MONOCLONAL ANTIBODY AGAINST HUMAN T CELL LEUKEMIA VIRUS p19 DEFINES A HUMAN THYMIC EPITHELIAL ANTIGEN ACQUIRED DURING ONTOGENY*

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A novel human retrovirus, human T cell leukemia virus (HTLV)[1] has recently been isolated from a variety of adult T cell leukemias and lymphomas that have so far generally exhibited a particular geographic distribution in the southern United States, West Indies, and Southern Japan (1-3; M. Popovic, manuscript in preparation). Specific antibodies to HTLV proteins have been found in virus-positive cases (4-9) often in family members (9) and much less frequently in the general population. To further study the incidence of HTLV in large patient populations, a monoclonal antibody to the HTLV structural protein, p19 (V. S. Kalyanaraman and R. C. Gallo, manuscript in preparation), has been used to examine T cells of patients and normal donors for evidence of HTLV expression (10).

The monoclonal anti-p19 immunoprecipitates a 19,000-dalton structural protein of HTLV. The antigen is located at the surface of fixed HTLV-infected T cells and is not detected in HTLV$^-$ malignant or normal T cells of any type (10). During a recent screen of anti-p19 reactivity with a large number of normal human tissues, we observed that anti-p19 reacted strongly with the epithelial component of normal human thymus. Thus, in this study, we characterized the specificity of anti-p19 binding to human thymus, and sought other HTLV antigens in normal thymic tissue. We demonstrated the extent of reactivity of anti-p19 with thymic epithelium as a function of the age of the donor of the thymus and showed that all anti-p19$^+$ thymic epithelial cells contain thymosin α_1 and thymopoietin. Moreover, no other HTLV proteins or proviral HTLV DNA were demonstrated in anti-p19$^+$ thymic epithelium. Thus, in normal human thymus, the antigen defined by anti-p19 is specific for the neuroendocrine component of thymic epithelium and is acquired during normal

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[1] *Abbreviations used in this paper:* DTT, dithiothreitol; HTLV, human T cell leukemia virus; MG, Myasthenia gravis; PBS, phosphate-buffered saline; PMSF, phenylmethylsulffonyl fluoride; RIP, radioimmunoprecipitation; SRBC, sheep erythrocyte; T ALL, T cell acute lymphoblastic leukemia; TNE, a buffer composed of 0.01 M Tris-HCl, pH 8.0, 0.1 M EDTA; TT, tetanus toxin.

thymic epithelial ontogeny. Our results clearly indicate that thymic epithelium is not generally infected with HTLV. Two other possibilities are discussed: (*a*) that the observations simply reflect a cross-reactivity between the HTLV and thymic epithelial antigens; and (*b*) that HTLV p19 is a host-encoded protein selectively expressed in normal thymus and also induced in HTLV-infected T cells and incorporated into the virus.

Materials and Methods

Monoclonal Antisera. Hybridoma 12/1-2, a cloned murine hybridoma line, produced the IgG1 monoclonal anti-HTLV p19 (10). The immunizing antigen was prepared as follows: cells producing HTLV were grown in RPMI 1640 medium containing 20% fetal calf serum. HTLV was concentrated from cell culture supernatants by continuous flow centrifugation through a sucrose gradient. The virus pool was subsequently centrifuged through 30% glycerol in a buffer composed of 0.01 M Tris-HCl, pH 8.0, 0.1 M EDTA (TNE) onto a 100% glycerol cushion, and rebanded on a 20–60% sucrose gradient in TNE. The virus was pooled based on its reverse transcriptase activity with poly$(A)_n$ dT_{15} as template (1) and its density. The latter peaked at ~1.17 g/ml. HTLV was disrupted by adding an equal volume of disruption buffer composed of 100 mM Tris-HCl, pH 7.9, 0.5% Triton X-100, 0.04 M dithiothreitol (DTT), 2 mM EDTA, 1 M KCl, and 2 mM phenylmethylsulfonyl fluoride (PMSF) and stirring for 1 h at 4°C. After centrifugation at 15,000 rpm for 30 min, the supernatant solution was used for immunizations. Monoclonal A2B5 binds to a GQ ganglioside antigen on all neuroendocrine vertebrate tissues (11), and in human thymus selectively binds to the entire neuroendocrine component of thymic epithelium (12).[2]

Polyclonal Antisera. Rabbit anti-thymosin α_1 (the generous gift of Dr. Allen Goldstein, George Washington University, Washington, DC) and anti-thymopoietin (the generous gift of Dr. Gideon Goldstein, Ortho Pharmaceutical, Raritan, NJ) were used as previously described (12). Goat anti-HTLV p24 was prepared against the homogeneously purified core protein (13). Its titer in a radioimmunoprecipitation (RIP) assay was 1:180,000 for 20% precipitation of the labeled antigen. It was used in indirect immunofluorescence at a 1:100 dilution, with an affinity-pure fluorescein-conjugated rabbit anti-goat IgG (H and L specific) (Tago, Inc., Burlingame, CA). Antibody to HTLV was raised in a goat using detergent-ether disrupted virus. In RIP assays, its titer for 20% precipitation of labeled HTLV p19 was 1:2,000. In immunofluorescence assays it was used at a 1:1,000 dilution with an affinity-purified fluorescein-conjugated rabbit anti-goat IgG.

