# Endogenous and Monoclonal Antibodies to the Rat Pancreatic Acinar Cell Golgi Complex

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ABSTRACT Normal, unimmunized mouse serum from several strains (BALB/c, C57/b, DBA/2, NZB, SJL, CD/1) contains an endogenous IgG antibody that localizes to the Golgi complex of rat pancreatic acinar cells. Treatment of pancreatic acini with 5 μM monensin resulted in the swelling and vacuolization of the Golgi cisternae, and in a corresponding annular staining by the mouse serum as observed by immunofluorescence, suggesting that the antigen recognized is on the Golgi complex cisternal membrane. The antiserum did not react with pancreatic secretory proteins, and its binding to smooth microsomal membranes was retained following sodium carbonate washing, supporting a Golgi membrane localization.

Advantage was taken of the existence of the endogenous murine antibody for the isolation of monoclonal antibodies directed to the Golgi complex of the rat pancreas. Two antibodies, antiGolgi 1 and antiGolgi 2, are described. Both antibodies are IgMs that recognize integral membrane proteins of the *trans*-Golgi cisternae, with lighter and patchy staining of the pancreatic lumen membrane, as observed both by light and electron microscopy. AntiGolgi 1 recognizes predominately a protein of molecular weight 103,000–108,000, whereas antiGolgi 2 shows a strong reaction to a 180-kd band as well as the 103–108-kd protein.

The problem of organelle biogenesis may reduce to the question of how membrane domains are created and maintained. To answer this question it is necessary to have specific markers for membrane antigens which are defined by their localization, rather than solely by their biochemical properties. Monoclonal antibody techniques offer researchers a relatively straightforward methodology for the acquisition of probes for cellular components that are biochemically undefined but that can be recognized by their desired location, or other characteristics.

In the course of devising techniques for screening monoclonal antibodies to various membrane domains, we observed an endogenous mouse serum antibody that recognizes integral membrane antigens of the rat pancreatic Golgi complex (1). The usefulness of such antibodies for our laboratory's studies of membrane biogenesis prompted us to exploit this discovery, and to isolate monoclonal antibodies directed to integral membrane proteins of the *trans*-Golgi cisternae. Investigators developing murine monoclonal antibodies should also be aware that this endogenous antibody is likely to contaminate ascites fluids containing other monoclonal antibodies of interest.

## MATERIALS AND METHODS

*Mouse Sera:* Balb/C, SJL, NZB, DBA/2, and C57/b mice were obtained from Jackson Laboratories (Bar Harbor, ME). CD/1 mice were from Charles Rivers Laboratories (Wilmington, MA). To obtain small quantities of normal serum for immunolocalization, mice were anesthetized (Metofane, Pitman-Moore, Washington Crossing, NJ) and bled from their retro-orbital sinuses. The blood was clotted overnight at 4°C and spun for 5 min at 18,000 *g*, after which the supernatant was retained. Larger quantities of mouse serum, diluted in phosphate-citrate buffer, were donated by J. Gershoni (Weizmann Institute, Rehovot, Israel). This was precipitated in 50% saturated ammonium sulfate, and dialyzed against 20 mM sodium phosphate buffer, pH 8.0, containing 5–10 mM EDTA. Recovery of the mouse serum IgGs was done on DEAE-Affigel Blue (Bio-Rad Laboratories, Richmond, CA) in the above buffer, with the IgM-containing fraction obtained after elution of the column with 0.2 M NaCl added to the buffer. An IgG fraction of mouse serum was also purchased from Miles Laboratories Inc., (Kankakee, IL) and an IgM fraction was obtained from Pel-Freeze Biologicals (Rogers, AR). Mouse IgG Fc molecules were purchased from Jackson Immunoresearch (Avondale, PA).

Mouse antiserum to pancreatic secretory proteins was obtained ~1 mo after intraperitoneal immunization of mice with 100 μg of alum-precipitated secretory proteins.

