Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 4999–5003, July 1987
Immunology

Cloning of human thymic subcapsular cortex epithelial cells with T-lymphocyte binding sites and hemopoietic growth factor activity

(interleukin 1/transformed cell line/CD2 antigen/macrophage colony-stimulating factor/lymphocyte function-associated antigen 3)

SHUKI MIZUTANI*†, SUZANNE M. WATT*‡, DAVID ROBERTSON*, SHABIR HUSSEIN§, LYN E. HEALY*, ANDREW J. W. FURLEY*, AND MELVYN F. GREAVES*

*Leukaemia Research Fund Centre, The Institute of Cancer Research, Chester Beatty Laboratories, 237 Fulham Road, London SW3 6JB, United Kingdom; and §Department of Pathology, Royal College of Surgeons, London WC2, United Kingdom

Communicated by John Humphrey, April 6, 1987 (received for review January 14, 1987)

ABSTRACT **The thymic microenvironment involves complex cell interactions among different types of epithelial cells, macrophages, tissue histiocytes, and immature and maturing T cells. We describe the isolation of a subset of thymic epithelial cells by selective primary culture followed by cotransfection with a simian virus 40 replication-origin-defective mutant and pSV2neo plasmid. The cloned cells have the composite immunophenotype that is unique to thymic subcapsular epithelial cells, suggesting that they may provide a model system *in vitro* for analyzing the earliest steps in T-cell differentiation. This possibility is supported by the finding that these epithelial cells express LFA-3-associated binding sites for T cells, secrete a macrophage hemopoietic growth factor, and synergize with macrophages in the production of interleukin 1.**

Critical steps in the early differentiation of T lymphocytes occur within the thymus. Bone marrow-derived cells migrating into this organ undergo extensive proliferation, clonal rearrangement of antigen-receptor genes, and an associated immunological "education" involving tolerance to self antigens and positive selection for antigen recognition in association with self molecules encoded by the major histocompatibility complex (1–4). The control of this complex process is not understood but almost certainly involves selective interactions with distinct elements of the thymic stromal environment and diffusible or cell-bound regulators or growth factors (5, 6).

The non-lymphoid stromal structure of thymic tissue consists of phenotypically distinct subsets of epithelial cells derived from pharyngeal pouch endoderm or branchial cleft ectoderm, mesenchymal cells, and bone marrow-derived histiocytes or interdigitating macrophages (2, 6–8). Some success has been reported in culturing rodent and human thymic epithelial cells (9–12). Culture supernatants from such cells can regulate T-cell phenotype or immunological function. However, cloned lines representing phenotypically and functionally distinct subtypes of human thymic epithelia have not been reported.

The subcapsular epithelium of thymus has a distinctive structure and is probably the first site of interaction with migrating T-cell precursors (2, 6, 8). We have sought to isolate and grow these epithelial cells from human fetal thymus by a combination of selective culture conditions and gene-transfection techniques. We report here the successful establishment of two such cell strains, which retain phenotypic properties specific for subcapsular cortex epithelium and express functional activities that may regulate early steps in T-cell differentiation.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Primary Epithelial Cell Culture. Epithelial cells were derived from thymus of 16-week-old human fetuses, obtained from the Royal Marsden Hospital (London). Thymic tissue was grown as described by Singer *et al.* (12).

Transfection of Primary Epithelial Cells. Epithelial cultures were transformed by calcium phosphate-mediated (13) cotransfection with the simian virus 40 (SV40) ori$^-$ mutant 6-1 (14, 15), which lacks 6 base pairs at the SV40 origin of DNA replication, and pSV2neo (16), a plasmid containing the bacterial neomycin-resistance gene (*neo*) under control of SV40 promoter and enhancer sequences. One month after transfection, cells were selected for neomycin resistance with G418 (GIBCO) at 1 mg/ml. Two clones (SM1 and SM2) were obtained from these transfections. The SM1 cells were subcloned by limiting dilution using primary irradiated epithelial cells as feeder layers, and the subclones SM1.1 and SM1.9 were selected for resistance to G418.

Antibody Analysis. Standard immunofluorescence techniques (49) were used to assess binding of antibodies (listed in Table 1) to viable epithelial cell suspensions or to preparations of cells fixed with paraformaldehyde, acetone, acetone/methanol, or Bouin's reagent. Thymopoietin and thymulin (FTS, facteur thymique sérique) were assessed on acetone-fixed preparations by use of a rabbit anti-thymopoietin antibody (courtesy of G. Goldstein, Ortho Diagnostics) and a monoclonal anti-thymulin antibody (17).