Acquisition and Preparation of Tissue. Normal fetal thymus tissue was obtained at the time of therapeutic abortion. In one case tissue was obtained from a 24-wk fetal product of a septic abortion. Normal thymic tissue from neonates or older subjects was obtained during corrective cardiovascular surgery. Myasthenia gravis (MG) thymic tissue was obtained at the time of therapeutic thymectomy. Fetal liver and spleen and adult skin, lymph node, spleen, adrenal, thyroid, prostate, stomach, kidney, uterus, parotid, breast, liver, colon, and brain were obtained at autopsy no later than 4 h after death. Pancreas and adrenal tissue were obtained from specimens taken during abdominal surgical procedures. 3 x 3-mm portions of all tissue types were snap frozen in an ethanol-dry ice slurry, and stored in liquid N_2 until used. Suspensions of thymocytes, normal peripheral blood cells, tissue tumor cells, or peripheral blood leukemic cells were prepared as described previously (14, 15).

Indirect Immunofluorescence Assay for Monoclonal Anti-p19 or A2B5 Reactivity. All frozen tissue was embedded in OCT compound (Scientific Products, McGraw Park, IL), 4-μm frozen sections were cut, and the sections were fixed for 5 min in cold acetone and stored at −100°C. Acetone-fixed tissue slides were incubated for 30 min in a moist chamber with a saturating amount of monoclonal antibody (1:1,000 of anti-p19 ascites, 1:500 of A2B5 ascites, and 1:1,000 or 1:500 of P3 × 63/Ag8 control ascites). After three rinses in cold phosphate-buffered saline (PBS), a

[2] Haynes, B. F., R. W. Warren, R. H. Buckley, J. E. McClure, A. L. Goldstein, F. W. Henderson, L. L. Hensley, and G. S. Eisenbarth. Demonstration of abnormalities in expression of thymic epithelial surface antigen in severe cellular immunodeficiency diseases. Manuscript submitted for publication.

saturating amount (1:100 final dilution) of affinity-pure fluorescein isothiocyanate-conjugated (fluorescein/protein 6.0) goat anti-mouse IgG (Tago, Inc.) was layered on the slide and incubated 30 min. After one rinse in PBS, the slides were briefly dipped in distilled water, allowed to air dry, overlayed with 30% glycerol in PBS, coverslipped, and read on a Nikon Optiphot microscope (Garden City, NY) (15). Double indirect immunofluorescence with anti-thymic hormone antisera and monoclonal antibodies A2B5 or anti-p19 was performed as described in detail (12).

Immunoperoxidase-Peroxidase Assay for Anti-p19 Reactivity. After preincubation of the tissue sections with 10% nonimmune goat serum, slides were incubated for 60 min at room temperature with 1:1,000 diluted anti-p19 or P3 ascitic fluid. A second 45-min incubation was performed with a 1:50 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG (N. L. Cappel Laboratories, Inc., Cochranville, PA). Slides were developed by incubation for 15 min with a solution of diaminobenzidine (0.5 mg/ml) and hydrogen peroxide (0.001%) in 0.05 M Tris buffer at pH 7.6 and were counterstained with hematoxylin (16).

Assay for Tetanus Toxin (TT) Binding to Thymus. TT binds to GD and GT gangliosides which, in the thymus, identify all A2B5$^+$ neuroendocrine thymic epithelial cells (12). Human thymus sections were also incubated with purified TT (kindly supplied by R. O. Thomson of the Wellcome Research Laboratories, Beckenham, England), 0.1 μg/ml diluted in PBS 1% albumin for 30 min at room temperature, washed three times in PBS, incubated with 1:100 dilution of monoclonal anti-TT antibodies 3DB and 3B3 (kindly supplied by V. R. Zurawski of Centocor, Inc. Malvern, PA), washed three times, and then incubated as described above for antibody A2B5 with fluorescein-conjugated anti-mouse IgG (12).[2]

Indirect Immunofluorescent Assay for Surface Binding of Anti-p19 and Antibody A2B5 to Viable Thymic Epithelial Cells. The thymocytes from fresh normal or MG thymus tissue were gently teased from thymic epithelium with forceps using mechanical dissociation, and the resulting thymocyte-free thymic epithelium was cut into 1 × 1-mm pieces and stained in suspension with either P3 × 63/Ag8 ascites as control (1:1,000 or 1:500), anti-p19 (1:1,000), or A2B5 (1:500) as previously described for cells in suspension (14). After staining with fluorescein-conjugated goat anti-mouse IgG, the tissue pieces were overlayed in 30% glycerol in PBS with a coverslip and viewed by fluorescent microscopy. Cell surface reactivity of p19 with a variety of lymphoid cells in suspension was determined by indirect immunofluorescence and cytofluorography using the Ortho 50H cytofluorograph (Ortho Instruments, Westwood, MA) as described (14).

Blocking of Anti-p19 Antibody Binding to Thymus Epithelium with Extracts of Thymic Epithelium or HTLV. 500 mg of purified MG thymic stroma (shown by indirect immunofluorescence to be strongly anti-p19$^+$) was homogenized in a Dounce homogenizer with 2.0 ml of extraction buffer containing 1% NP40, 2 mM PMSF, 10 mM iodoacetamide in 10 mM Tris-HCl buffer, pH 8.0. After homogenization, the extract was centrifuged at 27,000 rpm for 60 min, and the supernatant was collected. As controls, sheep erythrocyte (SRBC) membranes (previously shown to be anti-p19$^-$), 24-wk fetal thymus (shown to be 99% p19$^-$), and adult spleen (also p19$^-$), were similarly extracted and used at concentrations from 1.5 to 3.6 μg/ml. Disrupted HTLV was prepared and solubilized as previously described (13). HTLV p19 was also purified as previously described (13). Briefly, detergent disrupted HTLV was freed of nucleic acids by passage through DEAE-cellulose. The unadsorbed protein fraction was dialyzed against buffer A (50 mM Tris-HCl, pH 7.9, 1 mM DTT, 20% glycerol, and 0.1 mM PMSF) and passed through a column of phosphocellulose equilibrated with the same buffer. The column was successively washed with 0.7 and 1.2 M NaCl in buffer A. The 1.2 M NaCl wash contained nearly pure HTLV p19, which was further purified by high-pressure liquid chromatography (HPLC) using a C-18 column. The column was developed with a gradient of acetonitrile in 0.1% trifluoroacetic acid. HTLV p19 was eluted between acetonitrile concentrations of 60–75% and was lyophilized and reconstituted in 50 mM phosphate buffer, pH 7.5, before use. For blocking, 10 μl of either adult thymic epithelium (180 μg), fetal thymic epithelium (360 μg), adult spleen (360 μg), SRBC (75 μg) extract, 10 μl of purified HTLV p19 (2.75 μg), 20 μl of disrupted HTLV (37 μg), or 10 μl of extraction buffer were added to a subsaturating amount (1:25,000) of monoclonal anti-p19. The mixture was allowed to stand 15 min and was then added to 4-μm sections of thymus as described above. Inhibition of anti-p19 binding by the various extracts or buffer control was determined by lack of fluorescence of thymic epithelium.