*Immunofluorescence:* For the examination of the pancreas and most tissues, rats (Sprague-Dawley, Charles Rivers Laboratories) were killed by decapitation, and the organ of interest was dissected out and minced in 3% paraformaldehyde in PBS (containing 8 mM $Na_2HPO_4$, 1.5 mM $KH_2PO_4$, 3

THE JOURNAL OF CELL BIOLOGY · VOLUME 98 JUNE 1984 2035–2046

mM KCl, and 140 mM NaCl), pH 7.2. Tissues were fixed for from 2 h to several days at 4°C. In some cases animals were anesthetized with ether and perfused with the same fixative before dissection and further fixation, and 0.1% glutaraldehyde was occasionally added for use with the monoclonal antibodies. After fixation, the tissues were infiltrated with 1.2 M sucrose in 20 mM sodium phosphate buffer, pH 7.2, and sections of 0.5–1.0 μm were cut at −55° to −70°C on an ultramicrocryotome (E. I. DuPont de Nemours & Co., Inc., Sorvall Instruments Div., Newtown, CT; reference 2) and placed on gelatin-subbed slides. Slides with tissue sections under 2.3 M sucrose could be stored at −20° up to 12 mo with no loss of structural detail or antigenicity.

Indirect immunofluorescence was done by the general procedure of Coons and Kaplan (3). The sucrose overlaying the sections were washed away with 20 mM phosphate buffer, pH 8.0, containing 10–20 mM lysine (wash buffer) which was changed four times in 0.5 h. The primary antibody was diluted into 20 mM phosphate buffer, pH 8.0, containing 25% goat serum (Gibco Laboratories, Grand Island, NY) and 0.3 M NaCl (serum dilution buffer, SDB[1]) and applied to the sections for 1 h at 25°C. Sections were then washed with four changes of wash buffer in 0.5 h, and the rhodamine-conjugated second antibody (goat anti-mouse, Cappel Laboratories, Inc. Cochranville, PA) diluted at 1:300 in SDB was applied for 1 h. Sections were again washed, and put into coverslips under 10% glycerol in phosphate buffer, pH 8.0.

*Monensin Treatment:* Rat pancreata were digested to acini and small lobules by the method of Schultz et al. (4). These were then washed free of collagenase by repeated centrifugation through a cushion of 4% BSA and returned to the Krebs Ringer HEPES buffer used for dissociation. Monensin (gift of M. Marsh, Yale University) in ethanol was added to this buffer to a concentration of 5 μM, and the acini were incubated with continuous shaking and oxygenation every 10 min at 37°C for 2 h. Control acini were incubated with ethanol alone. After incubation, the tissue was fixed as above for immunofluorescence or with 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for processing with Epon embedding and electron microscopy.

*Tissue Fractionation:* Smooth microsomal, rough microsomal, and secretory granule fractions from rat pancreas were isolated by a modification of the method of Tartakoff and Jamieson (5). Pancreata (usually 10–15 g wet wt) were homogenized with six strokes at 2,000 rpm with a Brendler-type homogenizer in 6–10 vol of 0.3 M sucrose containing 0.2 mM, phenylmethylsulfonyl fluoride (Sigma Chemical Co., St. Louis, MO), 0.1 mM benzamidine (Aldrich Chemical Co., Milwaukee, WI), 10 μg/ml leupeptin (Sigma Chemical Co., 10 μg/ml pepstatin (Sigma), 20–50 U/ml Trasylol (Mobay Chemical Corp., NY), 50 μg/ml bacitracin (Sigma), 10 μM antipain (Sigma) and 5 mM beta-mercaptoethanol (Bio-Rad Laboratories). These inhibitors were also added to the 2 M sucrose used to make the sucrose gradients. For smooth microsomes, the homogenate was filtered through two layers of surgical gauze, and adjusted to 1.4 M sucrose by addition of 2 M sucrose. This was overlayed with 1.2 M sucrose and 0.3 M sucrose and spun at 100,000 $g_{av}$ for 1 h in a Beckman Ti 70 rotor (Beckman Instruments Inc., Palo Alto, CA). The smooth microsomes were collected at the 1.2–0.3 M sucrose interfaces. After recovery of the smooth or rough microsomes from the sucrose gradients, the microsomal fractions were mixed at 2,000 rpm in a Brendler-type homogenizer with an equal volume of 0.4 M sodium carbonate (~0.1 mg/ml final protein concentration) at 4°C (6–9), and membranes were recovered after centrifugation at 150,000 $g_{av}$ for 0.75 h. These membranes (1–2 mg) were resuspended in distilled water, and sonicated (Sonifier, Heat Systems Ultrasonics, Plainview, NY) for 30 s before storage at −20°. Secretory proteins were obtained by lysis of secretory granules in 0.2 M sodium carbonate followed by centrifugation at 200,000 $g_{av}$ for 0.5 h to remove granule membranes. Incubation medium of carbachol-stimulated lobules was centrifuged in the same manner (10).