Growth-Factor Assays. *Colony assays.* Epithelial cell clones were assayed for the production of hemopoietic stimulatory activities by the double-layer agar system of Gualtieri *et al.* (18). SM1, SM2, SM1.1, and SM1.9 cells and primary thymic epithelial cells served as feeder layers in this assay. Mouse L cells were used as a negative control. Adherent-cell-depleted, low-density (<1.077 g/ml) human fetal liver cells were used as a source of hemopoietic cells. Cultures were incubated for 7–14 days at 37°C in a 5% CO_2 atmosphere. An inverted microscope was used to count colonies of 50 or more cells. Cultures without feeder layers were used to determine the number of spontaneously forming colonies. Colonies were typed *in situ* (18) or were picked off and stained histochemically (19) and assayed for reactivity with monoclonal antibodies. Macrophage colony-stimulating factor (colony-stimulating factor 1, CSF-1) activity was measured by radioimmunoassay (20) of the SM1 day-7 and day-14 conditioned medium, which either was unconcentrated or was concentrated by Amicon filtration (PM10 membrane).

Abbreviations: SV40, simian virus 40; SV40 ori, SV40 origin of DNA replication; IL-1 and IL-2, interleukins 1 and 2; CSF-1, colony-stimulating factor 1 (macrophage colony-stimulating factor).

†Present address: The National Children's Medical Research Center, 3-35-31 Taishido, Setagaya-ku, Tokyo, Japan.
‡To whom reprint requests should be addressed.

Interleukin assays. Conditioned media from cocultures of SM1 and SM2 cells with low-density, adherent-cell-depleted fetal liver cells and from SM1, SM2, and primary thymic epithelial cells after 14 days of culture were analyzed for interleukin 1 (IL-1) activity (21, 22). Interleukin 2 (IL-2) production by SM1 and SM2 was assayed using Con A-stimulated mouse thymocytes (23) and CT6 cells (24). Medium plus 5% fetal bovine serum served as a negative control. Recombinant IL-1 (Cistron, Pine Brook, NJ) was the positive control.

Rosetting. Thymocyte rosettes with SM1 and SM2 cells, inhibition of the rosette reaction, and thymocyte binding to monolayers of SM1 and SM2 cells were assayed at 4°C as described (25).

Electron Microscopy. Cells grown on 35-mm tissue culture dishes were fixed for 2 hr at room temperature with 1% glutaraldehyde in phosphate-buffered saline and processed for transmission electron microscopy (26). Cultures for scanning electron microscopy were fixed overnight in 1% glutaraldehyde in cacodylate buffer and postfixed with 2% tannic acid for 1 hr at room temperature and with 1% OsO_4 for 1 hr at room temperature. After dehydration, samples were critical-point-dried in an EMscope CPD750, mounted on stubs, sputter-coated with gold by an EMscope SC500, and examined in a Hitachi S800 electron microscope.

RESULTS

Transformation of Thymic Epithelial Cells. Cotransfection of primary thymic epithelial cells with the SV40 ori$^-$ mutant 6-1 and pSV2neo provided two transformed colonies, SM1 and SM2. The SM2 cells are polygonal and appear to be morphologically homogeneous, whereas the SM1 cells consist of two morphologically distinct cell types. By limiting dilution, two subclones of SM1 were selected. The SM1.1 subclone (Fig. 1*A*) contains fibroblastoid cells, while the SM1.9 subclone (Fig. 1*B*) comprises the polygonal cells.

Characterization. Transfection of cells with SV40 ori$^-$ DNA was confirmed by the presence of nuclear large tumor (T) antigen in the original clones (Fig. 1*D*) and in the subclones. The epithelial nature of the cells was confirmed by cytokeratin staining using the Le61 antibody (Fig. 1*C*). SM1, SM2, and the subclones all have desmosomes (Fig. 2*A* and *C*) and tonofilaments (Fig. 2 *B* and *D*), which characterize cells as epithelial. The monoclonal antibodies used to define the type of epithelial cells isolated are presented in Table 1. Strong reactivity was found with anti-human Thy-1, whereas the anti-LFA-3 and anti-GQ ganglioside antibodies reacted to a lesser extent (Table 1; Fig. 3). Monoclonal antibody MR19 stained all the SM1 and SM2 cells, but no reactivity was observed with the thymic medulla-specific monoclonal antibody PE-35 or with antibodies (MR7, MR9, and MR13) to the thymic cortical epithelium and Hassall's bodies (Table 1). The SV40 ori$^-$-transformed cells express HLA-A, -B, and -C determinants but not HLA-DR, -DQ, or -DP. Lymphoid- and monocytoid-specific cell markers were uniformly negative (Table 1). These findings indicate that SM1 and SM2 are derived from subcapsular cortex epithelium. The presence of chromogranin is thought to be indicative of endocrine function within the neuroendocrine system and has been identified in rat thymic epithelium (40). Neither chromogranin nor the neurotransmitters vasopressin, neurophysin II, and synaptophysin were detected in the isolated clones by monoclonal antibodies (Table 1). There was no staining with antibodies to the thymic hormones thymopoietin and thymulin.