Radioimmunoassay for Binding of Purified HTLV p19 to Monoclonal Anti-p19. A solid-phase radioimmunoassay was carried out as previously described (17). Briefly, ascites fluid containing monoclonal anti-p19 (12/1-2) was diluted 1:1,000 in PBS/Tween and duplicate 50-μl aliquots were incubated in purified HTLV p19-coated microtiter wells for 45 min at 37°C. Unbound proteins were aspirated, and the plate was washed three times with PBS/Tween. An appropriate dilution of rabbit anti-mouse IgG in PBS/Tween was added to each well. After incubation and washing as above, iodinated protein A (50,000 cpm/50 μl PBS/Tween) was added to each well. Wells were subsequently incubated, washed, and counted in a gamma counter.

DNA Blotting of Thymus and Thymus Epithelial DNA with Cloned HTLV Probes. Sequences derived from the 5′ and 3′ termini of HTLV (450 and 500 nucleotides, respectively) have been molecularly cloned in pBR322 (V. Manzari and R. C. Gallo, unpublished results). These were labeled with ^{32}P by nick translation (18) and used as probes. High molecular weight DNA from thymus, thymic epithelium, normal thymocytes, and two HTLV-positive cell lines was digested with the restriction endonuclease Kpn I and blot hybridized to labeled HTLV probe as described (19).

Results

Reactivity of Anti-p19 with Thymic Epithelium. As previously reported (10), anti-p19 did not react with antigens of normal thymocytes. However, when anti-p19 was assayed for reactivity on frozen thymus 4-μm tissue sections from a 30-mo-old normal child, the entire subcapsular cortex and medullary thymic epithelium (as defined by A2B5 and TT reactivity [12][2]) was anti-p19$^+$ (Fig. 1 A and B). However, in contrast to A2B5 or TT reactivity, anti-p19 reactivity was variable from thymus to thymus in the amount of thymic epithelium labeled. Ontogenic study of normal thymic tissue from 8 wk fetal gestation through 60 yr of age (Table I) demonstrated that the reactive antigen was acquired during thymic ontogeny. Whereas the entire thymic epithelium component of all fetal and adult thymuses tested were A2B5$^+$, no anti-

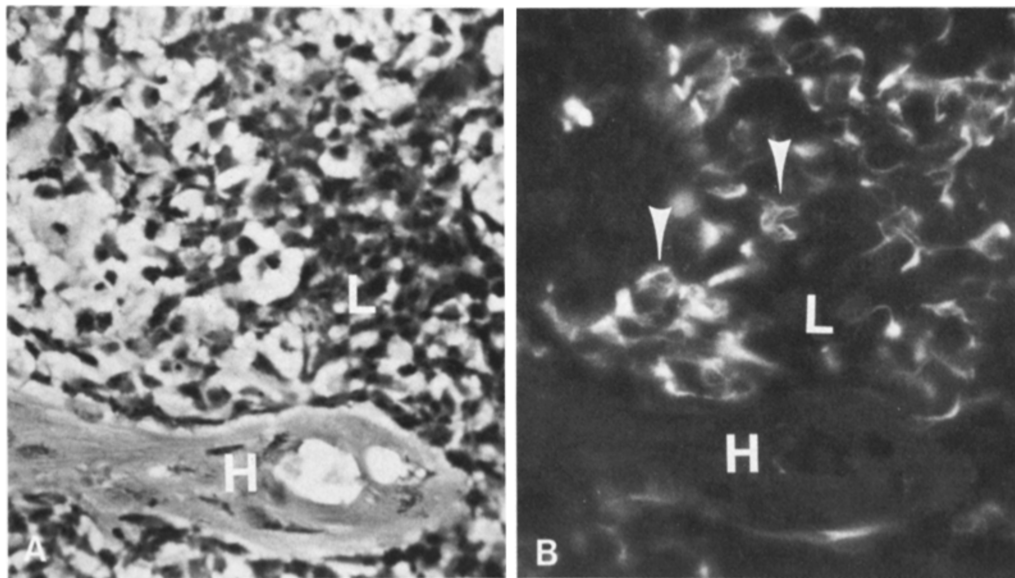

FIG. 1. Monoclonal anti-p19 reacts with thymic epithelium of normal thymus. (A) Light micrograph of hematoxylin and eosin-stained frozen section of thymic medulla. Structure labeled H is a Hassall's corpuscle. A foci of medullary lymphoid cells is labeled L. (B) Fluorescent micrograph of the same field showing the anti-p19 reactive epithelial cells (arrows) around anti-p19$^-$ lymphoid cells (L) (× 400).