Rat pancreas plasmalemma fractions were prepared by the method of Rosenzweig et al. (11), and washed with sodium carbonate as above.

*Monoclonal Antibody Production:* Young male litter-mate mice (BALB/c, Jackson Laboratories) were immunized intraperitoneally with 100 μg of a plasmalemma fraction of rat pancreas which had been precipitated by the addition of 3% formalin (12). After 1 mo, sera from the mice were screened by immunofluorescence, and the mouse whose immunostaining of the Golgi complex was most enhanced compared with the "preimmune" state was chosen for fusion. This mouse was injected intravenously with 100 μg of smooth microsomal membranes precipitated with formalin as above, and the spleen was removed for fusion 3.5 d later. Spleen cells were removed and fused with P3 × 63Ag8U.1 myeloma cells (13) (gift of I. Mellman, Yale University), plated into 88 1.77 $cm^2$ wells (Costar 24-well plates, Costar, Data Packaging, Cambridge, MA), and cultured according to standard procedures (14). Hybridoma tissue culture supernatants were screened by enzyme-linked immunosorbent assay (ELISA) on smooth microsomal membranes with myeloma supernatant as control, and the top 25 responses were screened further by immunofluorescence. Cultures showing the best responses by both measures were cloned by limiting dilution (15) until all cultures were homogenously positive by ELISA, and clones were injected into pristane-primed mice for generation of ascites fluid. Ascites fluid was spun at 100,000 $g_{av}$ for 0.5 h to eliminate aggregates and fat, but no further attempt was made to purify the antibodies so obtained, which were stored at −20°C in 50% glycerol and 0.1% Na azide.

*ELISA Analyses:* In general, the method of Engvall and Perlmann (16) for the ELISA was followed. The fractions were adsorbed onto microtiter plate wells (EIA 1/2 area, Costar) at 5 μg/50 μl in PBS for at least 1 h. Plates could be stored for ≥9 mo at −20°C. Any remaining binding sites on the wells were blocked with 0.5% BSA (type 5, Sigma) in PBS for 0.5 h, and this was used for all subsequent washes. The primary antibody was allowed to react in the wells for 1 h at 25°C, and the amount of bound antibody was detected using a goat $F(ab)'_2$ anti-mouse secondary antibody conjugated to horseradish peroxidase (Boehringer Mannheim, Indianapolis, IN), or in cases where a rabbit anti-mouse second antibody was used, with a staphlyococcus protein A–horseradish peroxidase conjugate (gift of J. Gershoni). The *o*-phenylaminediamine (Sigma) reaction product was read at 492 nm in a Titertek Multiskan (Flow Laboratories, McLean, VA). Routine controls included wells without antigen or primary antibody (whose values were blanked to zero) and wells without primary antibody.