T-Lymphocyte Binding. Human cortical thymic epithelial cells in primary culture bind T cells via the LFA-3 molecule on epithelial cells and the CD2/gp50 molecule on T cells (27, 30). Our subcapsular thymic epithelial cell clones also exhibit this characteristic. Fifty to seventy percent of the SM2 cells formed rosettes with juvenile thymocytes (Table 2). A431, a non-thymic epithelial cell line, also formed rosettes, whereas embryonic fibroblasts did not (Table 2). The rosette reaction could be inhibited by pretreating the SM1 and SM2 cells with a monoclonal antibody to LFA-3 or the thymocytes with a monoclonal antibody to CD2 (Table 2). Significant inhibition was not observed with anti-Thy-1 or anti-CD7. Attachment of thymocytes to monolayers of SM1 and SM2 cells was also observed (Fig. 4). Transmission electron micrographs showed the close association of thymocytes with the epithelial cell strains.

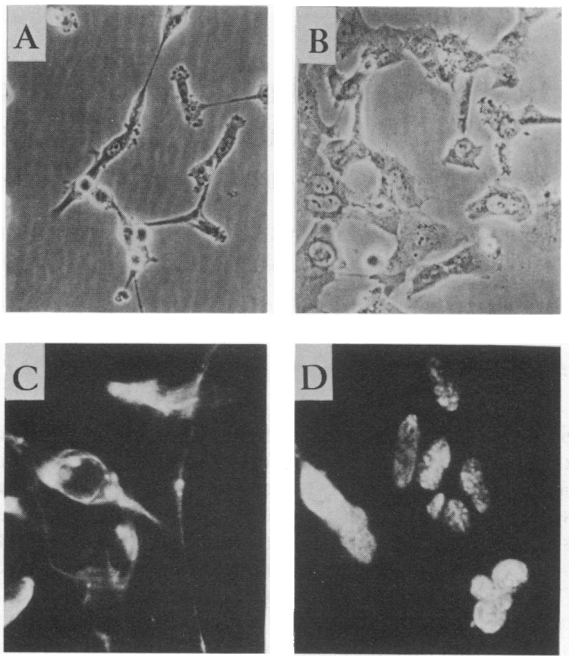

FIG. 1. Growth of SM1 subclones SM1.1 (*A*) and SM1.9 (*B*) in monolayer cultures. Immunofluorescence staining of SM1 cells for cytokeratin (*C*) and for SV40 large tumor (T) antigen (*D*). Clone SM2 is similar to SM1.9.

Subcapsular Cortex Epithelial Cells Support Hemopoietic Cell Growth. Two types of colonies can be stimulated by the SV40 ori$^-$-transformed epithelial cell strains when fetal liver is used as a source of hemopoietic progenitors in a double-layer agar assay; one type consists of large, tightly packed cells, and the other comprises more diffuse colonies of smaller cells (Table 3). Cytochemical staining revealed that both colony types contained nonspecific esterase (α-naphthyl

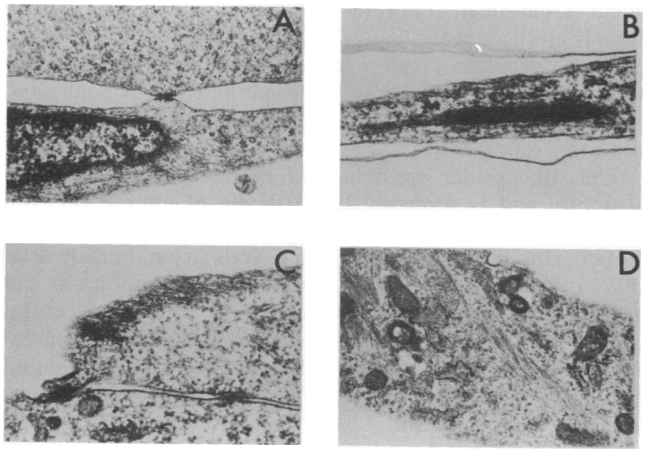

FIG. 2. Electron micrographs of SM1 (*A* and *B*) and SM2 (*C* and *D*) cells showing the presence of desmosomes (*A* and *C*) and tonofilaments (*B* and *D*).