TABLE I

Reactivity of Anti-p19 Monoclonal Antibody with Normal Thymus Tissue as Determined by Indirect Immunofluorescence

Thymus number	Age	Pattern of reactivity of anti-p19 antibody
1	8 wk gestation	All areas of thymic epithelium were nonreactive.
2	15 wk gestation	Most cortical and medullary areas were nonreactive. Occasional linear arrays of p19$^+$ dendritic cells were located in the subcapsular cortical region of rare thymic lobules.
3	24 wk gestation	Most cortical and medullary areas were nonreactive. Occasional linear arrays of p19$^+$ dendritic cells were located in the subcapsular cortical region of rare thymic lobules.
4	4 mo (postnatal)	All subcapsular cortical areas of each lobule rimmed by linear arrays of p19$^+$ dendritic cells. Occasional p19$^+$ cells were scattered about upper cortex. Most medullary areas contained no p19$^+$ cells.
5, 6	12 mo	All subcapsular cortical areas of each lobule were rimmed by linear arrays of p19$^+$ dendritic cells. Occasional p19$^+$ cells were scattered about upper cortex. Only occasional to few medullary areas contained p19$^+$ cells.
7	30 mo	All subcapsular cortical areas of each lobule were rimmed by linear arrays of p19$^+$ dendritic cells. Occasional p19$^+$ cells were scattered about upper cortex. Most medullary areas contained many p19$^+$ cells.
8	60 yr	Streaks of p19$^+$ epithelium were present throughout thymic fibrofatty stromal remnant.

p19$^+$ epithelium was present until 15 wk gestation, and these anti-p19$^+$ cells were limited to only a few areas of the subcapsular cortex (Fig. 2A and B). By birth, the entire subcapsular cortical thymic epithelial region was anti-p19$^+$, whereas the A2B5$^+$ medullary component of thymic epithelium was anti-p19$^-$ (Fig. 2C). By 30 mo of age, the entire A2B5$^+$ neuroendocrine component of thymic epithelium was anti-p19$^+$ (Fig. 2D, E). In 15 normal thymuses tested (ages ranging from birth to 68 yr), the pattern of anti-p19 reactivity listed in Table I held true. In addition, in thymic tissue from six MG patients (ages of donors ranging from 7 to 68 yr), the entire A2B5$^+$ neuroendocrine thymic epithelium was anti-p19$^+$. Assay for anti-p19 reactivity on rabbit or mouse thymic epithelium was negative. Specificity of anti-p19 was demonstrated by blocking of a nonsaturating amount of anti-p19 (1:25,000) by anti-p19$^+$ thymic epithelium membrane extracts, but not by anti-p19$^-$ SRBC fetal thymus or adult spleen membrane extracts (Table II).

Extracts of whole HTLV and high pressure liquid chromatography (HPLC)-purified HTLV p19 antigen were incubated with subsaturating amounts of monoclonal anti-p19 (1:25,000–1:50,000) before incubation with thymic tissue sections. Both HTLV whole extract and purified HTLV p19 antigen completely inhibited the binding of anti-p19 to thymic epithelium (Table II). To show directly that the monoclonal anti-p19 bound HPLC-purified HTLV p19, binding of anti-p19 to varying amounts of plate bound purified p19 antigen in a solid phase radioimmunoassay was performed. The results are shown in Fig. 3.

A goat antiserum to disrupted HTLV reacted in indirect immunofluorescence assay to normal neuroendocrine thymic epithelium in a pattern similar to monoclonal anti-

