Characterization of a Renal Tubular Epithelial Cell Line Which Secretes the Autologous Target Antigen of Autoimmune Experimental Interstitial Nephritis

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Abstract. Proximal tubular epithelial cells from mice which develop autoimmune interstitial nephritis were found to express the nephritogenic target antigen, 3M-1. Anti-3M-1 mAbs (α3M-1-Ab) were used to positively select for 3M-1-secreting tubular epithelium and, after stabilization in culture, this new cell line (MCT) was examined for the production of several moieties important to either immune interactions or to the development of extracellular matrix. Alkaline phosphatase–staining MCT cells also express epithelial growth factor receptors with a K_d of 0.87 nM and an epithelial growth factor receptor constant (R_o) of 2.1×10^4 receptors/cell. MCT culture supernatants contain greater amounts of laminin, and types IV and V procollagens compared to types I and III procollagens, and growing MCT cells on type I collagen matrix causes them to preferentially secrete even more type IV and V procollagen.

The 30,000-M_r 3M-1 antigen could be immunoprecipitated from biosynthetically labeled MCT cell supernatants with α3M-1-Ab. An identical-sized moiety was isolated by immunoaffinity chromatography from collagenase-solubilized mouse kidney tubular basement membranes. The 3M-1 antigen can be found on the MCT cell surface by radioimmunoassay, or deposited in a linear array in the extracellular matrix surrounding the MCT cells in culture by immunofluorescence. Mature messenger RNA species for both class I and class II major histocompatibility complex (MHC) molecules were detected by Northern hybridization, and their corresponding cell surface gene products were detected by cytofluorography of MCT cells stained with haplotype-specific antibodies. Both the cell surface 3M-1 and the small amounts of detected class II MHC molecules appear to be biologically functional, as MCT cells can support the proliferation of 3M-1-specific, class II MHC-restricted helper T cells in culture. These findings suggest that MCT cells provide all the necessary biological parameters for interfacing both as the target of a nephritogenic immune response, and as a potential source for new extracellular matrix which develops as a fibrogenic response to interstitial nephritis.

PATHOLOGIC damage from autoimmunity is largely the result of phlogistic antibodies, mononuclear cells, or soluble factors which are directed towards parenchymal structures in some combination of effect. The precise cellular source of these target antigens, however, is usually not known, and as a consequence, relatively little attention has been focused on specific immune interactions with tissue-derived cells. Even if important relationships could be established, many of these cells, unfortunately, have yet to be isolated or formally stabilized in culture. Nevertheless, one such cell type of growing experimental interest is parenchymal epithelium which may express or secrete antigenic epitopes of autoimmune interest (3, 12, 25, 26). Recent studies have also implicated an important role for immune-relevant, associative-recognizing, or growth-promoting molecules which may either modulate at the target cell level (2, 18, 29, 51) or influence the development of new extracellular matrix that may irrevocably alter parenchymal structure and function (42, 50, 56).

We have been studying autoimmune reactions to renal tubular basement membranes that produce an interstitial nephritis called anti-tubular basement membrane (αTBM)[1]

Portions of this work have appeared previously in abstract form (1988. Kidney Int. 33:149A).

1. *Abbreviations used in this paper*: αTBM, tubular basement membrane; α3M-1-Ab, anti-3M-1 antibodies; EGF, epithelial growth factor; MHC, major histocompatibility complex; RTA, renal tubular antigen.

disease (58). Autoimmunity is induced with heterologous antigen in adjuvant (34), and is directed against an autologous renal basement membrane glycoprotein (3M-1) which has been purified to homogeneity by immunoaffinity chromatography (7). Mice immunized to develop interstitial nephritis produce αTBM antibodies (α3M-1-Ab) (8), and a complex nephritogenic T cell response (28, 36) leading to a progressively destructive tubuloepithelial lesion associated with fibrosis and renal failure (34). We now report that the tubular epithelium producing this target antigen has been established as a cultured cell line (MCT). These cells express several moieties which may be important either to immune interactions or to the development of extracellular matrix.

Materials and Methods

Preparation of MCT Epithelial Cells

Kidneys were removed from naive SJL mice (H-2^s) and proximal tubules were microdissected (52) and briefly digested in 0.5 mg/ml purified collagenase (Worthington Biochemical Corp., Freehold, NJ) and 15 μg/ml DNAse I (Sigma Chemical Co., St. Louis, MO) for 45 min at 37°C. Washed cell suspensions were briefly exposed to 25 μl of α3M-1-Ab ascites fluid for 30 min at 4°C, followed by three washes with 0.1% BSA in PBS, and second incubation on petri dishes precoated with affinity-purified anti-mouse IgG (1 mg/ml) for 5–10 min at 22°C (46). The nonadherent cells were gently washed off and the antibody-adherent cells were released by temperature shift at 4°C. The resulting MCT cell suspension was washed and laid down on type I collagen-coated flasks in hormonally defined K-1 serum-free media (52). Cells were serially passaged using 0.05% EDTA. The cells were established in long-term culture by transformation with a nonreplicating, noncapsid-forming SV-40 virus (strain Rh 911, 10^8 IU/ml; provided by Dr. Barbara Knowles, Wistar Institute, Philadelphia, PA). The MCT cells were passaged every 48–72 h and were carried in RPMI-1640 media with 200 μg/ml streptomycin, 192 IU/ml penicillin, and 10% FCS, or in serum-free media of 50:50 Ham's F12/DME with 5 μg/ml transferrin, 5 μg/ml insulin, and 5×10^{-8} M hydrocortisone for selected experiments.

Cytofluorography for Detection of Major Histocompatibility Complex (MHC) Antigens

1×10^6 MCT cells were cultured for 48 h and removed from plastic flasks by incubating at 37°C with 0.02% EDTA in PBS for 10 min. 50 μl of cells (12×10^6/ml) in PBS with 0.1% BSA were incubated with 5 μl of ascites containing a primary anti-MHC antibody at 1:100 final dilution in staining buffer on ice for 30 min. Primary anti-MHC antibodies were class I haplotype-specific αK^s (39), class I haplotype-control αK^k (38), class II haplotype-specific αI-A^s (20), and class II haplotype-control αI-A^k (obtained from David Sachs, National Cancer Institute, Bethesda, MD). MCT cells were then washed and incubated with fluoresceinated F(ab')$_2$-rat anti-mouse IgG (Jackson Immuno Research Laboratories, Inc., Avondale, PA) diluted 1:1 with FCS for 30 min on ice followed by three washes. Stained cells were resuspended in staining buffer at 5×10^5 cell/ml and fluorescence was recorded on a FACStar cytofluorograph (Becton-Dickinson, Mountain View, CA). 10,000 live gated cells were analyzed in each run.