Table 1. Immunophenotype of SV40 ori$^-$-transformed thymic epithelial cells

Antibody			Reactivity* with thymic subcapsular cortex epithelium	
Specificity (ref.)		Name	*In situ*	SV40 ori$^-$ cells
Thy-1 glycoprotein	(27)	Thyl	+	+
GQ gangliosides	(2)	A_2B_5	+	+ (54–83%)
Thymic epithelium, a†	(6)	RFD4	+‡	–
LFA-3	(28)	TS2/9	+	+
Thymic epithelial subsets†	(29)			
a		909 (MR19)	+	+
b		910 (1st 8.18)	–	–
c		908 (MR13)	–	–
d		899 (MR9)	–	–
d		899 (MR7)	–	–
Thymic medullary epithelium	(30)	PE-35	–	–
Cytokeratin	(31)	Le61	+	+
Vimentin	(§)	Clone 9	+	+
Factor VIII	(¶)	VIII R:Ag	–	–
Macrophages	(6)	RFD7	–	–
	(32)	3.9	–	–
	(32)	C.7	–	–
Transferrin receptor	(33)	OKT9	+	+
EGF receptor	(34)	R1		+
HLA-A and -B	(35)	B7/8	+	+
HLA-DP	(36)	B7/21		–
HLA-DQ	(36)	Genox 3.53		–
HLA-DR/DP/DQ	(36)	DA2	–	–
HLA-DR/DQ	(36)	DA6.164		–
HPCA-1/gp100–120	(37)	BI.3C5	–	–
CALLA/gp100	(38)	AL2	–	–
T-lymphocyte subsets	(39)	Anti-Leu-6 (CD1), -Leu-5 (CD2), -Leu-4 (CD3), -Leu-3a (CD4), -Leu-2 (CD8), -Leu-9 (CD7)	–	–
Chromogranin	(40)	ND		–
Synaptophysin	(41)	SY38		–
Vasopressin	(‖)	ND		–
Neurophysin II	(**)	WP13-3-72		–
T200/LCA	(42)	F8.11.13	–	–
Thymus, e†	(2)	TE-7	–	–
SV40 large tumor antigen	(43)	SV-40	–	+

LFA-3, lymphocyte function-associated antigen 3; factor VIII, blood coagulation factor VIII; EGF, epidermal growth factor; HPCA 1, hemopoietic progenitor cell antigen 1; gp*n*, glycoprotein of n kDa; CALLA, common acute lymphoblastic leukemia antigen; LCA, leukocyte common antigen; T antigen, large tumor antigen; ND, no designation.

*+, >98% of cells positive unless otherwise shown; –, <2% of cells positive.

†Reactive with subcapsular cortex and medulla (a), Hassal's corpuscles and a subset of medulla (b), Hassal's corpuscles (c), cortex (d), or mesoderm-derived fibrous connective tissue and vessels in thymus (e).

‡Positive only in postnatal thymus.

§Sanbio, Uden, Holland.

¶Royal Free Hospital, London.

‖Wellcome Diagnostics, Beckenham, U.K.

**RIA, Washington, U.K.

butyrate esterase)- and acid phosphatase-positive cells, indicative of their monocyte/macrophage nature. Morphologically, the more diffuse colonies contained small monocytes, while the tightly packed colonies appeared to be activated macrophages. The latter stained with RFD-7 and anti-HLA-DR monoclonal antibodies. None of the hemopoietic colonies formed contained either intracellular terminal deoxynucleotidyltransferase or T3, markers characteristic of T-cell precursors (44). Macrophage colony growth was also observed when primary thymic epithelial cells were used as the feeder layer, albeit in higher numbers than was observed using either SM1 or SM2.

Radioimmunoassay of SM1 conditioned media revealed CSF-1 concentrations of 62 ± 20 units/ml (n = 3; S.M.W. and E. R. Stanley, unpublished data).

SM1 and SM2 culture supernatants were assayed by L. Vodinelich and colleagues (Celltech, Slough, U.K.) for mycoplasma by DNA fluorescent staining (Hoechst staining kit), 6-methylpurine deoxyriboside cytotoxicity, and microbiological culture (45), as well as for γ-interferon (using Celltech assay systems), IL-2, IL-1, and macrophage-activating factor (46), and were found to be uniformly negative in all cases.

IL-1 Production. Fig. 5 shows that although IL-1 was present in the primary epithelial cell cultures (bar H), no IL-1 was detected in conditioned medium from the SM2 or SM1 cell strains (bars B and E) when compared with negative controls (bars A and D). However, when SM1 and SM2 cells were cocultured with fetal liver cells for 14 days in order to allow macrophage maturation and activation, IL-1 was detected in the culture supernatants (bars C and F).

