibodies all inhibited binding of TE cells to thymocytes. By using indirect immunofluorescence assays, we determined that cultured TE cells were 90% LFA-3 positive and CD-2 negative, whereas thymocytes were 10% LFA-3 positive and 98% CD-2 positive. Pretreatment of TE cells with anti-LFA-3 but not anti-LFA-2 inhibited TE-thymocyte binding. In contrast, pretreatment of thymocytes with anti-CD-2 but not anti-LFA-3 antibodies inhibited TE-thymocyte binding. Thus TE cell-thymocyte binding is blocked by antibodies to the CD-2 (T11) antigen on thymocytes and by an antibody to the LFA-3 antigen on TE cells. Because the CD-2 antigen has been implicated in T cell activation, these data suggest that a natural ligand for T cell activation via the CD-2 molecule is present on human thymic epithelial cells.

Although the thymus is necessary for generation of functionally mature T lymphocytes, the specific roles that thymic microenvironment components play in promotion of normal T cell maturation are not understood (1–4). We have previously developed an in vitro system for the long-term growth of human thymic epithelial (TE)[3] cells (5). Moreover, we have shown TE cells can activate autologous thymocytes in vitro, and serve as accessory cells for phytohemagglutinin (PHA)-induced thymocyte activation (6).

Recently, we have described binding of both cortical and medullary thymocytes to autologous and allogeneic cultured TE cells by using a TE-thymocyte rosette-forming assay (7). In that study, TE-thymocyte binding was not blocked by antibodies to major histocompatibility complex (MHC) class I and class II antigens (7). To additionally define molecules involved in human TE-thymocyte binding in vitro, we have investigated the ability of monoclonal antibodies against a wide variety of epithelial and lymphocyte antigens to inhibit TE-thymocyte binding. In this report, we identify two molecules, the CD-2 molecule (LFA-2, T11, E-rosette receptor) on thymocytes, and the LFA-3 molecule on TE cells, which appear to be involved in human TE-thymocyte binding in vitro.

MATERIALS AND METHODS

Monoclonal antibodies. Antibodies against the lymphocyte function associated antigen-1 (LFA-1) α and β subunits, (TS1/22 and TS1/18, respectively), LFA-2 (TS2/18, TS1/8), and LFA-3 (TS2/9) antigens were raised against HLA-DR specific human cytotoxic T lymphocyte clones (8) and were used as ascites or purified antibody. Antibody LM-2 was raised against granulocytes and detects the Mac-1 α subunit (Miller and Springer, manuscript in preparation). Antibodies against human TE cells (TE-3, TE-4, TE-8, TE-15, TE-16, and TE-19) were raised against human thymic stroma and define discrete stages of TE cell maturation (9–11). Antibody 8AD6 against early T cell precursors was generated in our laboratory by using the DU.528 human stem cell line (12) as immunizing antigen and lymphocyte hybrid techniques as described (10). Other anti-T cell antibodies were obtained through the Second International Workshop on Leukocyte Differentiation Antigens (13). Antibody 3F10 reacts with non-polymorphic MHC class I determinants (14). Antibody L243 reacts with non-polymorphic MHC class II determinants (15) and was obtained from the American Type Culture Collection (ATCC; Rockville, MD). Anti-IgG-Fc receptor antibody (KFc79) was the gift of Dr. T. Mohanakumar, Medical College of Virginia, Richmond, VA; anti-thymulin antibodies E8C10 and 1H6H10 (16, 17) were the gift of Dr. M. Dardenne, Paris. Anti-keratin monoclonal antibody AE1 (18) was provided by Dr. T.-T. Sun, New York University School of Medicine, New York.

In vitro culture of human thymic epithelial cells. Thymus tissue was obtained at the time of corrective cardiovascular surgery from 15 patients aged 7 mo to 16 yr. Human TE cell cultures were initiated by an explant technique, were propagated in enriched medium, and were subcultured as described (5). The A431 cell line (19) was obtained from Dr. J. DeLarco, National Institutes of Health, and was passaged in Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, NY) supplemented with 10% fetal calf serum (GIBCO), 100 μg/ml streptomycin, 100 IU/ml penicillin, and 0.25 μg/ml amphotericin B (GIBCO).