Immunofluorescence

In other experiments culture dish-adherent MCT cells were acetone fixed and directly stained with monoclonal α3M-1-Ab (7), anti-cytokeratin antibody, and appropriate class-specific control antibody, followed by species and class-specific fluorescein-labeled F(ab')$_2$ anti-IgG. After the final wash, each mount was covered with phenylenediamine in glycerol and examined with a Carl Zeiss Inc. microscope (Thornwood, NY) filtered for fluorescein-isothiocyanate.

Cell-binding Radioimmunoassay

Purified monoclonal α3M-1-Ab (57R-2/IgG) (9) and normal control IgG were both iodinated with ^{125}I (sp act, $10–12 \times 10^6$ cpm/μg). 25,000 MCT cells were grown overnight in a 96-well flat-bottom polyvinyl chloride plate. The wells were flicked clear of media and incubated with serial dilutions of radiolabeled antibody (100,000 cpm/50 μl) in 1% BSA/PBS. After a 2-h incubation at 22°C, the wells were washed in 1% BSA/PBS and counted for gamma emissions.

Biosynthetic Labeling of MCT Cells and the Immunoprecipitation of 3M-1

MCT cells were biosynthetically labeled with 10 μCi of ^{3}H-leucine/ml of RPMI-1640 containing 3% dialyzed FCS, antibiotics, ascorbic acid, and β-aminopropionitrile. After 36 h, the medium was harvested and dialyzed against deionized water containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 10 mM *N*-ethylmaleimide. The labeled MCT cells were also lysed with cell lysis buffer (150 mM NaCl, 10 mM Hepes, 4 mM EDTA, 2.5% NP-40, 2.5% SB-14, 1 mM PMSF, and 10 mM *N*-ethylmaleimide, pH 7.5). The resultant lysate was spun for 45 min at 4°C in a microcentrifuge at 10,000 rpm. The cell lysate was then dialyzed as described above for the labeled media. The dialyzed media and cell lysate were lyophilized for concentration and then resuspended in water. Aliquots of this preparation were immunoprecipitated with either monoclonal or polyclonal monospecific LEW rat α3M-1-Ab (7, 9), followed by biotinylated secondary antibody, and streptavidin-agarose (Gibco Laboratories, Grand Island, NY/Bethesda Research Laboratories, Gaithersburg, MD) according to the manufacturer's instructions. Precipitates were disrupted with Laemmli sample buffer containing 5% 2-mercaptoethanol and run through an 8% SDS-PAGE gel (24). Gels were fixed in methanol/acetic acid, soaked in EN^{3}HANCE (New England Nuclear, Boston, MA), dried and exposed to Xomat AR film (Eastman Kodak Co., Rochester, NY) at −70°C for 2 wk.

Preparation of Murine Kidney 3M-1

Crude renal tubular antigen (RTA) from SJL mice was isolated by a differential sieving technique (34). Highly enriched basement membrane fragments were lyophilized and stored at −20°C. Solubilized RTA was made from these lyophilized membranes using bacterial collagenase (CLS IV; Cooper Biomedical, Inc., Malvern, PA) in protease inhibitors (21). 3M-1 was purified out of this crude preparation using immunoaffinity chromatography with α3M-1-Ab coupled to Sepharose 4B (7). The eluate from this column was run under reducing conditions on an 8% SDS-PAGE gel, and potential bands were developed with silver stain (33).

Assay of T Cell Proliferation

A CD4$^+$, H-2^s cloned helper T cell line (M30) was carried weekly at 10^4 cells/ml in RPMI-1640 with 10% FCS, 20 μg/ml solubilized rabbit renal tubular antigen, 5×10^6 irradiated H-2^s splenocytes as feeders, and 10% supernatant from MLA-144 cells as a source of IL-2 (16). 10^5 T cells at end passage were incubated with various combinations of 3×10^5 irradiated MCT cells, 5×10^6 syngeneic irradiated spleen cells, or 3M-1 antigen at 10 μg/ml and cocultured for 72 h in 2 ml RPMI-1640 with 10% FCS. During the last 4 h of culture, 100 μl of cells were cultured with 1 μCi of ^{3}HTdR (Amersham Corp., Arlington Heights, IL) in triplicate. Cells were then harvested onto glass-fiber filter paper and the radioactivity assayed as a measure of proliferation by counting filters dissolved in scintillation cocktail for beta emissions.

Assay of MCT Cell Proliferation

10^4 MCT cells were cultured in 200 μl 50:50 Ham's F12/DME in flat-bottom microtiter plates with varying amounts of transferrin/insulin. After several days, incubation wells were pulsed with 1 μCi ^{3}HTdR for 6 h. Media was removed and the cells were released with trypsin, harvested, and counted as for T cells above.

Radioimmunoassay of Secreted Matrix Proteins

2×10^6 MCT cells were plated in flasks in serum-free media supplemented with K-1 additives and 50 μg/ml of ascorbic acid and β-aminopropionitrile. After 48–72 h, the supernatants were harvested and assayed for soluble matrix proteins by radioimmunoassay (vide infra). Adherent matrix was prepared by first removing the cells from their flasks with 0.02% EDTA in PBS. 0.1 mg/ml pepsin (Sigma Chemical Co.) in 0.5 M acetic acid was then used to limit digest-adherent matrix proteins by overnight shaking at 25°C. The solubilized material was precipitated at 0°C in 20% wt/vol NaCl and resuspended in 1.0 ml 0.5 M acetic acid before radioimmunoassay. Stan-

dard collagens (obtained from Dr. Nicholas A. Kefalides) types I (31) and IV (5) were demonstrated pure by SDS-PAGE (5); type III (Sigma Chemical Co.; 49) ran at 295,000 M_r unreduced and 95,000 M_r reduced on SDS-PAGE; type V (obtained from Dr. J. Madri, Yale University, New Haven, CT) was purified as described (41) and laminin from Englebreth-Holm swarm sarcoma (23) was obtained from Bio-Rad Laboratories (Richmond, CA). Standards were reconstituted at 1–10 mg/ml in 0.5 M acetic acid and diluted to 10–30 μg/ml into 0.1 M PO_4 buffer, pH 8.0, from which they were dispensed into polyvinyl chloride plates at various concentrations for comparison with supernatants and pepsin-digested matrix proteins from the MCT cells. Plates were dried overnight at 4°C, blocked with 4% BSA, and incubated with a 1:100 dilution of rabbit anti-type I, type IV, or type III (1, reviewed in reference 30) collagen antibodies (gifts of Dr. Antonio Martinez-Hernandez, Thomas Jefferson University, Philadelphia, PA), anti-type V collagen antibody (27; gift of Dr. Heinz Furthmayr, Yale University), or rat anti-laminin antibody (prepared in our laboratory). Binding of these primary antibodies was measured by using ^{125}I-labeled anti-rat or anti-rabbit antibodies (10^5 cpm/well) in 4% BSA. Binding of matrix and supernatants was compared to known standards and data were expressed in micrograms of protein/flask.