DISCUSSION

The thymic stromal cell strains that we have isolated from human fetal thymus are clearly derived from the subcapsular

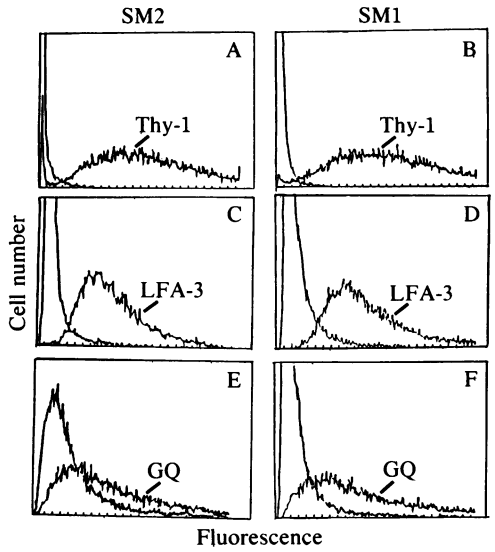

FIG. 3. Flow cytofluorometric analysis (FACS IV; Becton Dickinson) of SM1 and SM2 cells, using monoclonal anti-Thy-1 (*A* and *B*), anti-LFA-3 (*C* and *D*), and anti-GQ ganglioside (*E* and *F*) antibodies. For the negative controls (left histogram in each panel), the first antibody was replaced with an irrelevant antibody of the same immunoglobulin subclass. Fluorescein-conjugated second antibody was used in all cases. Photomultiplier gains were set at 16 (*C–F*) or at 1 (*A* and *B*).

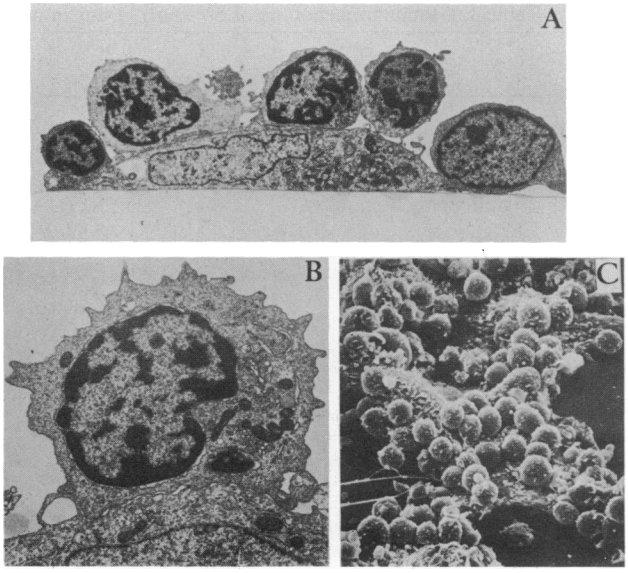

FIG. 4. Attachment of thymocytes to SM1 cells. SM1 cells were grown on 24-well dishes prior to addition of thymocytes for 1.5 hr at 4°C. The nonadherent thymocytes were removed and the remaining cells were fixed with glutaraldehyde and processed for transmission (*A* and *B*) or scanning (*C*) electron microscopy. (*A*, ×2600; *B*, ×24,000; *C*, ×700.)

cortex epithelium. This is indicated (*i*) by ultrastructural features; (*ii*) by their expression of cytokeratin and of determinants recognized by anti-Thy-1, MR19, and A_2B_5 antibodies (2, 27, 29); and (*iii*) by their lack of reactivity with a number of monoclonal antibodies (2, 6, 29, 30, 39) specific for macrophages, thymic fibroblasts, Hassall's corpuscles, and cortical and medullary epithelia.

The mechanisms by which thymic subcapsular cortex epithelial cells promote the differentiation and proliferation of thymocytes are not understood. They could interact directly with T-cell precursors or activate these precursors by the production of lineage-specific or multilineage growth factors. Putative thymic hormones such as thymopoietin, thymosin, and thymulin may be implicated in mediating some of these events (5, 6). Neither the subclones, SM1.1 and SM1.9, nor the two original clones, SM1 and SM2, produce detectable levels of thymopoietin or thymulin when analyzed with monoclonal antibodies. Two plausible explanations for these observations are that SV40 transformation affects hormone production or that the cells isolated do not normally secrete such factors. The findings that subcapsular epithelial cells do, however, produce and release CSF-1, which activates macrophage precursors, and that they also synergize with macrophages to produce IL-1 have important implications for both thymus histogenesis and T-cell development. The macrophage population of the thymus almost certainly makes a critical contribution to T-cell ontogeny and clonal diversity via, for example, IL-1 release (47) or tolerance induction (48). IL-1 binding to T cells appears to provide an activation signal resulting in the subsequent expression of IL-2 receptors. Our observations suggest that production of CSF-1 or other hemopoietic factors by subcapsular epithelial cells could play a role in the expansion of a small number of macrophage progenitors known to colonize the thymus at approximately the same time as the lymphoid precursors enter this organ during thymic embryogenesis (7) and could also serve to activate thymic macrophages to secrete IL-1.