Thymocytes. Thymocytes were gently teased from thymus tissue and were purified by centrifugation through Ficoll-Hypaque (20). Cells were frozen slowly in RPMI 1640 (GIBCO) containing 20% fetal calf serum (FCS), 7.5% dimethylsulfoxide (Sigma Chemical Co., St., Louis, MO), and 10 μg/ml gentamicin (Schering, Kenilworth, NJ), and were stored in liquid nitrogen. To thaw, thymocytes were incubated at 37°C for 1 hr in RPMI 1640 containing 30% fetal calf serum, 100 μg/ml deoxyribonuclease I (Sigma), and 10 μg/ml gentamicin.

Immunofluorescence. Indirect immunofluorescence was performed on cells in suspension, on frozen tissue sections, and on cytocentrifuge preparations as described in detail (21). P3X63/Ag8 ascites fluid was used as a negative control in all assays.

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[3] Abbreviations used in this paper: LFA, lymphocyte function-associated antigen; TE, thymic epithelial.

TABLE I

Monoclonal antibodies that inhibit TE-thymocyte binding[a]

Antibody	Specificity	Percent inhibition ± SEM
TS2/9	LFA-3	91 ± 5
TS1/8	CD-2, LFA-2	91 ± 4
TS2/18	CD-2, LFA-2	91 ± 1
35.1	CD-2	96 ± 0
T11/3Pt2H9	CD-2	93 ± 3
T11/ROLD2-1H8	CD-2	93 ± 3
T11/7T4-7A9	CD-2	83 ± 7
T56	CD-2	44 ± 10
D66	CD-2	59 ± 14
T12/3Pt12B8	CD-6	15 ± 5
12.1.5	CD-6	12 ± 3

[a] Thymocytes were pretreated with monoclonal antibody as described in *Materials and Methods*, and TE or A431 cells were added. The data are expressed as mean percent inhibition of epithelial cell-thymocyte binding. The data represent mean ± SEM of three experiments.

TABLE II

Monoclonal antibodies that did not inhibit TE-thymocyte binding[a]

Antibody	Specificity
3F10	Anti-HLA class I
L243	Anti-HLA class II (Ia)
NA1/34	CD-1
T3	CD-3
T4	CD-4
6-2	CD-5
2H4	CD-5
3A1	CD-7
T8	CD-8
F1089-4-7	T200
8AD6	Pan-thymocyte
TS1/22	LFA-1
TS1/18	LFA-1
TE-3	Cortical thymic epithelium
TE-4	Thymic subcapsular cortex and medulla
TE-8	Thymic Hassall's bodies
TE-15	Thymic Hassall's bodies
TE-16	Thymic Hassall's bodies
TE-19	Thymic Hassall's bodies
E8C10	Anti-native thymulin
1H6H10	Anti-(synthetic) thymulin
LM-2	MAC-1, CD-11
KFc79	Anti-human Fc receptor for IgG

[a] The ability of antibodies to inhibit thymocyte-TE and thymocyte-A431 rosette formation was tested in two separate experiments as described in *Materials and Methods*. Monoclonal antibodies listed effected less than 3% inhibition of TE-thymocyte rosette formation.

TE-thymocyte binding assay. Assays of TE-thymocyte binding were performed as described (7). Briefly, 2×10^6 thymocytes and 1.5×10^5 TE cells or A431 cells were combined in 200 μl of RPMI 1640 supplemented with 5% FCS (RPMI-FCS) at 4°C, were centrifuged for 5 min at 250 × G at 4°C, and were incubated for 30 min at 4°C. Cells were gently resuspended, and coded samples were counted under light microscopy (150× magnification). TE cells or A431 cells that bound three or more thymocytes were scored as positive (7). For each assay, 200 TE cells were counted, and each determination was performed in duplicate or triplicate. We have previously shown that thymocytes bind to the epidermoid carcinoma A431, with binding properties identical to those observed with TE cells (7). Therefore we have used A431 cells to compare with normal cultured TE cells, and performed experiments with A431 and TE cells in parallel.