Epidermal Growth Factor (EGF) Binding Studies

Purified rat EGF and ^{125}I-labeled EGF were generously provided by Richard Savage, Jr. (Department of Biochemistry, Temple University School of Medicine, Philadelphia, PA). The EGF had been isolated from submaxillary glands of adult male rats by the method of Savage and Cohen (43). Purified EGF was radioiodinated with carrier-free Na^{125}I, using a modification of the Hunter and Greenwood procedure (32). EGF was radiolabeled to a specific activity range of 150–200 μCi/μg. Greater than 90% of the radiolabeled hormone was precipitated by 7% TCA. Radioactivity was measured in an autogamma spectrometer (Packard Instrument Co., Downers Grove, IL) with a counting efficiency of 73%.

Binding studies were conducted using confluent monolayers of MCT cells in 24-multiwell plates (Costar, Data Packaging Corp., Cambridge, MA) as previously described (44). Cells were seeded at a density of 1×10^5 cells/well in 1.0 ml of culture medium and grown to confluency (3 d). The binding reaction was carried out in 0.2 ml of MEM containing 0.2% (wt/vol) BSA and 25 mM Hepes, pH 7.4. The specific radioactivity of the ^{125}I-EGF was adjusted to 3 μCi/μg by the addition of nonlabeled EGF and the final concentration of EGF incubated with cells ranged from 0.08 to 1.5 nM. Nonspecific binding was determined in the presence of a 1,000-fold excess of unlabeled EGF and always was <10% of the total bound cell radioactivity. After incubation for 40 min at 37°C, the plates were drained and flooded three times with ice cold PBS to remove unbound EGF. Cells were dissolved in 1 ml of 10% NaOH for 10 min at room temperature and the amount of radioactivity was determined. The data was analyzed by the method of Scatchard (45) to determine the concentration of EGF required for half-maximal binding and the apparent number of receptors.

Electron Microscopy

MCT cells were detached using 0.5% trypsin at room temperature for 3 min and collected in 3% glutaraldehyde in 0.1 M $NaPO_4$ buffer with 0.026 M NaCl for 3 h, postfixed in 1% O^sO_4 in 0.1 M $NaPO_4$ for 1 h, dehydrated in graded ethanol solutions, and embedded in Medcast–Araldite via propylene oxide (1). Ultrathin sections were mounted on uncoated grids, stained with lead citrate and uranyl acetate, and photographed on a Zeiss model EM10 photomicroscope.

Northern Hybridization

10^8–10^9 MCT cells were washed in cold PBS, and RNA was extracted by guandinium thiocyanate (6). 10–15 μg of RNA was denatured by heating in formamide/formaldehyde/EDTA at 65°C for 5 min, diluted in SDS/glycerol sample buffer, and subjected to electrophoresis on 1.5% agarose gels in 2.2 M formaldehyde overnight at 30 V (4). The gels were blotted onto Nytran (Schleicher and Schuell, Inc., Keene, NH) baked for 2 h at 80°C, and prehybridized at 42°C for 6 h in buffer (0.25 ml/cm^2) containing 50% formamide, 5× Denhardt's solution, 0.1% SDS, 150 μg/ml salmon sperm DNA, and 5× SSPE. A 0.6 kb Pst-1 murine class I MHC fragment in pUC 13 (10) or a 5.6 kb Eco RI murine class II A_β fragment in pBr322 (47) were nick translated to a specific activity of 0.5–1.0×10^9 cpm/μg and added to hybridization fluid of 50% formamide, 2× Denhardt's solution, 150 μg/ml salmon sperm DNA, 0.1% SDS, and 5× SSC for incubation at 42°C for 24 h. The filters were twice washed for 15 min in 6× SSC, 0.5% SDS at 22°C, and once in 0.1× SSC, 0.5% SDS at 65°C for 1 h. The filters were then autoradiographed with intensifying screens at −70°C.

Results

Establishment of MCT Cells as Epithelium

When our MCT cells were first stabilized in culture, we wanted to determine whether they were of epithelial origin. This is because immune destruction in the model of autoimmune αTBM disease seems to be directed, in part, against renal tubular epithelial cells. By morphologic criteria, using electron microscopy, MCT cells (Fig. 1) show numerous surface projections consistent with villi of proximal tubular epithelium. They also express desmosomes or tight junctions which are thought to be unique features of most epithelium (Fig. 1, *inset*). Finally, MCT cells stained positive for alkaline phosphatase, and, by immunofluorescence in monolayer culture, they stained positive for cytokeratin (data not shown).

Primary cultures of renal tubular epithelial cells have typically been established, without serum, in media supplemented with insulin, transferrin, hydrocortisone, and triiodothyronine (K-1 additives) (52, 53, 57). Fibroblasts, by their absolute need for serum, can be overgrown by epithelial cells if the beginning cultures are serum-free. We suspected that the growth of MCT cells in serum-free medium would suggest an epithelial, as opposed to fibroblastic, origin. In fact, when supplemented with 100% K-1 additives in serum-free media, MCT showed exponential growth (Fig. 2) which was decreased in a dose-dependent fashion by reducing the K-1 additives. Our cultured renal fibroblasts from SJL mice cannot be passaged in serum-free media (data not shown).