The subcapsular cortex epithelium also has unique structural and phenotypic features that are conserved in the transformed cells and are likely to play a key role in cell interactions and T-lymphocyte differentiation and clonal diversification. Precursor T cells at all stages of differentiation can bind to primary epithelial cells derived from the thymic cortex via an interaction involving the LFA-3 mole-

Table 2. Inhibition of thymocyte rosettes by pretreatment of cells with various antibodies

Cells	% inhibition				
	Untreated	Anti-LFA-3	Anti-Thy-1	Anti-CD7	Anti-CD2
	Experiment 1				
SM1	66 ± 2	6 ± 2	60 ± 2	68 ± 4	4 ± 2
SM2	60 ± 3	10 ± 9	63 ± 3	60 ± 13	5 ± 1
A431	49 ± 5	2 ± 1	55 ± 1	53 ± 8	3 ± 2
Flow 5000	5 ± 2	ND	ND	ND	ND
	Experiment 2				
SM1	48 ± 3	10 ± 2	52 ± 18	48 ± 12	14 ± 10
SM2	77 ± 5	12 ± 4	58 ± 12	50 ± 4	11 ± 3
A431	49 ± 5	15 ± 3	36 ± 17	36 ± 17	10 ± 6
Flow 5000	11 ± 1	ND	ND	ND	ND

Values are means ± SD of triplicate samples for each thymus donor; ND, not done. Thymuses from infants less than 1 year old were used. Flow 5000 cells are human embryo fibroblasts.

Table 3. Stimulation of growth of human fetal liver cell colonies

Feeder layer	Macrophage colonies per 10^5 cells plated	
	Day 7	Day 14
None	0 ± 0	0 ± 0
Mouse L cells	0 ± 0	0 ± 0
SM1	71 ± 31	112 ± 8
SM1.1	175 ± 76	348 ± 134
SM1.9	96 ± 46	107 ± 53
SM2	103 ± 51	150 ± 23

Values are means ± SD of four replicate cultures. Stromal cells (2×10^5) were plated in 35-mm dishes 24 hr before X-irradiation (3000 rads; 1 rad = 0.01 Gy). Fetal liver cells (28,000; density < 1.077 g/ml) were plated in agar above the feeder layer. Colonies of ≥50 cells were scored at days 7 and 14.

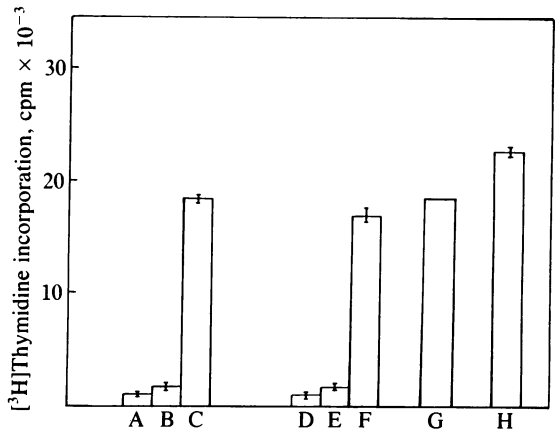

FIG. 5. Analysis of IL-1 production. IL-1 activity was analyzed in conditioned media by stimulation of [^{3}H]thymidine incorporation by D.10 cells. Bars A and D: negative controls with unconditioned medium (Dulbecco's modified Eagle's medium plus 5% fetal bovine serum). Bars B and E: conditioned media from SM2 and SM1 cell cultures, respectively. Bars C and F: conditioned media from cocultures of fetal liver monocytes with SM2 and SM1 cells, respectively. Bar G: unconditioned medium supplemented with recombinant IL-1 (positive control). Bar H: conditioned medium from primary thymic epithelial cell cultures. All samples were diluted 1:8 prior to analysis. IL-1 activity was not detected in undiluted samples of unconditioned medium or of SM1 or SM2 conditioned medium.

cule on the epithelial cells and the CD2/gp50 structure on T cells (28). The SV40 ori$^-$-transfected subcapsular epithelial cells express LFA-3-associated binding sites for T cells. Binding of thymocytes to these epithelial cells also appears to involve LFA-3–CD2 interactions.