Screening of monoclonal antibodies and titering of all antibodies was done by ELISA on smooth microsomal membranes, as was the typing of antibodies using subclass specific second antibodies (Litton Bionetics, Kensington, MD). In instances when pancreatic secretory proteins were to be tested by ELISA, it was found necessary to include 0.2 M *N*-acetylglucosamine, 0.2 M galactose, or 0.2 M alpha-methyl mannoside (all obtained from Sigma) to inhibit binding by the secretory proteins of the horseradish peroxidase conjugate. Neither fucose nor sialic acid at 0.2 M was effective in preventing this "nonspecific" binding. Sodium carbonate washed membrane fractions were devoid of any sugar-competable binding of either primary antibody or of the secondary antibodies. Neuraminidase (type X, Sigma) digestion of smooth microsomal membranes was carried out in the ELISA plate in 0.2 M Na acetate buffer, pH 5.0, with 0.04 M $CaCl_2$ and 0.5% BSA for 21 h at 37°C (17). One-half and two units per well were used, and the buffer minus the enzyme served as the control.

*Electron Microscopic Immunolocalization:* Rat pancreata were minced, and fixed in either 3% paraformaldehyde and 0.1% glutaraldehyde or 3% paraformaldehyde alone in 0.1 M sodium phosphate buffer, pH 7.4, with 0.2 M NaCl. This concentration of salt in the fixative greatly facilitated the preservation of the Golgi complexes. Fixation was for ~18 h at 4°C, after which the tissue was dehydrated at −30°C through a graded series of ethanols and infiltrated with Lowicryl K4M (Polaron, Watford, UK) (18, 19). Polymerization was carried out by long-wavelength UV light (15 W, 360 nm) at −30°C for 8 h and at 4°C for 18 h. Silver-gold sections were cut, and placed on Formvar- and carbon-coated nickel grids.

Nonspecific binding sites on the tissue were quenched at room temperature by floating sections on SDB for 5 min before the application of the ascites fluid for 3 h at 4°C. Sections were washed with PBS prior to application of the affinity-purified rabbit anti-mouse second antibody (Cappel Laboratories, Inc., 40 μg/ml) in SDB for 1 h at 4°C. After washing, Protein A-gold was applied (20-nm size, produced by citrate reduction; reference 20) for 1 h at 4°C. After final washing, staining of the sections was done by placing grids on a saturated solution of gallic acid (21) for 10–15 min, followed by uranyl acetate (1 min) and lead citrate (1 min). Observations were made on a Siemens 102 electron microscope. Control sections were incubated with the second antibody and Protein A-gold without any primary antibody, with low-titer mouse serum as primary antibody, or with commercially obtained IgM at 1 mg/ml.

*Antigen Identification:* Smooth microsomal membrane proteins were run on discontinuous SDS polyacrylamide slab gels (22). The configuration of the lanes of the gels was of two very wide lanes flanking a narrow lane, with the microsomal proteins in the wide lanes and molecular weight standards (Bio-Rad) in the center lane. We transferred each of the lanes to two layers of nitrocellulose membrane (BA 85, Schleicher & Schuell Inc., Keene, NH), in 8 mM Tris-60 mM glycine under conditions described by Gershoni and Palade (23), taking care to keep all filters equally aligned on all gel lanes. The center-lane filters were stained with amido black and re-swollen with water before drying to serve as the molecular weight key for the autoradiograms. In later experiments, prestained molecular weight standards (BRL, Bethesda, MD) were transferred to filters to serve as references. The filters to be overlayed (usually these were small strips of the larger lanes, to facilitate comparison between antibodies) were quenched overnight at 25°C in 20 mM Tris-HCl, pH 7.5, with 150 mM NaCl and 10% fetal calf serum (24). Incubation with the primary antibodies and the $^{125}$I-labeled (Iodogen, Pierce Chemical Co., Rockford, IL; reference 25) goat $Fab'_2$ anti-mouse (Boehringer-Mannhein) or $^{125}$I-sheep anti-

[1] *Abbreviations used in this paper:* ELISA, enzyme-linked immunosorbent assay; kd, kilodalton; SDB, serum dilution buffer.

mouse (Cappel) second antibodies was done in the same buffer, at 4°C. After antibody incubations the filters were washed with the same Tris-saline buffer without serum at 4°C, where the pH of the buffer shifted to 8.