Specific binding of ^{125}I-EGF by growing MCT cells showed EGF receptors (R_o) equal to 2.1×10^4/cell with a K_d of 0.87 nM (Fig. 3) which are very similar to the R_o and K_d reported for primary cultures of rabbit proximal tubule cells (37) of 1.7×10^4 receptors/cell and 0.3 nM, respectively. Epithelial cells and fibroblasts both secrete collagens, however, the former are thought to secrete predominately type IV collagen while the latter secrete primarily type I (22). To determine if MCT cells secreted matrix proteins, we compared the secretion of collagens and laminin into confluent culture supernatants (Fig. 4 *A*) or into matrix (Fig. 4 *B*) by solid-phase radioimmunoassay. Clearly, more laminin and collagenous proteins were secreted into the culture media than were deposited into the matrix. The production of relatively larger amounts of type IV and V collagen and laminin into the supernatants is consistent with MCT cells being of epithelial origin. MCT cells grown on artificial type I collagen matrix demonstrated a marked increase in type IV and V collagen and laminin in the supernatants (Fig. 5), compared to supernatants from cells grown on plastic. Thus by electron microscopic morphology, serum growth requirements, and matrix protein secretion profiles, MCT cells appear to be of epithelial origin.

MCT Cell Expression of 3M-1 Protein

For renal tubular MCT cells to be an immunologic target in murine interstitial nephritis, they would have to produce the unique glycoprotein antigen (3M-1) of αTBM disease (7). To assess production of 3M-1 initially, we assayed cultured MCT cells for binding by ^{125}I-labeled α3M-1 (αTBM) anti-

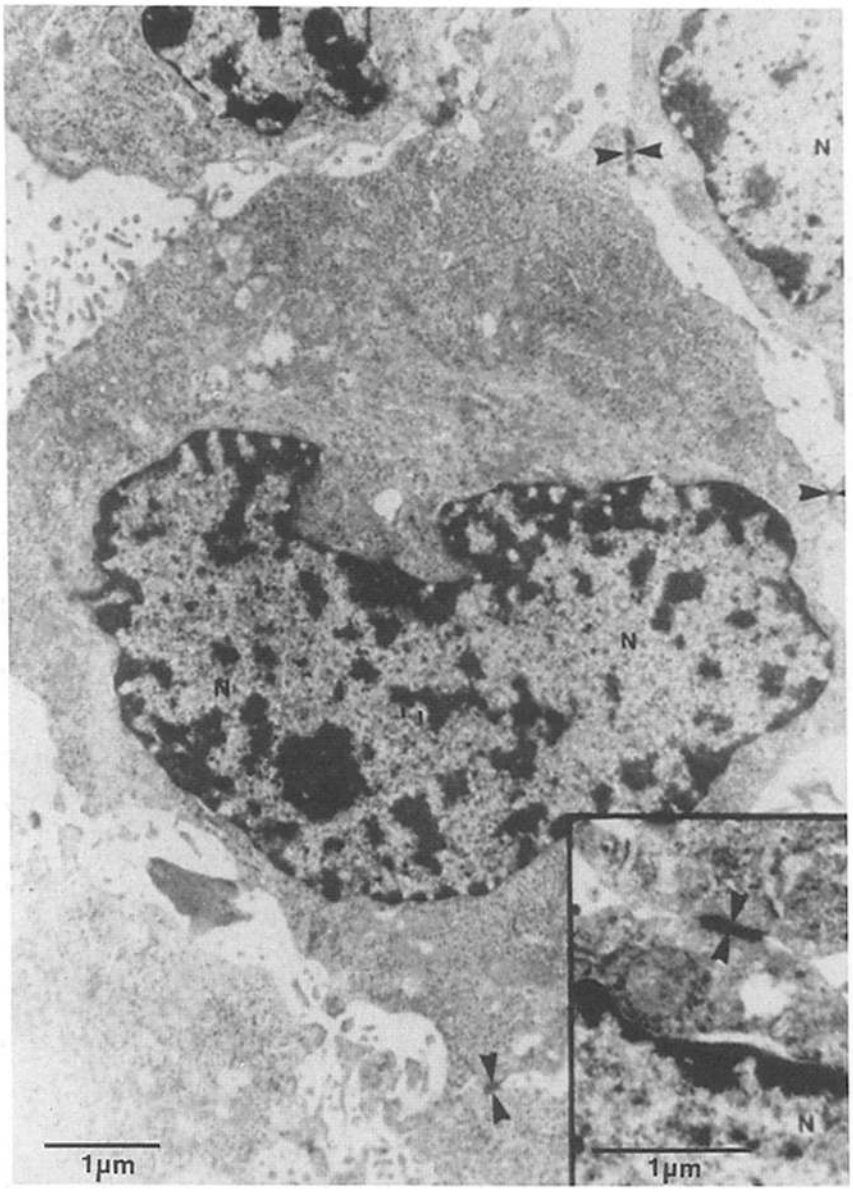

Figure 1. Electron microscopy of MCT cells. Cells were detached with trypsin, fixed with glutaraldehyde, stained with lead citrate–uranyl acetate, and photographed with a Zeiss EM10 photomicroscope. *Arrowheads*, desmosomes.

body (Fig. 6). There was specific binding by α3M-1-Ab over control rat IgG. To ascertain whether this binding was to a unique determinant, we attempted to immunoprecipitate a single moiety with monoclonal α3M-1-Ab from biosynthetically labeled MCT cells. Indeed, the results indicate that only one 30,000-M_r protein was immunoprecipitated by this antibody (Fig. 7 *A*; identical results were also obtained with monospecific, polyclonal LEW α3M-1-Ab [data not shown]). Cell lysates also contained this 30,000-M_r moiety (data not shown), suggesting that the murine 3M-1 antigen was not degraded in the extracellular culture supernatant. To verify that this unique protein from MCT cells was the same antigen as in syngeneic mouse kidney, collagenase-digested SJL mouse kidney basement membranes were subjected to immunoaffinity chromatography with monoclonal α3M-1-Ab. Electrophoresis of this eluate on SDS-PAGE and silver staining of the gel again revealed a single band at 30,000 M_r (Fig. 7 *B*).

In vivo, however, α3M-1-Ab reveal a pattern of linear deposition around the tubular basement membrane (7), and no pattern suggestive of 3M-1 being simply a cell surface glycoprotein. To clarify the spatial distribution of 3M-1 in growing MCT cells, fixed cells were stained with monoclonal α3M-1-Ab and developed by fluorescence microscopy. Fluorescence was seen as linear strands in the extracellular matrix (Fig. 8 *A*). When MCT cells were permeabilized to allow visualization of the intracellular compartment, bright fluorescence was observed intracellularly with α3M-1-Ab (Fig. 8 *B*) as compared with control antibodies (Fig. 8 *C*). It appears that MCT cells synthesize a 30,000-M_r protein that is present in large amounts intracellularly, and is secreted into the extracellular matrix. Antibodies which immunoprecipitate this protein also purify from mouse kidney collagenase-solubilized basement membranes a moiety of identical M_r, implying that this epithelial cell line produces an antigen that is similarly found in native kidney.