Our data support the view that activation of precursor T cells could occur within the thymus by contact interaction with subcapsular epithelial cells and response to IL-1 produced by epithelial cell–macrophage interactions. Availability of transformed derivatives of the subcapsular cortex thymic epithelium should aid in further understanding these regulatory interactions and may provide an assay system for the so far elusive circulating pre-T-cell progenitors.

We thank Drs. C. Marshall and H. Patterson for their advice and help concerning transfection procedures; Prof. S. Lawler, Dr. L. Wong, and Mr. C. Lincoln for the thymic tissue; Drs. Y. Gluzman and M. Fried for the SV40 ori$^-$ DNA; Dr. R. Newbold for the pSV2neo plasmid; Drs. F. Bach, J. Polak, D. Lane, B. Lane, M. Ritter, N. Hogg, G. Janossy, T. Springer, and B. Haynes for antibodies; Mr. G. Harrison for technical assistance; Mr. D. Claugher for the stereoscan photographs; Ms. L. Altass for flow cytometry; and Miss Geraldine Parkins for typing the manuscript. We are particularly grateful to Drs. B. Haynes, T. Springer, S. Chang, M. Ritter, and E. R. Stanley for providing valuable advice and preprints prior to publication. This work is supported by the Leukaemia Research Fund of Great Britain.