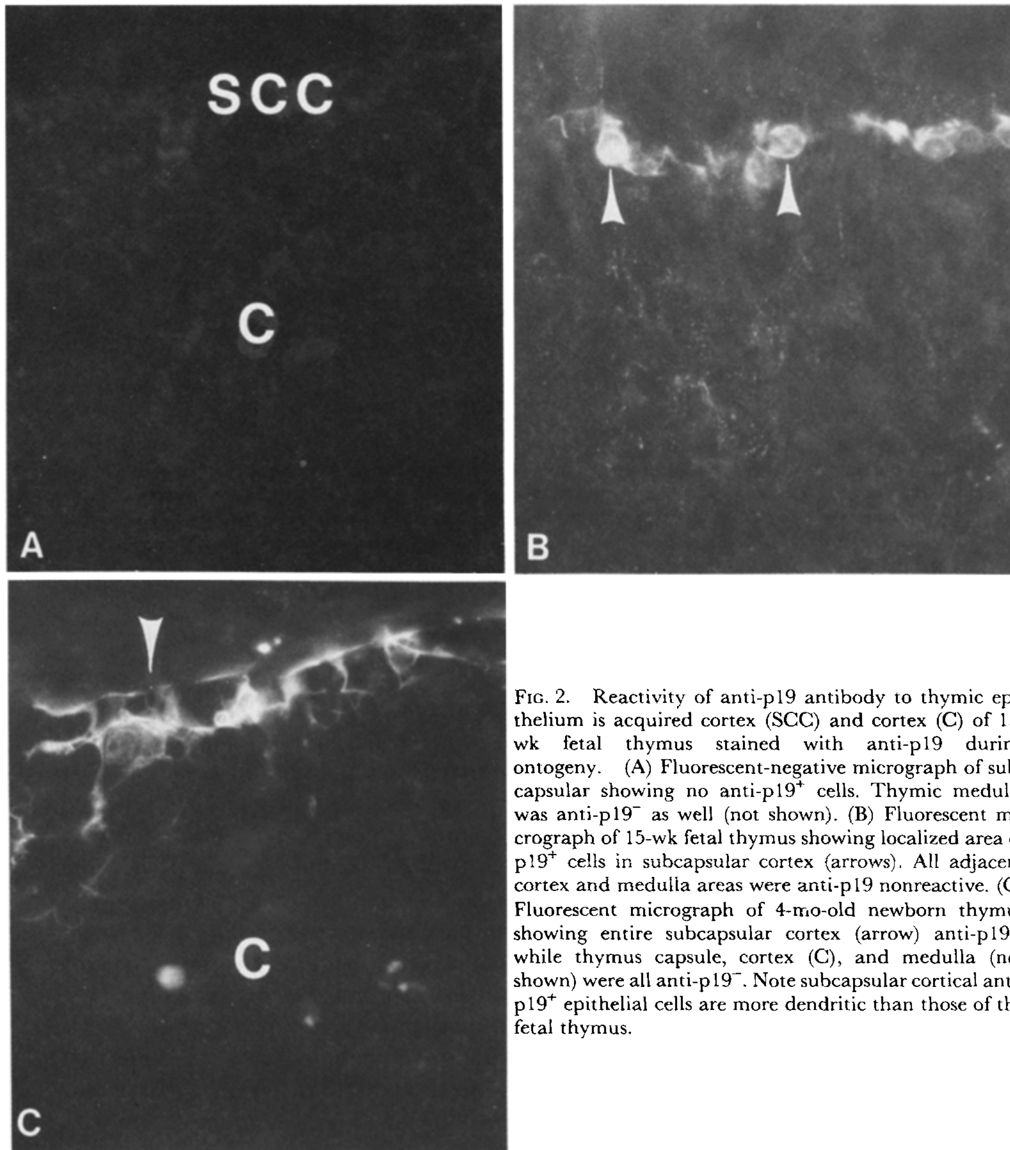

FIG. 2. Reactivity of anti-p19 antibody to thymic epithelium is acquired cortex (SCC) and cortex (C) of 15-wk fetal thymus stained with anti-p19 during ontogeny. (A) Fluorescent-negative micrograph of subcapsular showing no anti-p19$^+$ cells. Thymic medulla was anti-p19$^-$ as well (not shown). (B) Fluorescent micrograph of 15-wk fetal thymus showing localized area of p19$^+$ cells in subcapsular cortex (arrows). All adjacent cortex and medulla areas were anti-p19 nonreactive. (C) Fluorescent micrograph of 4-mo-old newborn thymus showing entire subcapsular cortex (arrow) anti-p19$^+$, while thymus capsule, cortex (C), and medulla (not shown) were all anti-p19$^-$. Note subcapsular cortical anti-p19$^+$ epithelial cells are more dendritic than those of the fetal thymus.

19 (not shown). In addition, this antibody also precipitated soluble purified HTLV p19 in RIP assays (not shown). Thus, the monoclonal anti-p19 and the polyclonal goat anti-HTLV antibody bound to both normal thymic epithelium and purified HTLV p19 antigen.

Lack of Reactivity of Anti-p19 with Other Human Tissues. Because TT and A2B5 bind to all neuroendocrine tissues, a broad screen of anti-p19 reactivity with many types of hematopoietic and neuroendocrine tissues was performed (Table III). In contrast to TT and A2B5, anti-p19 did not react with any normal neuroendocrine or other human tissues tested other than thymic epithelium. However, the basal layer of tonsil, adenoid, and esophagus squamous epithelium did react with anti-p19.

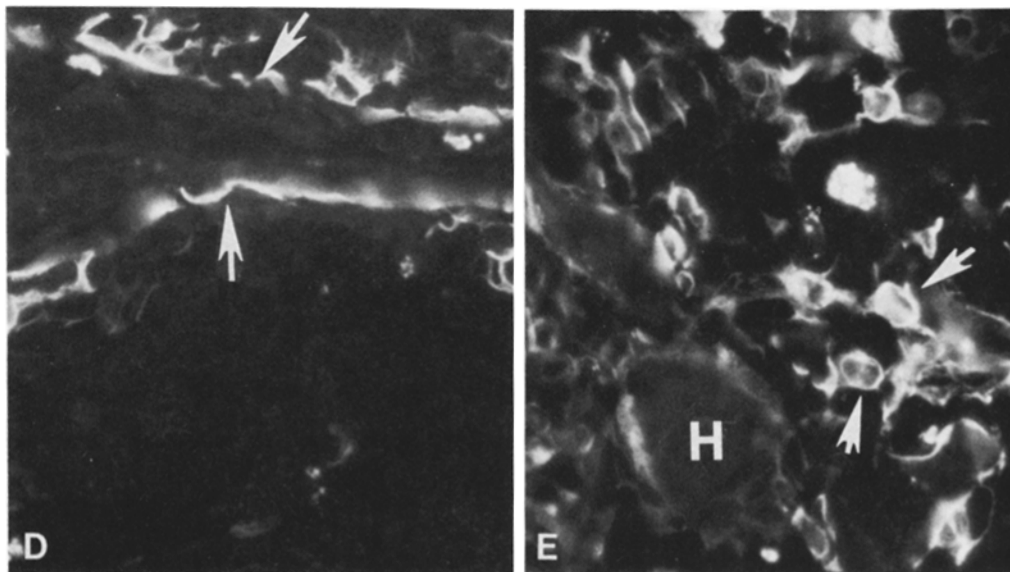

FIG. 2. (D and E) Fluorescent micrograph of 30-mo thymus showing (D) entire subcapsular cortex epithelium of two adjacent thymic lobules (arrows) anti-p19^{+} and (E) entire medullary epithelium anti-p19^{+} (arrows). H denotes Hassall's corpuscles (× 400).