Inhibition of TE-thymocyte binding by using monoclonal antibodies. Either 2×10^6 thymocytes or 1.5×10^5 TE cells or both were preincubated with saturating amounts of monoclonal antibodies (ascites fluid or purified antibody at a 1/100 dilution) in 200 μl RPMI-FCS (4°C for 30 min), and cell suspensions were assayed in the TE-thymocyte binding assay as described above. Control cells were sham-treated by incubation with RPMI-FCS without antibody. Percent inhibition of TE-thymocyte binding was determined by the equation:

$$\text{Percent inhibition of TE-thymocyte binding} = \left[1 - \frac{\%\text{ TE-thymocyte rosettes with antibody}}{\%\text{ TE-thymocyte rosettes with RPMI-FCS}}\right] \times 100$$

In reciprocal washing experiments, 1×10^6 TE cells, 2×10^6 A431 cells, or 40×10^6 thymocytes were preincubated with a 1/100 dilution of antibody in 400 μl of RPMI-FCS for 30 min at 4°C. Remaining free antibody was removed by washing cells twice (250 × G, 5 min, 4°C) in 15 ml of RPMI-FCS. Cells were then examined for TE-thymocyte binding as described. Assays for inhibition of TE-thymocyte binding with antibody 3F10 (anti-MHC class I) and with antibody L243 (anti-MHC class II) were performed both in the presence of excess (unbound) antibody and after washing out excess antibody.

RESULTS

Screen of a panel of monoclonal antibodies for ability to inhibit TE-thymocyte binding. A large panel of anti-lymphocyte and anti-epithelial monoclonal antibodies was tested for ability to inhibit binding of thymocytes to cultured TE or A431 cells. Of the antibodies tested, only those directed against the CD-2 (LFA-2, T11, E-rosette receptor) molecule and the LFA-3 molecule inhibited TE-thymocyte and A431-thymocyte rosette formation (Table I). Anti-LFA-2 antibodies TS1/8 and TS2/18, as well as other anti-CD-2 antibodies T11/3Pt2H9, T11ROLD2-1H8, T11/7T47A9, and 35.1 blocked greater than 80% of TE-thymocyte or A431-thymocyte binding. The anti-CD2 antibodies D66 and T56 partially blocked TE-thymocyte binding (59 and 44% inhibition, respectively) (Table I). D66 and T56 have been shown to bind a subset of CD-2 positive peripheral blood T cells and to bind to a distinct epitope on the CD-2 molecule (13, 22). We have also observed that the monoclonal antibody TQ1, which inhibits E-rosette formation (22) and was found by Knowles to bind to the CD-2 molecule (23), completely inhibited thymocyte-A431 binding (not shown). We observed a slight inhibition (12 to 15%) of A431-thymocyte binding in the presence of CD-6 antibodies (Table I). The possible significance of this inhibition is currently being investigated.

Anti-LFA-1 (TS1/22, TS1/18) and anti-T8 antibodies that are known to inhibit binding of cytotoxic T lymphocytes (CTL) to target cells (24, 25), and anti-T3 antibodies that inhibit CTL-mediated killing at a post-conjugation step (26) did not have any effect on TE-thymocyte binding (Table II). Other monoclonal antibodies that did not inhibit TE-thymocyte binding are shown in Table II.

As previously reported (7), antibodies against HLA class I molecules (3F10) and HLA class II molecules (L243) had no effect on TE-thymocyte binding (Table II). To demonstrate that the TE-thymocyte binding observed in the presence of anti-HLA antibodies was not due to bridging of TE cells to thymocytes by bivalent anti-HLA antibodies, thymocytes and TE cells were pretreated with anti-HLA antibodies, followed by addition of anti-LFA-2 antibody before mixing thymocytes with TE cells. As shown in Table III, CD-2 antibody TS1/8 completely inhibited TE-thymocyte binding in the presence of anti-HLA class I or class II antibodies. Similar results were obtained with the A431 cell line (not shown).