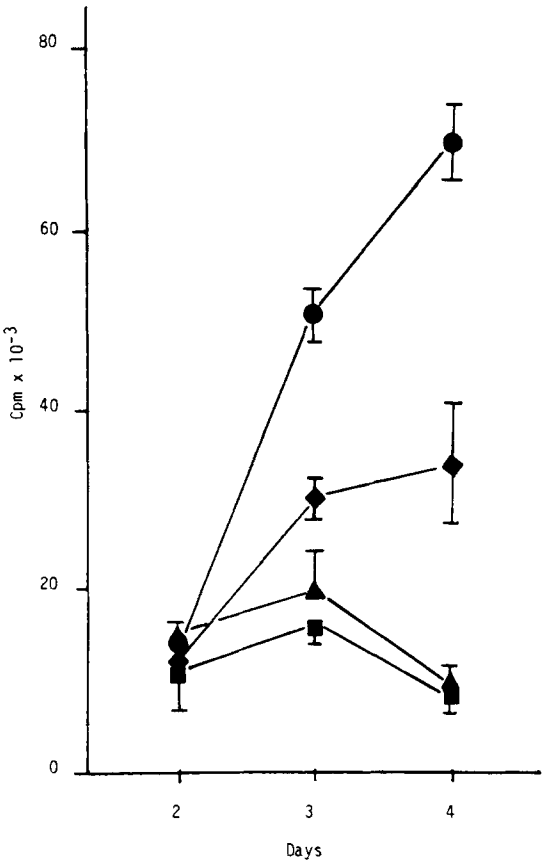

Figure 2. Modulation of MCT cell proliferation by transferrin/insulin. 10^5 MCT cells were cultured in 50:50 Hams F12/DME (serum-free media) in 200-μl, 96-well, flat-bottom plates with varying concentrations of transferrin/insulin: (●) 5 μg/ml, (♦) 1 μg/ml, (▲) 0.2 μg/ml, and (■) 0 μg/ml. At the indicated times, the cultures were pulsed with 1 μCi ^{3}HTdR for 6 h after which the cells were detached with trypsin and collected onto filter paper for counting in scintillation fluid. Data are expressed as mean cpm ± SEM.

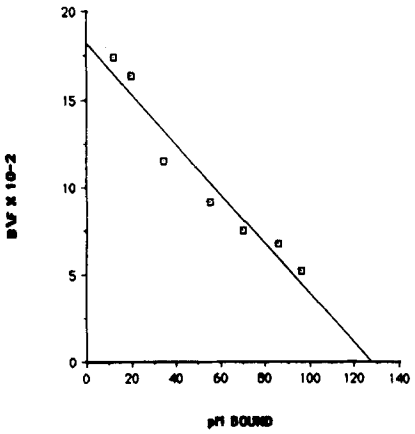

Figure 3. EGF Receptor Scatchard Analysis. 1×10^5 cells were grown to confluence and incubated with various concentrations of ^{125}I-labeled EGF alone, and with 1,000-fold excess cold EGF. After washing, cells were counted for radioactivity and data analyzed by the methods of Scatchard. R_0 (EGF receptor sites per cell) = 21,003; K_d (dissociation constant for EGF with its receptor) = 0.87 nM.

MCT Cell Expression of MHC Molecules

T cells which recognize 3M-1 antigen do so in the context of MHC determinants (34). Helper cells from different strains of mice susceptible and nonsusceptible to this autoimmune disease, for example, are restricted by class II MHC antigens. Nonsusceptible mice also express effector T cells which are restricted by class II determinants, while susceptible strains produce effector T cells which are restricted by class I MHC antigens. Thus, the expression of classes of MHC antigen, at the target cell level, may be potentially relevant to the development of interstitial injury (36). We, therefore, wished to determine the extent of expression of these MHC antigens on our MCT cells. Suspensions of MCT cells

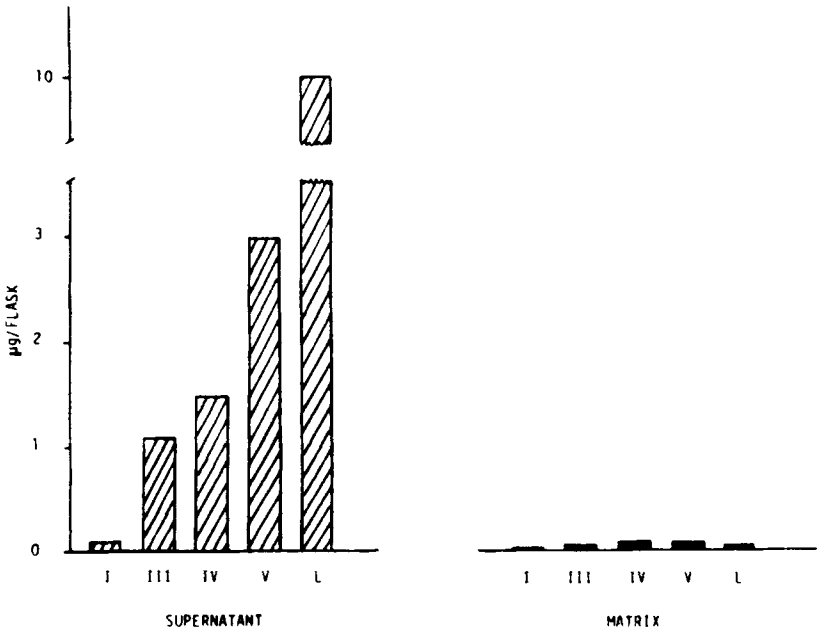

Figure 4. Measurement of matrix protein in MCT cells by radioimmunoassay. 2×10^6 MCT cells were cultured in 5-ml T25 flasks in serum-free media supplemented with 5 μg/ml transferrin/insulin and 50 μg/ml ascorbic acid/β-amino propionitrile for 48 h after which the supernatants were collected and cell-free matrix released from the flask by pepsin digestion. Supernatants or matrix proteins in duplicate were used to line polyvinyl chloride plates and binding of adsorbed type-specific, anti-collagen or laminin antibody was compared to binding of standard amounts of applied collagens or laminin measured by incubating wells with species-specific ^{125}I-labeled second antibody.