TABLE II
Specificity Controls of Monoclonal Anti-p19 Binding to Normal Thymic Epithelium

Reagent*	Reactivity of anti-p19 with thymic epithelium‡
Anti-p19 (1:50,000)	Positive
Anti-p19 (1:50,000) + 2.75 μg purified HTLV p19 antigen	Negative
Anti-p19 (1:50,000) + 36 μg solubilized whole HTLV extract in 0.5% Triton X	Negative
Anti-p19 (1:50,000) + 0.5% Triton X control	Positive
Anti-p19 (1:25,000) + 180 μg Thymic epithelial stromal extract in 1% NP-40	Negative
Anti-p19 (1:25,000) + 75 μg SRBC stromal extract in 1% NP-40§	Positive
Anti-p19 (1:25,000) + 360 μg 24-wk fetal thymus extract in 1% NP-40	Positive
Anti-p19 (1:25,000) + 360 μg adult spleen extract in 1% NP-40	Positive
Anti-p19 (1:25,000) + 1% NP-40 control	Positive

* P3 × 63/Ag8 ascitic fluid at 1:25,000 or 1:50,000 dilution, either alone or with each of the tissue extracts, purified HTLV-associated p19 antigen, or controls, gave no fluorescent staining pattern of any portion of normal human thymus. Blocking experiments were performed as outlined in Materials and Methods.

‡ As determined by indirect immunofluorescence.

§ Nonidet P-40.

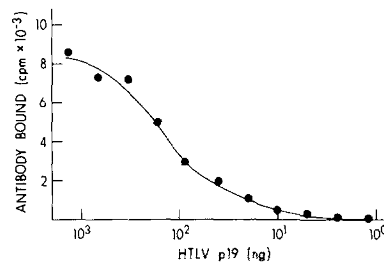

FIG. 3. Monoclonal anti-p19 antibody binds purified HTLV p19 antigen. Varying amounts of purified HTLV p19 (275 μg/ml) were bound to plastic microtiter wells and assayed for reactivity with anti-p19 as described in Materials and Methods.

TABLE III

*Localization of Anti-p19 Reactivity on Normal Tissue or Normal or Malignant Hematopoietic Cells**

Cell type	Surface p19 reactivity	Cytoplasmic anti-p19 reactivity‡
HUT 102 (HTLV+ CTCL line)	−	+
HTLV+ ATL cultured PBL	−	+
HUT 78 HTLV− CTCL§ line	−	−
HSB-2 T ALL cell line	−	−
Fresh normal PBL‖	−	−
Fresh thymocytes	−	−
Fresh thymocytes	−	−
Fresh AML PBL	−	−
Fresh hairy cell PBL	−	−
Fresh T cell CLL	−	−
Fresh CTCL leukemia	−	−

* The following tissues (number tested) did not bind anti-p19 monoclonal antibody as determined by indirect immunofluorescence or immunoperoxidase staining techniques on 4-μm frozen tissue sections: fetal spleen (1), fetal liver (1), adult lymph node (4), spleen (1), liver (1), skin (6), adrenal (2), pancreas (1), thyroid (1), brain (2), colon (1), breast (1), prostate (1), stomach (1), kidney (1), parotid (1), and uterus (1).

‡ Fresh leukemia cells cultured 7 d in T cell growth factor supplemented with RPMI 1640 + 20% fetal calf serum medium.

§ Cutaneous T cell lymphoma.

‖ Peripheral blood leukocyte.

Characterization of Surface vs. Intracytoplasmic Reactivity of Anti-p19. Because HTLV p19 is not exposed on the surface of HTLV-infected malignant T cells, but can only be visualized in fluorescent assays using fixed cells (10), reactivity of anti-p19 was tested on one normal and two MG subjects' fresh viable purified thymic epithelial cells in suspension. We found both anti-p19 and antibody A2B5 reacted with a cell surface rim pattern on viable thymic epithelial cells (not shown).

Next, a comparison was made of anti-p19 reactivity on both fixed and living cells of various types including normal and malignant HTLV$^+$ or HTLV$^-$ cells (Table III). While fixed cultured PB cells from a patient with Japanese HTLV$^+$ adult T cell leukemia and the cutaneous T cell lymphoma HTLV$^+$ HUT 102 cell line reacted with anti-p19, surface reactivity for these anti-p19$^+$ living cells with anti-p19 by cytofluorography was absent. As a control, a series of other types of malignant cells

were anti-p19 non-reactive by fluorescent assays on both fixed and living cells (Table III).

All Anti-p19 Reactive Thymic Epithelial Cells Contain Thymopoietin and Thymosin α_1. Anti-p19 reacted with both subcapsular cortical and medullary thymic epithelium in all thymic tissue from donors over the age of 30 mo. Since this reactivity pattern was identical to the pattern seen with A2B5 and TT, and since all A2B5$^+$ and TT$^+$ thymic epithelial cells contain thymopoietin and thymosin α_1 (12), we wanted to confirm that all anti-p19$^+$ epithelial cells as well contained thymopoietin and thymosin α_1. Using double indirect immunofluorescence with rabbit anti-thymosin α_1 or anti-thymopoietin developed with rhodamine-conjugated goat anti-rabbit IgG and monoclonal anti-p19 developed with fluorescein-conjugated goat anti-mouse IgG, we demonstrated that all anti-p19$^+$ thymic epithelial cells contained these two thymotrophic hormones (Fig. 4A and B).

Because all anti-p19$^+$ thymic epithelial cells contained thymosin α_1, using double immunofluorescence we next determined whether anti-p19$^+$ malignant T cells contained thymosin α_1. We found that both the HTLV$^+$, HUT 102 cell line and the HTLV$^-$, HUT 78 cell lined contained thymosin α_1, whereas only the HUT 102 HTLV$^+$ cell line was anti-p19$^+$ (data not shown). Thus, although malignant HUT 102 T cells do produce thymosin α_1, there was no direct correlation between anti-p19 reactivity and the presence of thymosin α_1 in malignant T cells.