Inhibition of TE-thymocyte binding by anti-CD-2 and anti-LFA-3 antibodies was concentration dependent, with maximum inhibition occurring at a 1/1000 dilution of TS1/8 ascites fluid (anti-CD-2) and at a 1/1000 dilution of TS2/9 ascites fluid (anti-LFA-3) (Fig. 1).

To determine the site of inhibition by anti-CD-2 (TS1/8) and anti-LFA-3 (TS2/9) antibodies, experiments were performed by using either TE cells, thymocytes, or both that had been preincubated with antibody, washed, and

TABLE III

Epithelial-thymocyte binding was not inhibited in the presence of Anti-MHC class I or class II (Ia) antibodies[a]

TE	Thymocytes	Anti-CD-2	Percent Rosettes	Percent Inhibition
—	—	–	25	0
—	—	+	2	92
Anti-MHC class I	Anti-MHC class I	–	25	0
Anti-MHC class I	Anti-MHC class I	+	2	92
Anti-Ia	Anti-Ia	–	27	0
Anti-Ia	Anti-Ia	+	2	92

[a] Thymocytes and TE cells were preincubated with either media (—), anti-HLA-class I (3F10), or anti-HLA-class II (Ia) (L243) antibodies for 30 min at 4°C. Either media (—) or anti-CD-2 antibody (TS1/8) in saturating amounts was added to thymocyte suspensions, and after 5 min more incubation, TE cells were added. The data are expressed as mean percent rosette formation and also as % inhibition of binding. The data are from one representative experiment of three experiments.

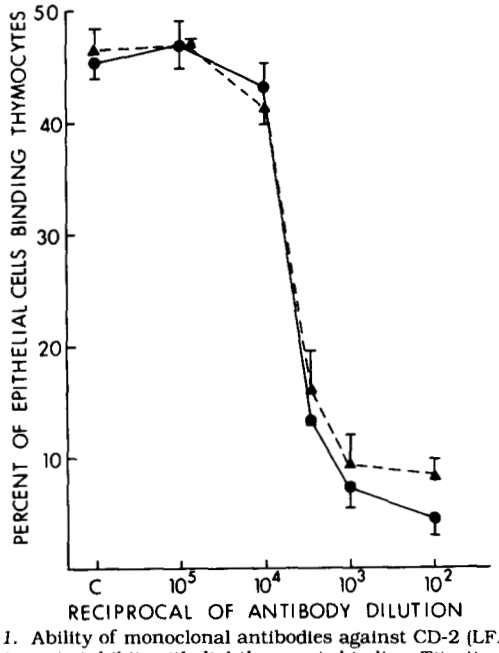

Figure 1. Ability of monoclonal antibodies against CD-2 (LFA-2) and LFA-3 antigens to inhibit epithelial-thymocyte binding. Titration of monoclonal antibody TS1/8 against CD-2 (LFA-2) antigen (●) and antibody TS2/9 against LFA-3 antigen (▲) for inhibition of T cell-epithelial cell binding was tested by using A431 cells and allogeneic thymocytes. The results are expressed as percent rosettes ± SEM and represent the mean of triplicate points. *C* indicates the level of control binding when no antibody was added.

TABLE IV

Effect of pretreatment of thymocytes and TE cells with anti-CD-2 or anti-LFA-3 antibodies on epithelial cell-thymocyte binding[a]

Pretreatment		Percent TE-Thymocyte Rosettes	Percent Inhibition
TE	Thymocyte		
—	—	31	0
—	Anti-CD-2	2	94
Anti-CD-2	—	28	10
Anti-CD-2	Anti-CD-2	2	94
—	Anti-LFA-3	32	0
Anti-LFA-3	—	2	94
Anti-LFA-3	Anti-LFA-3	2	94