1. Zinkernagel, R., Callahan, C., Althage, A., Cooper, S., Klein, P. & Klein, J. (1978) *J. Exp. Med.* **147**, 882–896.
2. Haynes, B. F. (1984) *Adv. Immunol.* **36**, 87–142.
3. Rothenberg, E. & Lugo, J. P. (1985) *Dev. Biol.* **112**, 1–17.
4. Lo, D. & Sprent, J. (1986) *Nature (London)* **319**, 672–675.
5. Oates, K. K. & Goldstein, A. L. (1984) *Trends Pharmacol. Sci.* **5**, 347–352.
6. Janossy, G., Bofill, M., Trejdosiewicz, L. K., Willcox, H. N. A. & Chilosi, M. (1986) in *Current Topics in Pathology: The Human Thymus*, ed. Muller-Hermelink, H. K. (Springer, Berlin), Vol. 75, pp. 89–125.
7. Le Douarin, N. M., Dieterlen-Lievre, F. & Oliver, P. D. (1984) *Am. J. Anat.* **170**, 261–299.
8. Van de Wijngaert, F. P., Kendall, M. D., Schurrman, H.-J., Rademakers, L. H. P. H. & Kater, L. (1984) *Cell Tissue Res.* **237**, 227–237.
9. Itoh, T., Aizu, S., Kasahara, S. & Mori, T. (1981) *Biomed. Res.* **2**, 11–19.
10. Beardsley, T. R., Pierschbacher, M., Wetzel, G. D. & Hays, E. F. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6005–6009.
11. Glimcher, L. H., Kruisbeek, A. M., Paul, W. E. & Green, I. (1983) *Scand. J. Immunol.* **17**, 1–11.
12. Singer, K. H., Harden, E. A., Robertson, A. L., Lobach, D. F. & Haynes, B. F. (1985) *Hum. Immunol.* **13**, 161–176.
13. Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52**, 456–467.
14. Gluzman, Y., Sambrook, J. F. & Frisque, R. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3898–3902.
15. Nagata, Y., Diamond, B. & Bloom, B. R. (1983) *Nature (London)* **306**, 597–599.
16. Southern, P. J. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1**, 327–341.
17. Berrih, S., Savino, W., Azoulay, M., Dardenne, M. & Bach, J.-F. (1984) *J. Histochem. Cytochem.* **32**, 432–438.
18. Gualtieri, R. J., Shadduck, R. K., Baker, D. G. & Quesenberry, P. J. (1984) *Blood* **64**, 516–525.
19. Bancroft, J. D. & Cook, H. C. (1984) *Manual of Histological Techniques* (Churchill Livingstone, Edinburgh).
20. Stanley, E. R. & Guilbert, L. J. (1981) *J. Immunol. Methods* **42**, 253–284.
21. Kay, J., Porcelli, S., Tite, E., Jones, B. & Janeway, C. A. (1983) *J. Exp. Med.* **158**, 836–856.
22. Trechsel, U., Murphy, J. & Reynolds, J. (1982) *Biochim. Biophys. Acta* **720**, 364–369.
23. Coutinho, A., Larsson, E., Bronisk, K. & Anderson, J. (1979) *Eur. J. Immunol.* **9**, 587–592.
24. Gillis, S., Fern, M. M. & Smith, K. A. (1978) *J. Immunol.* **120**, 2027–2030.
25. Singer, K. H., Wolf, L. S., Lobach, D. F., Denning, S. M., Tuck, D. T., Robertson, A. L. & Haynes, B. F. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6588–6592.
26. Kuhn, G. (1981) *J. Histochem. Cytochem.* **29**, 84–86.
27. Ritter, M. A., Sauvage, C. A. & Cotmore, S. F. (1981) *Immunology* **44**, 439–446.
28. Sanchez-Madrid, F., Krensky, A. M., Ware, C. F., Robbins, E., Strominger, J. L., Burakoff, S. J. & Springer, T. A. (1982) *Immunol. Today* **79**, 7489–7493.
29. Schurrman, H. J., Ritter, M. A., Brekhuizen, R., Ladyman, H. & Larché, M. (1987) in *Leucocyte Typing III: White Cell Differentiation Antigens*, eds. McMichael, A. J., Beverley, P. C. L., Cobbold, S., Crumpton, M. J., Gilks, W., Gotch, S. M., Hogg, N., Horton, M., Ling, N., MacLennan, I. C. H., Mason, D. Y., Milstein, C., Spiegelhalter, D. & Waldmann, H. (Oxford University Press, Oxford), in press.
30. Takahashi, T., Ueda, R., Song, X., Nishida, K., Shinzato, M., Namikawa, R., Ariyoshi, Y., Ota, K., Nagatsu, T., Imaizumi, M., Abe, T. & Takahashi, T. (1986) *Cancer Res.* **46**, 4770–4775.
31. Lane, E. B. (1982) *J. Cell Biol.* **92**, 665–673.
32. Hogg, N., McDonald, S., Slusarenko, M. & Beverley, P. C. L. (1984) *Immunology* **53**, 753–767.
33. Sutherland, R., Delia, D., Schneider, C., Newman, R., Kemshead, J. & Greaves, M. F. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4515–4519.
34. Waterfield, M. D., Mayes, E. L. V., Stroobant, P., Bennett, P. L. P., Young, S., Goodfellow, P. N., Banting, G. S. & Ozanne, B. (1982) *J. Cell. Biochem.* **20**, 149–161.
35. Brodsky, F. M., Parham, P., Barnstable, C. J., Crumpton, M. J. & Bodmer, W. F. (1979) *Immunol. Rev.* **47**, 3–61.
36. Katz, F. E., Davis, L., Myers, C. D. & Greaves, M. F. (1985) *Exp. Hematol.* **13**, 1182–1187.
37. Tindle, R. W., Nicols, R. A. B., Chan, L. C., Campana, D., Catovsky, D. & Birnie, G. D. (1984) *Leuk. Res.* **9**, 1–11.
38. Lebacq-Verheyden, A.-M., Ravoet, A.-M., Hazin, H., Sutherland, D. R., Tidman, N. & Greaves, M. F. (1983) *Int. J. Cancer* **32**, 273–278.
39. Bernard, A., Boumsell, L. & Hill, C. (1984) in *Leukocyte Typing* eds. Bernard, A., Boumsell, L., Dausett, J., Milstein, C. & Schlossman, S. F. (Springer, Berlin), pp. 354–363.
40. Hogue-Angeletti, R. & Hickey, W. F. (1985) *Science* **230**, 89–90.
41. Lloyd, R. V. & Wilson, B. S. (1983) *Science* **222**, 628–630.
42. Dalchan, R., Kirkley, J. & Fabre, J. W. (1980) *Eur. J. Immunol.* **10**, 737–744.
43. Harlow, E., Crawford, L. V., Pim, D. C. & Williamson, N. M. (1981) *J. Virol.* **39**, 861–869.
44. Furley, A. J., Mizutani, S., Weilbaecher, K., Dhaliwal, H. S., Ford, A. M., Chan, L. C., Molgaard, H. V., Toyanaga, B., Mak, T., van den Elsen, P., Gold, D., Terhorst, C. & Greaves, M. F. (1986) *Cell* **46**, 75–87.
45. McGarrity, G. J. (1982) in *Advances in Cell Cultures*, ed. Maramorosch, K. (Academic, New York), Vol 2, pp. 99–131.
46. Vodinelich, L. (1987) in *Interleukins and Interferons—A Practical Approach*, eds. Clemens, M., Gearing, A. & Morris, A. (IRL, Oxford), in press.
47. Durum, S. K., Schmidt, J. A. & Oppenheim, J. J. (1985) *Annu. Rev. Immunol.* **3**, 263–287.
48. Ready, A. R., Jenkinson, E. J., Kinston, R. & Owen, J. J. T. (1984) *Nature (London)* **310**, 231–233.
49. Janossy, G. (1981) in *The Leukemic Cell*, ed. Catovsky, D. (Churchill-Livingstone, New York), pp. 129–183.