Reactivity of Anti-p19 in Abnormal Thymic Tissue. Since anti-p19 reactivity is acquired during thymic ontogeny, we looked to see if the antigen recognized was present in abnormal thymic tissue from patients with thymic dysplasia (severe combined im-

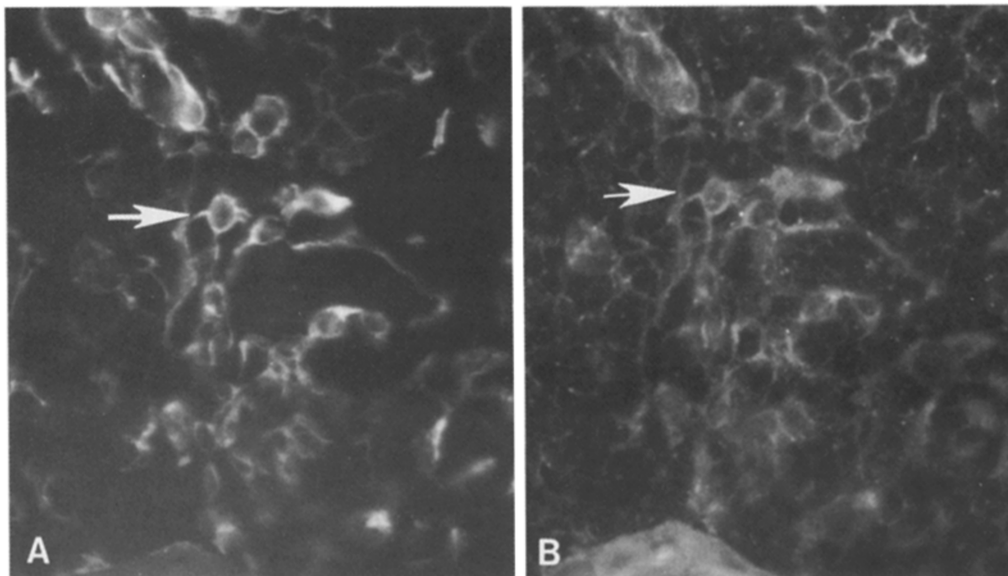

FIG. 4. All anti-p19$^+$ thymic epithelial cells contain thymosin α_1. (A) 30 mo-old thymus was incubated with anti-p19 antibody followed by fluoresceinated goat anti-mouse IgG and viewed for green fluorescein excitation. Arrow shows anti-p19$^+$ epithelial cells. (B) Same field as in (A). Counter-stained with rabbit anti-thymosin α_1 and rhodaminated-goat anti-rabbit IgG and viewed for red rhodamine excitation. Arrow shows thymosin $\alpha_1{}^+$ epithelial cells. Identical results were obtained using anti-thymopoietin instead of anti-thymosin α_1 (not shown) (× 400).

TABLE IV
*Reactivity of Anti-p19 in Abnormal Thymic Tissue**

Thymic tissue source	A2B5	TT	p19
SCID‡	+	−	+
SCID	+	−	−
Nezelof's syndrome	−	+	−
Thymoma	+	ND§	−
Thymoma	+	ND	+
T ALL‖	+	ND	+

* As determined by indirect immunofluorescence assay.
‡ Severe combined immunodeficiency disease.
§ Not done.
‖ Thymus autopsy tissue was from a T ALL patient whose leukemic PB cells in culture with T cell growth factor-supplemented media were HTLV $p19^-$ and HTLV $p24^-$.

munodeficiency disease and Nezelof's syndrome)[2] thymic epithelial malignancies (thymoma), and thymus lymphoid malignancy ($HTLV^-$ T cell acute lymphoblastoid leukemia [T ALL]). As expected, we found both reactive and nonreactive dysplastic and malignant thymic epithelial tissues (Table IV).

Assay for the Presence of HTLV Proteins or HTLV DNA in Normal or MG Thymic Epithelium. Because p19 is an HTLV structural protein, the question arose as to whether normal thymic epithelium was infected with, and thus a revervoir for, HTLV. To study this question, a goat anti-p24 antiserum that reacts with a cytoplasmic antigen in HTLV-infected T cells and immunoprecipitates the 24,000-dalton HTLV internal core protein (14) was used. It did not react by indirect immunofluorescence with any thymic tissue previously shown to have strongly anti-$p19^+$ thymus epithelium. Finally, DNA from normal and MG-purified thymic epithelium, normal whole thymus, normal thymocytes, and DNA from an HTLV-infected cell line were extracted, digested with Kpnl, and probed via hybridization with the 5′ (450 N) and 3′ (500 N) sequence of HTLV (V. Manzari and R. C. Gallo, unpublished results). DNA from the cell line infected with HTLV showed distinct bands of hybridization with the HTLV probes, whereas the normal thymic epithelium, whole thymus, and thymocyte DNA did not (not shown).

Discussion

In this study, we show that monoclonal antibody 12/1-2 (anti-p19) binds not only to HTLV p19, which is expressed in HTLV-infected T cells, but to normal human thymic epithelium. Furthermore, the thymic epithelial-reactive antigen is acquired during thymic ontogeny. Specificity studies demonstrated that binding of the monoclonal anti-p19 to thymic epithelium was completely inhibited by thymic epithelium extracts, whole HTLV extracts, and purified HTLV p19 antigen (Table II). Moreover, a goat anti-HTLV anti-serum bound to normal thymic epithelium.