[a] Thymocytes and TE cells were pretreated with either media (—), anti-CD-2 (TS1/8), or anti-LFA-3 (TS2/9), were washed, and were tested for ability to form rosettes. The results are expressed as mean percent of cells rosetting with thymocytes and also as percent inhibition of epithelial-T cell binding. The data are from one representative experiment of three experiments.

then tested for binding in the TE-thymocyte binding assay (Table IV). Inhibition of thymocyte binding to A431 cells by anti-LFA-2 and anti-LFA-3 antibodies was also tested. Inhibition of TE-thymocyte binding occurred when TE cells were pretreated with anti-LFA-3 (94% inhibition) or thymocytes were pretreated with anti-CD-2 (94% inhibition). Inhibition of binding of thymocytes to A431 was similarly inhibited when thymocytes were pretreated with anti-CD-2 and when A431 cells were pretreated with anti-LFA-3 (not shown). Previously we have shown that the ability of thymocytes to bind to TE cells was trypsin sensitive (7). By using indirect immunofluorescence assays and cytofluorograph analysis, we observed that the loss in ability of trypsinized thymocytes to bind to TE cells paralleled the trypsin-induced loss of CD-2 molecules from the thymocyte cell surface (data not shown).

Expression of LFA-2, (CD-2) and LFA-3 antigens by thymocytes and TE cells in vivo and in vitro. By using indirect IF assays with antibodies TS1/8, 35.1, and TS2/9, we investigated the distribution of LFA-2 (CD-2) and LFA-3 antigens on TE cells, thymocytes, and A431 epithelial cells in vitro, and on frozen sections of normal human thymus. As previously reported (27), resting thymocytes were less than 10% LFA-3$^+$, and greater than 95% CD-2$^+$ (Fig. 2*A* and *B*). Cultured thymic epithelial cells were 98% LFA-3$^+$ (Fig. 2*C*) and CD-2 negative. The A431 cell line was 99% LFA-3$^+$ (Fig. 2*D*) and did not express CD-2 antigen. Figures 3*A* and 3*B* show a cytocentrifuge preparation of cultured human TE cells in vitro in double IF assay labeled with anti-LFA-3 (Fig. 3A) and rhodamine-conjugated goat anti-mouse immunoglobulin, and the same cell reacted with fluoresceinated anti-keratin antibody AE-1 (Fig. 3*B*). Figure 3C shows a CD-2 negative TE cell bound to CD-2-positive autologous thymocytes, whereas Figure 3*D* shows an LFA-3-positive TE cell bound to LFA-3 negative autologous thymocytes.

To confirm that thymic epithelial cells expressed LFA-3 antigens in vivo, we performed double immunofluorescence assays on frozen sections of human thymus by

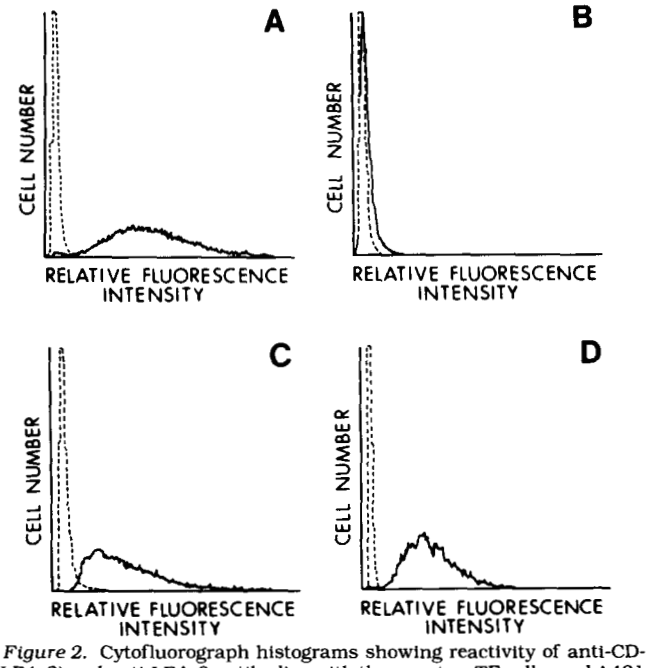

Figure 2. Cytofluorograph histograms showing reactivity of anti-CD-2 (LFA-2) and anti-LFA-3 antibodies with thymocytes, TE cells, and A431 epithelial cells. *Panel A* shows anti-LFA-2 antibody (TS1/8) reactivity with thymocytes. *Panel B* shows anti-LFA-3 antibody (TS2/9) reactivity with thymocytes. *Panel C* shows anti-LFA-3 antibody (TS2/9) reactivity with TE cells. *Panel D* shows anti-LFA-3 antibody (TS2/9) reactivity with A431 epithelial cells.