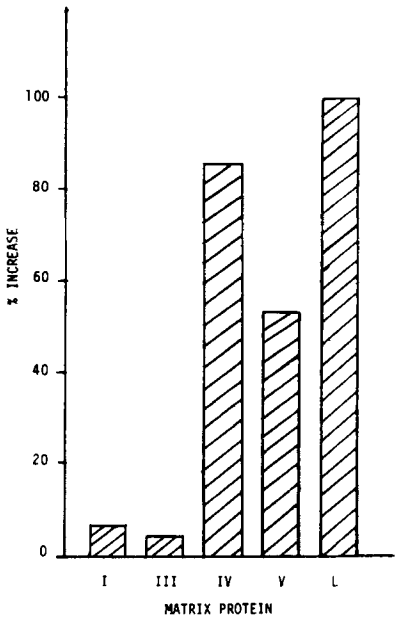

Figure 5. Effect of artificial extracellular matrix on secretion of matrix proteins by MCT cells. Cells were cultured as in Fig. 3 in an untreated flask or in one coated with type I collagen matrix. Matrix proteins in supernatants were then measured by radioimmunoassay. The data are expressed as percent increase in production when cultured on Type I matrix compared to plastic.

were accordingly incubated with haplotype-specific class I or class II antibodies whose binding was assayed with species-specific fluoresceinated second antibodies by cytofluorography. As shown in Fig. 9, there is abundant class I expression (Fig. 9 *A*; 70% cells positive, mean channel fluorescence 125, background 32 arbitrary units) and a small but detectable amount of class II on the cell surface (Fig. 9 *B*; 10% cells positive, mean channel fluorescence 30, background 22).

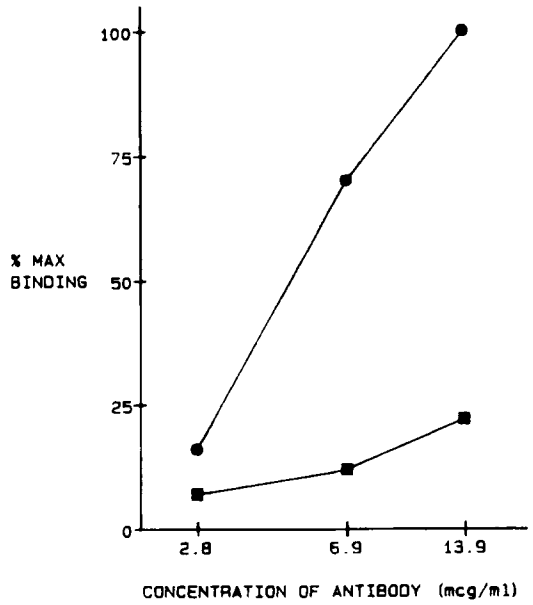

Figure 6. Binding of α3M-1-Ab to MCT cells. 10^5 MCT cells were dispensed in polyvinyl chloride plates and ^{125}I-labeled α3M-1-Ab (●), or control Brown Norway or rat IgG (■) of equivalent specific activity were added in the indicated concentrations. After washing, wells were harvested, counted, and data expressed as maximum binding.

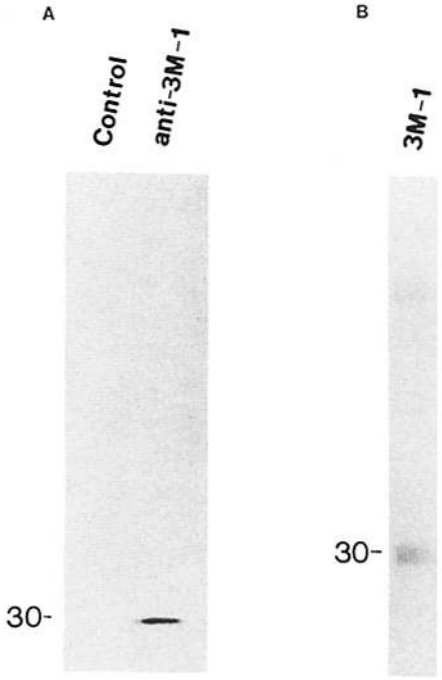

Figure 7. Comparison of secreted 3M-1 from MCT cells with 3M-1 isolated from mouse kidney. MCT cells cultured in RPMI-1640 supplemented with 3% FCS, 5 μg/ml transferrin/insulin, and 50 μg/ml ascorbic acid/β-amino propionitrile were pulsed with 10 μCi [^{3}H]leucine/ml for 36 h after which the supernatant was lyophilized, resuspended in water, and immunoprecipitated with α3M-1-mAb to 3M-1, followed by biotinylated secondary antibody and Streptavidin agarose. Precipitates were resolved by 8% SDS-PAGE and the dried gel was autoradiographed (*A*). Crude RTA was prepared by differential sieving and collagenase digestion. This RTA was passed over an α3M-1-Ab–Sepharose 4B column and the eluate was run under reducing conditions on an 8% SDS-PAGE gel with subsequent silver staining (*B*).

We were also curious as to the nature of the mRNAs expressed for MHC molecules, as a foundation for future studies on regulation of MHC expression at the molecular level (15). We prepared total cellular RNA from MCT cells, size fractionated it on agarose gels, and blotted it onto nitrocellulose. [^{32}P]cDNA probes for class I MHC antigen hybridized to a mature-sized message at 1.8 kb (Fig. 10 *A*) and class II hybridized to a mature-sized message at 1.4 kb (Fig. 10 *B*). Thus, in the unstimulated state, both class I and class II messages are detectable.