Total lipid extraction of smooth microsomal membranes was done with the assistance of the laboratory of R. K. Yu (Yale University). 1 mg (protein) of smooth microsomal membranes was sequentially extracted with chloroform/methanol 1:1 (10 vol) and 2:1 (20 vol). These extracts were pooled and the solvents were evaporated under $N_2$. The remaining lipids were resolvated in $CHCl_3$/MeOH 1:2 and lipids were separated from residual proteins over a silicic acid column (26).

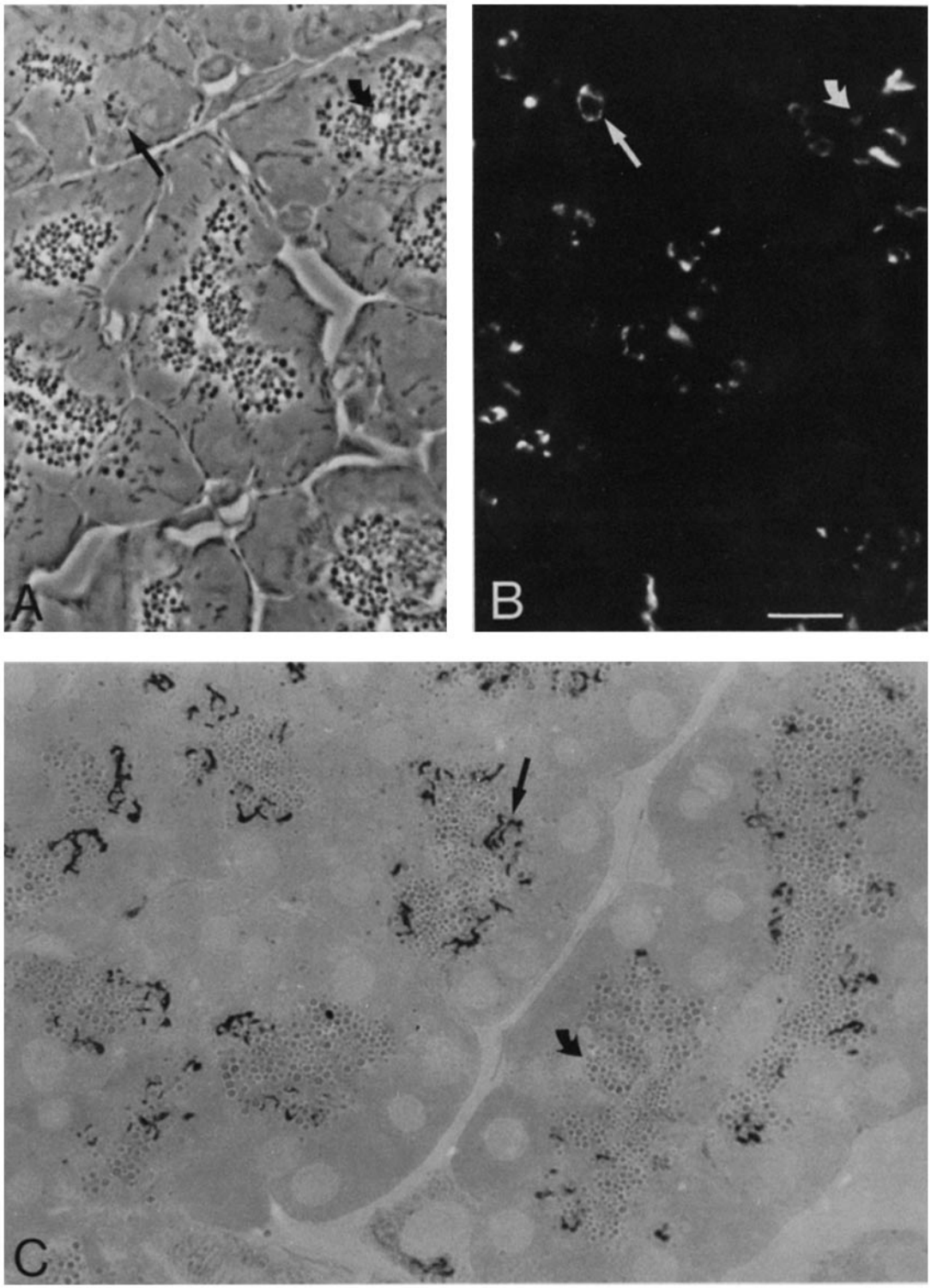


FIGURE 1 Immunolocalization of endogenous antibodies on rat pancreatic acinar cells. (*A*) Phase-contrast photomicrograph of a 500–1,000-nm-thick frozen section. (*B*) Localization of normal mouse serum (ammonium sulfate precipitated and concentrated). Long arrow indicates a Golgi complex area surrounding some secretory granules. Curved arrow points to a lumen, which is partially and lightly stained. (C) Epon-embedded section (2 μm thick) of pancreas osmium-stained by the method described by Friend (27). The Golgi complexes are darkly stained (long arrow), the granules are lightly stained (curved arrow). Tissue courtesy of D. Ingber (Harvard Medical School). Bar, 10 μm. × 1,150.

## RESULTS

### *Mouse Serum Antibody*

When first observed by immunofluorescence, the apparent Golgi complex localization of unimmunized mouse serum was barely above the threshold for detection on sections of rat pancreas. However, with the improvement of our immunofluorescence technique, the staining became unambiguously localized to the supranuclear region of the acinar cells where the Golgi complexes are found. Sera from all mice we have tested (Balb/c, SJL, CD/1, NZB, DBA/2, C57/b) show the same pattern of localization, as does at least one commercial mouse IgG fraction. In no instance is any variability of localization seen under the immunofluorescence conditions described, i.e., no staining is observed of secretory granules, lumen content, nuclei, or regions of the cell containing rough endoplasmic reticulum, etc. However, when the serum antibody is concentrated following ammonium sulfate precipitation, faint staining of the lumen border is visible (Fig. 1, *A* and *B*).