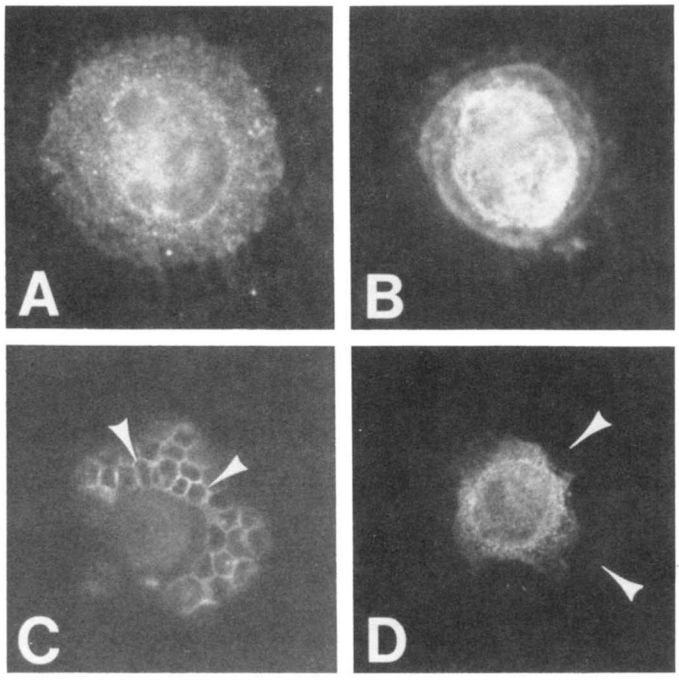

Figure 3. Phenotypic analysis of TE cells and thymocytes in autologous TE-thymocyte rosettes. *Panels A* and *B* show the same TE cell binding, both anti-LFA-3 antibody (TS2/9) visualized with rhodamine-conjugated goat anti-mouse immunoglobulin (Panel A) and directly fluoresceinated anti-keratin monoclonal antibody (AE1) (*Panel B*). In indirect imunofluorescence assays of cytocentrifuge preparations of autologous TE-thymocyte rosettes, anti-LFA-2 antibody (*Panel C*) bound to thymocytes (*arrows*), but not to the central TE cell, and anti-LFA-3 (TS2/9) bound to TE cells (*Panel D*), but not to surrounding thymocytes (*arrows*) (×400).

using a directly fluoresceinated anti-keratin antibody AE-1 to identify epithelial cells along with anti-LFA-3 detected by a rhodamine conjugated goat anti-mouse Ig. Anti-LFA-3 reacted with thymus in an epithelial pattern in thymic cortex and medulla. Figure 4 shows cells which react with both AE-1 (Fig. 4*A*) and anti-LFA-3 (Fig. 4*B*). Occasional cells were seen that reacted with anti-LFA-3 and not AE-1 (*small arrow*). All appropriate controls were negative (not shown).