To assure ourselves that the cell surface gene products for 3M-1 and class II MHC antigens are biologically relevant, we also determined whether MCT cells could induce and support the proliferation of M30 lymphocytes, a cloned CD4$^+$ helper/inducer class II MHC-restricted, 3M-1-reactive T cell line (17, 28). As demonstrated in Table I (Experiment 1), M30 lymphocytes proliferate to 3M-1 only if they are cocultured with syngeneic feeder spleen cells which provide a source of class II MHC. They do not proliferate in response to 3M-1 alone. In Table I (Experiment 2), MCT cells

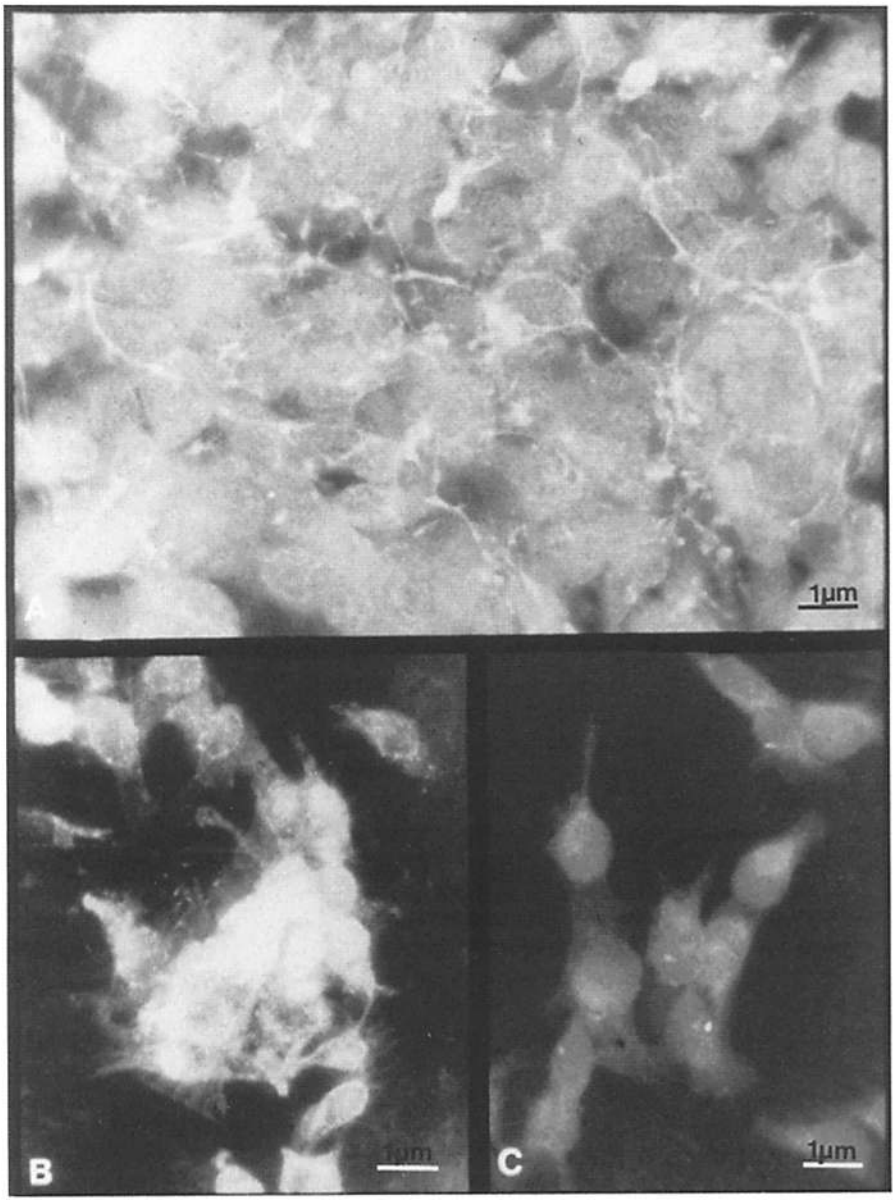

Figure 8. Localization of 3M-1 in MCT cell monolayers by immunofluorescence. Confluent MCT cells were fixed in acetone and labeled with α3M-1-mAb followed by class-specific fluorescein labeled $F(ab')_2$ anti-IgG with no pretreatment (*A*) or with digitonin permeabilization to allow access of the intracellular compartment before α3M-1-Ab (*B*) or control antibody (*C*) incubation. After washing, subsequent to fluoresceinated antibody development, each mount was covered with phenylenediamine in glycerol and examined with a Zeiss microscope filtered for fluorescein–isothiocyanate.

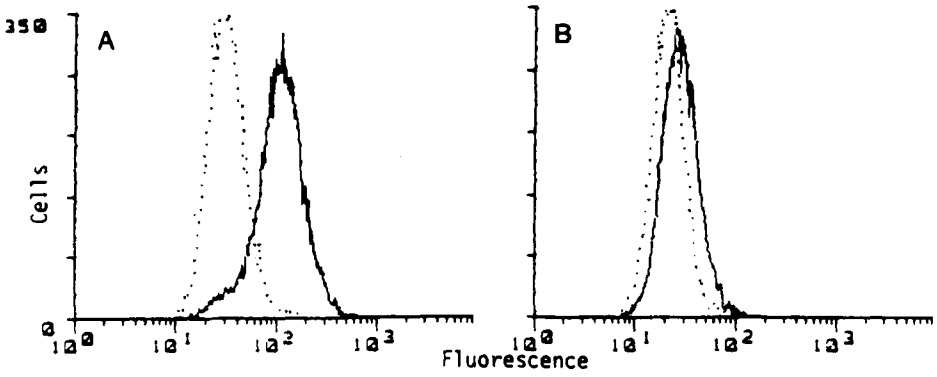

Figure 9. Cell surface MHC expression by MCT. MCT cells were released by 0.02% EDTA in PBS and 5×10^5 cells were incubated with class I MHC (*A*) or class II (*B*) specific and control antibodies after which the cells were incubated with fluoresceinated $F(ab')_2$–rat IgG. Stained cells were analyzed by cytofluorography with the fluorescence of 10,000 live gated cells recorded. *Dotted lines*, fluorescence histograms of cells incubated with control antibody; *solid lines*, cells incubated with haplotype-specific antibodies.

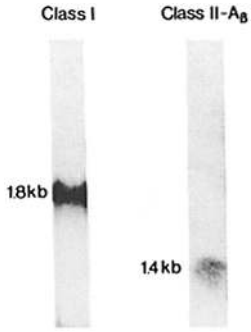

Figure 10. Northern blot analysis of MCT MHC. 10 μg/lane of MCT RNA was resolved by electrophoresis in 1.5% agarose gels. The gels were blotted onto Nytran, baked, and (after prehybridization) were hybridized to nick-translated ^{32}P-labeled class I or class II probe at 0.5 × 10^9 cpm/μg sp act for 16 h at 42°C. Blots were washed as described and autoradiographed.

alone will also support M30 proliferation, implying MCT provides a source of both biologically relevant 3M-1 and class II MHC antigens (17).