These observations gave rise to several hypotheses regarding the nature of the reactive antigen on thymic epithelium. (*a*) Thymic epithelium may be infected during ontogeny with HTLV. The observations that anti-$p19^+$ thymic epithelium is HTLV p24 negative, that no HTLV proviral DNA sequences can be detected with HTLV c-DNA probes by Southern blot hybridization techniques, and that all thymus tissues

tested from donors older than 8 wk gestation were anti-p19$^+$ provide strong evidence against this. Moreover, epidemiologic data strongly suggest that HTLV-associated T-cell leukemia is horizontally passed as an infectious disease and not by vertical transmission (4–9). Molecular hybridization studies have verified this and proven that the infection is postzygotic (3, 21). The observation that all normal, myasthenic, and T ALL thymuses tested were anti-p19$^+$ also mitigates against p19 expression on thymic epithelium reflecting HTLV infection. (*b*) Anti-p19 recognizes a structural protein of HTLV and also may bind to a "cross-reactive," host-derived, and therefore non–HTLV-associated antigen on thymic epithelium. This possibility is suggested by the specificity data (Table II) and by the lack of both HTLV proviral sequences and HTLV p24 antigen in anti-p19$^+$ thymic epithelium. (*c*) Monoclonal anti-p19 may recognize a 19,000-dalton host-encoded protein that is induced by HTLV infection of cells, contaminates HTLV preparations, and co-purifies with virally encoded HTLV p19. Studies are underway to characterize the reactive thymic epithelial antigen. If this antigen has a mass of 19,000 daltons, this possibility must be considered more carefully. (*d*) Finally, HTLV p19, although a viral structural protein, may be encoded by the host rather than the virus. Thus, monoclonal anti-p19 would specifically react not only with HTLV p19 but also with a host protein induced to be expressed by HTLV infection in malignant T cells that is also normally expressed on thymic epithelium. This host protein would be incorporated into the HTLV virion structure during viral morphogenesis. Precedents for such a phenomena have been observed in adenovirus-transformed mouse cells where a host protein (p53) becomes associated with a 58,000-dalton product of the adenovirus Elb region (21). This p53 host protein also binds to SV-40 T antigen in SV-40 transformed cells (21–23). In animals with SV-40-induced tumors and Abelson leukemia virus-induced leukemia, anti-p53 serum antibodies are present (21–23). This latter observation is particularly interesting as it has been clearly documented that HTLV-infected patients with Japanese and United States adult T cell leukemia make anti-p19 antibodies that compete with monoclonal anti-p19 for binding to HTLV (17). Natural antibodies to HTLV p19 have also been demonstrated by RIP assays.[3]

While reaction of anti-p19 has not been detected with any other normal epithelial or neuroendocrine tissues (Table III), we have observed binding of monoclonal anti-p19 to prostate adenocarcinoma cells, though not to normal prostate tissue. Moreover, HPLC-purified HTLV p19 blocked the binding of monoclonal anti-p19 to prostate carcinoma-associated p19 antigen in a manner similar to blocking anti-p19 reactivity with thymic epithelium (R. S. Metzgar, R. C. Gallo, and B. F. Haynes, manuscript in preparation). This observation again suggests either a host origin for the monoclonal anti-p19 antigen or a cross-reactive phenomenon, and as well suggests other epithelial tumors will be found to express this antigen.

Thus, the data clearly indicate that the normal thymic epithelial anti-p19 reactive antigen is of host origin. At present we cannot resolve the issue whether p19 carried by HTLV and found in HTLV-infected cells is of viral or host origin. However, its presence in thymic epithelium surface membranes and acquisition during thymic ontogeny is of considerable interest. It is well recognized (24) that thymic epithelium is critical to the normal induction of cell maturation. Recently (25), it has been suggested that thymic epithelium of AKR mice induces amplified expression of

[3] Schuepbach J., and R. C. Gallo. Manuscript submitted for publication.

murine retrovirus genes by thymocytes and induces preleukemic phenotypic changes in thymocytes. In humans, Borzy et al. (26) reported the induction of fatal lymphoma after transplantation of cultured thymic epithelium in children with severe combined immunodeficiency disease. Taken together, these data suggest that the study of an antigen either shared by cross-reactivity between HTLV and thymic epithelium, or induced in host T cells by HTLV and co-expressed on normal thymic epithelium, may well provide important information regarding normal and aberrant thymic epithelial-thymocyte interaction.

Summary

Using monoclonal antibody 12/1-2 against a 19,000-dalton human T cell leukemia virus (HTLV) protein (anti-p19), previously demonstrated to be reactive with HTLV-infected human cells, but not in numerous other uninfected cells, we found a reactive antigen to be expressed on the neuroendocrine component of human thymic epithelial cells but not on any other normal epithelial or neuroendocrine human tissues. Moreover, this reactive antigen is acquired on neuroendocrine thymic epithelium during thymic ontogeny—first appearing on fetal thymic epithelial cells between 8 and 15 wk gestation. While only a portion of thymic epithelial cells in the subcapsular cortical region of 15- and 24-wk fetal thymuses contained anti-p19$^+$ epithelial cells, the entire subcapsular cortical region of newborn thymus epithelium was anti-p19$^+$. By age 3 yr, normal subjects' entire subcapsular cortical and medullary thymic epithelium was anti-p19$^+$. Using antibody against HTLV core protein, p24, and c-DNA probes for HTLV DNA, neither HTLV-specific p24 protein nor proviral DNA could be demonstrated in anti-p19$^+$ thymic epithelial tissue. However, thymic epithelial extracts, disrupted HTLV extracts, as well as purified HTLV p19 antigen all inhibited the binding of anti-p19 antibody to thymic epithelium. Thus, anti-p19 may recognize a determinant on an HTLV-encoded 19,000-dalton structural protein that is shared by human thymic epithelium. Alternatively, anti-p19 defines a host encoded protein that is selectively expressed by normal thymic epithelium, and is induced to be expressed in HTLV-infected malignant T cells.

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