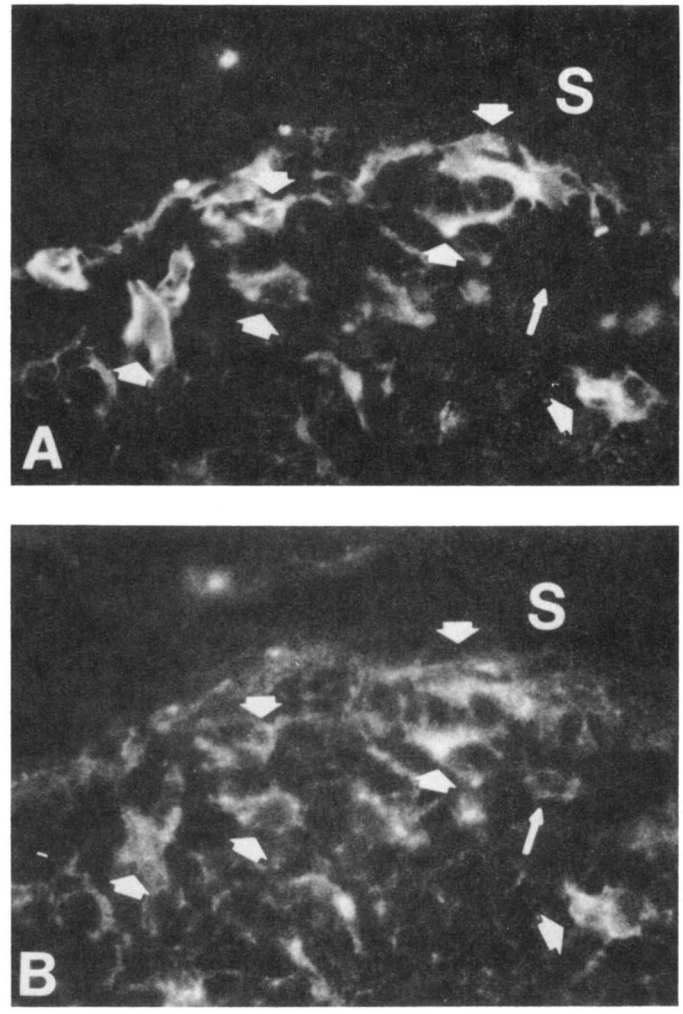

Figure 4. Reactivity on human thymus of anti-keratin antibody AE-1 (*Panel A*) and anti-LFA-3 antibody TS2/9 (*Panel B*) in double immunofluorescence assay on the same frozen section. Antibody AE-1 was directly conjugated to fluorescein, whereas, antibody α LFA-3 was detected with rhodamine-conjugated goat anti-mouse Ig. The *solid arrows* show AE-1$^+$ cells in *Panel A* that are anti-LFA-3$^+$ in *Panel B*. The *long arrow* shows an AE-1$^-$ cell in *Panel A* that is positive for anti-LFA-3 *Panel B*. *S* indicates interlobular septum (×400).

DISCUSSION

In this study, we have shown that antibodies against the CD-2 antigen (T11, LFA-2, E-rosette receptor) on thymocytes and the LFA-3 molecule on TE cells inhibit the binding of human thymocytes to autologous and allogeneic thymic epithelial cells. These antibodies previously have been shown to inhibit binding of cytotoxic T lymphocytes (CTL) to target cells (24); Anti-LFA-2 antibodies inhibited binding at the level of the CTL, whereas anti-LFA-3 antibody inhibited CTL-target binding at the level of the target cell (27). In this study, indirect immunofluorescence assays on sections of frozen thymus tissue, and cytofluorograph analysis of thymocyte and TE cell suspensions confirmed the presence of CD-2 antigen on thymocytes and LFA-3 antigen on thymic epithelial cells in vitro and in vivo.

It is of interest that the TE-thymocyte binding reported here did not appear to be mediated by MHC class I or class II (Ia) antigens. Antibodies to MHC class I and class II antigens did not inhibit binding, nor have quantitative differences been observed between autologous and allogeneic TE-thymocyte binding (7). It is important to note that although in vivo human TE cells express Ia antigens, cultured TE cells are Ia negative (5). However, treatment of cultured TE cells with interferon-γ (IFN) in vitro (28) or co-cultivation of TE cells with thymocytes (6) induces expression of Ia antigens and increases the level of expression of class I MHC antigens on TE cells. However, IFN-γ-treated TE cells that have been induced to express Ia antigens bind to thymocytes to the same degree as do Ia- TE cells, and anti-CD-2 antibodies inhibit completely the ability of Ia$^+$ TE cells to bind to thymocytes (Wolf, L., Haynes, B., Singer, K. unpublished observations). Taken together these data suggest that TE-thymocyte binding may not be related to MHC binding, nor to the generation of T cell MHC restriction, but rather TE cells may act as an intrathymic source of an endogenous ligand of the CD-2 antigen and promote thymocyte activation.