Discussion

We have stabilized a renal tubular epithelial cell line in continuous culture which appears to express a variety of functions that will make it a relevant and useful immunologic target for cell interaction studies in an autoimmune renal disease. This MCT cell secretes an extracellular matrix whose composition resembles basement membranes since it contains predominantly types IV and V collagens and laminin. The cells also synthesize and secrete into the matrix, a 30,000-M_r glycoprotein, identical by radioimmunoassay and immunoaffinity chromatography with 3M-1, the autologous target antigen of experimental αTBM disease. We are currently sequencing cDNA clones encoding 3M-1 mRNA that were isolated from a cDNA library prepared from MCT cells. Presumably this 3M-1 protein is linked to collagen in an extracellular process since collagenase is needed to isolate the antigen. The MCT cells, furthermore, express both class I and class II gene products, and the small amount of class II so detected appears to be functional as MCT cells are able to stimulate the proliferation of class II restricted 3M-1-reactive T cells. The in vitro demonstration of biologically active class II MHC gene and gene product expression may argue for antigen presentation capability of renal epithelium in vivo, a function traditionally limited to antigen-presenting cells. This stimulation, in preliminary studies, is blocked by antibodies to 3M-1 antigen or to the CD4-associative recognition molecule (Hines, W. H., T. P. Haverty, E. G. Neilson, and C. J. Kelly, manuscript in preparation). The bidirectional modulation of class II expression by antigen-specific and nonspecific factors (15) also suggests a potential regulatory role for epithelial class II MHC expression in immune injury.

Besides secreting various proteins, epithelial cells are also usually capable of generating and preserving compositional asymmetry between separate fluid phase domains (11, 13). We did not measure vectorial solute or water transport in MCT cells, nor did we characterize the asymmetric distribution of such enzymes as aminopeptidase or Na/K-ATPase (54, 57). Rather, we were interested in the secretory properties of MCT cells and selected the demonstrated presence of desmosomes and cytokeratin as evidence of their epithelial descent. Although the cells were immortalized by SV-40, they behave quite similarly to primary rabbit proximal cultures as evidenced by similar affinities and levels of EGF receptor expression (37).

For the majority of the described experiments, MCT cells were grown on plastic alone, without an artificial extracellular matrix. Malignant cells require or provide an extracellular matrix to flatten into monolayers and proliferate (55). Bovine collecting tubule explants can generate either fibroblast or epithelial populations, depending upon whether the cells are cultured on basement membrane produced by bovine corneal endothelial cell lines or on one from a mouse-derived endodermal cell line, respectively (14). Furthermore, corneal epithelium cultured without an artificial extracellular matrix spontaneously forms blebs which are blocked by the addition of soluble collagen, laminin, or fibronectin (48). Our culture of MCT cells on a type I collagen matrix increased the secreted amounts of types IV and V collagens and laminin. This, however, did not qualitatively alter the profile of secretion of matrix-relevant proteins by these cells. Culture on a collagen matrix, rather than causing a fundamental differentiation of MCT, probably allows them to amplify existing biochemical processes. A variety of growth promoting or inflammatory cytokines, however, have been shown, in preliminary studies, to alter the profile of matrix protein secretion in MCT epithelium. Steady state mRNA levels for type IV collagen are several-fold higher than for type I (unpublished observations), supporting our gene product secretion profile.

Table I. Proliferation of M30 Lymphocytes Cocultured with MCT Cells

	M30 helper T cells*	Irradiated MCT cells‡	Irradiated spleen feeders§	3M-1‖	^{3}HTdR¶
					cpm
Experiment 1	+	−	−	+	42 ± 7
	+	−	+	−	113 ± 59
	+	−	+	+	5,157 ± 262
Experiment 2	−	+	+	−	124 ± 50
	+	−	+	−	50 ± 6
	+	−	+	+	7,603 ± 252
	+	+	+	−	4,127 ± 1,324
	+	+	−	−	6,509 ± 196

* 10^5 helper cells added to each well where indicated.
‡ 3 × 10^5 MCT cells set down on well floor 24 h before coculture (2,500 rads).
§ 5 × 10^6 syngeneic spleen feeders (2,500 rads) added where indicated.
‖ 10 μg/ml used where indicated.
¶ All wells (in triplicate) were pulsed with 1 μCi ^{3}HTdR overnight on day 3 of culture and processed in a cell harvester for scintillation counting.

This is the first demonstration of an epithelial cell culture system which appears to both secrete the target antigen of an autoimmune disease and support the proliferation of antigen-reactive T cells. There are other intriguing, but less well-defined examples of parenchymal tissue demonstrating some antigen-presenting capabilities, such as cultured thyroid cells which can stimulate syngeneic T cell clones from patients and normal controls (25, 26). Astrocytes can present exogenous myelin basic protein to encephalitogenic T cell lines in experimental allergic encephalomyelitis (12). Finally, aberrant pancreatic islet cell class II expression has been shown in diabetes (3, 29), where it may be critical in the pathogenesis of immune-mediated injury.

A final common pathway of autoimmunity in many organ systems is the induction of fibrogenesis and irreversible scarring. The factors influencing the progression from immune destruction to fibrogenesis are still obscure at the parenchymal cell level. An obvious cell potentially responsible for interstitial fibrogenesis is the fibroblast, which can proliferate in response to antigen-reactive T cell supernatants in experimental interstitial nephritis (35). Lymphocyte factors that either stimulate or inhibit fibroblast collagen production have also been identified (19, 40). It is certainly possible that some of the fibrogenesis observed in experimental interstitial nephritis is due to lymphokine activation of local renal fibroblasts. However, since the MCT epithelial cells can also produce types I and III procollagens, we suspect that part of the fibrogenic response could be contributed by the tubulo-epithelium in this disease. This hypothesis is particularly attractive since MCT cells produce the antigen and both classes of MHC molecules which could focus the immune system to this epithelium. Immune mononuclear cells may also secrete factors which modulate the level of MHC gene and/or gene product expression in MCT cells (15). Such alterations might influence MHC-restricted lymphocyte–epithelial interactional events. To the extent the regulation of antigen, MHC molecule expression, and matrix-relevant protein secretion can be elucidated in an epithelial cell line such as MCT, conclusive biochemical mechanisms of autoimmunity may be more completely understood with subsequent work.

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