Meuer et al. (29) have demonstrated that a combination of antibodies against the $T11_2$ and $T11_3$ epitopes on the CD-2 (T11) molecule leads to IL 1 independent peripheral T cell activation. In contrast, CD-2 molecule triggering of thymocytes leads only to IL 2 receptor expression, with no thymocyte proliferation unless exogenous IL 2 is pro-

vided (30). The nature of the endogenous ligand in the thymus that triggers thymocytes via the CD-2 molecule to express IL 2 receptors is not known. Hunig (31, 32) has suggested that a 42,000 M_r glycoprotein expressed on sheep red blood cells, as well as sheep leukocytes binds to the CD-2 antigen in E-rosetting. An analogous molecule may be present on human cells. Milanese et al. (33) reported a 10,000 M_r T cell product (IL-4A) that activates peripheral T lymphocytes by binding to the CD-2 molecule. We have recently shown that TE-thymocyte binding leads to activation of mature thymocytes, induction of Ia antigens on TE cells, and potent TE accessory cell function for additional thymocyte activation by PHA (6). In addition, we have shown both cortical and medullary TE cells contain IL 1 in vivo and produce IL 1 in vitro (34).

Recently, we have demonstrated that TE-thymocyte binding results in thymocyte IL 2 receptor expression, and a low level of thymocyte activation that is augmented in the presence of exogenous IL 2. Moreover, TE cell-induced IL 2 receptor expression on thymocytes and TE accessory cell function for PHA-induced thymocyte activation are both inhibited by the addition of CD-2 antibody to thymocyte-TE co-cultures (Denning, S., L. Wolf, T. Springer, K. Singer, B. Haynes, unpublished observations). Thus we propose that human TE-thymocyte binding is mediated on thymocytes via the CD-2 molecule and that a cell membrane ligand for the CD-2 molecule in thymus is expressed on human TE cells. These observations suggest a possible role for TE cells in thymocyte activation by the CD-2 molecule.

The simplest and most attractive interpretation of the involvement of CD-2 (LFA-2) on thymocytes and of LFA-3 on TE cells in thymocyte-TE cell binding is a receptor-ligand interaction between CD-2 (LFA-2) and LFA-3. The CD-2 antigen has recently been shown to bind directly to the LFA-3 molecule on human erythrocytes (35). $CD\text{-}2^+$ thymocytes did not bind to $LFA\text{-}3^+$ cultured epidermal cells (7) or to $LFA\text{-}3^+$ corneal epithelial cells (Wolf, L., B. Haynes, and K. Singer, unpublished observations), which argues against direct binding. However, both LFA-2 and LFA-3 exhibit M_r heterogeneity on different cell types (27), and it is possible that glycosylation of LFA-3 on cultured epidermal or corneal cells is inappropriate for binding to LFA-2. Alternatively, the surface charge or other properties of epidermal or corneal cells may differ from thymic epithelial cells and hinder thymocyte binding. Although we cannot at present rule out blocking of TE-thymocyte binding by nonspecific effects on the cell membrane with anti-CD-2 and anti-LFA-3 antibodies, the fact that many other antibodies to antigens known to be expressed by thymocytes and TE cells did not block binding makes this possibility unlikely. Thus we propose that LFA-2 (CD-2) may interact directly with LFA-3. Certainly the ability of TE cells to act as a functional CD-2 ligand in triggering thymocyte proliferation (6) argues for direct involvement of the CD-2 antigen in TE-thymocyte binding, and studies are underway to address this question directly. Isolation and additional characterization of the molecules involved in TE-thymocyte binding should provide insight into mechanisms of human intrathymic T cell activation and maturation.

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