The consistent lace-like localization of the antigen to an area in the acinar cell that is apical to the nucleus and rough endoplasmic reticulum and at the basal fringe of the granule zone was taken to be *prima facie* evidence that the Golgi complex was being recognized, as the pattern of immunofluorescence mimics that of the classical osmium impregnation of the Golgi complex (Fig. 1 *C;* reference 27). To gain direct evidence on this point, we took advantage of the cellular effects of the drug monensin, which causes a marked dilation of elements of the Golgi complex (28, 29). Treatment of dissociated pancreatic acini with 5 $\mu$M monensin caused large vacuoles to be formed in the supranuclear zone occupied by the Golgi complexes (Fig. 2, *A* and *B*). These vacuoles are also seen by light microscopy, and correspond with rings of fluorescence where the anti-Golgi antibody is localized (Fig. 3, *A* and *B*). In control acini, treated with the ethanol vehicle used for the monensin, the localization of the mouse serum antibody to the unperturbed Golgi complex appeared as before (Fig. 3, *C* and *D*).

The absence of localization of the apparent anti–Golgi complex antiserum to other acinar compartments holding secretory proteins is not due merely to a lack of access, since mouse antisera raised to pancreatic secretory proteins are capable of localizing to secretory granules and the content of the lumens, as well as to the Golgi complex area (Fig. 4, *A* and *B*). In this latter case, however, it cannot be said how much of the apparent Golgi complex localization is due to the endogenous activity present in mouse serum, and how much to the immune response to secretory proteins.

That it is the antibody component of mouse serum that is binding to the Golgi complex region is established by the specificities of the anti-mouse second antibodies used for detection in immunofluorescence and ELISA. In whole mouse serum the endogenous polyclonal anti-Golgi antibod-

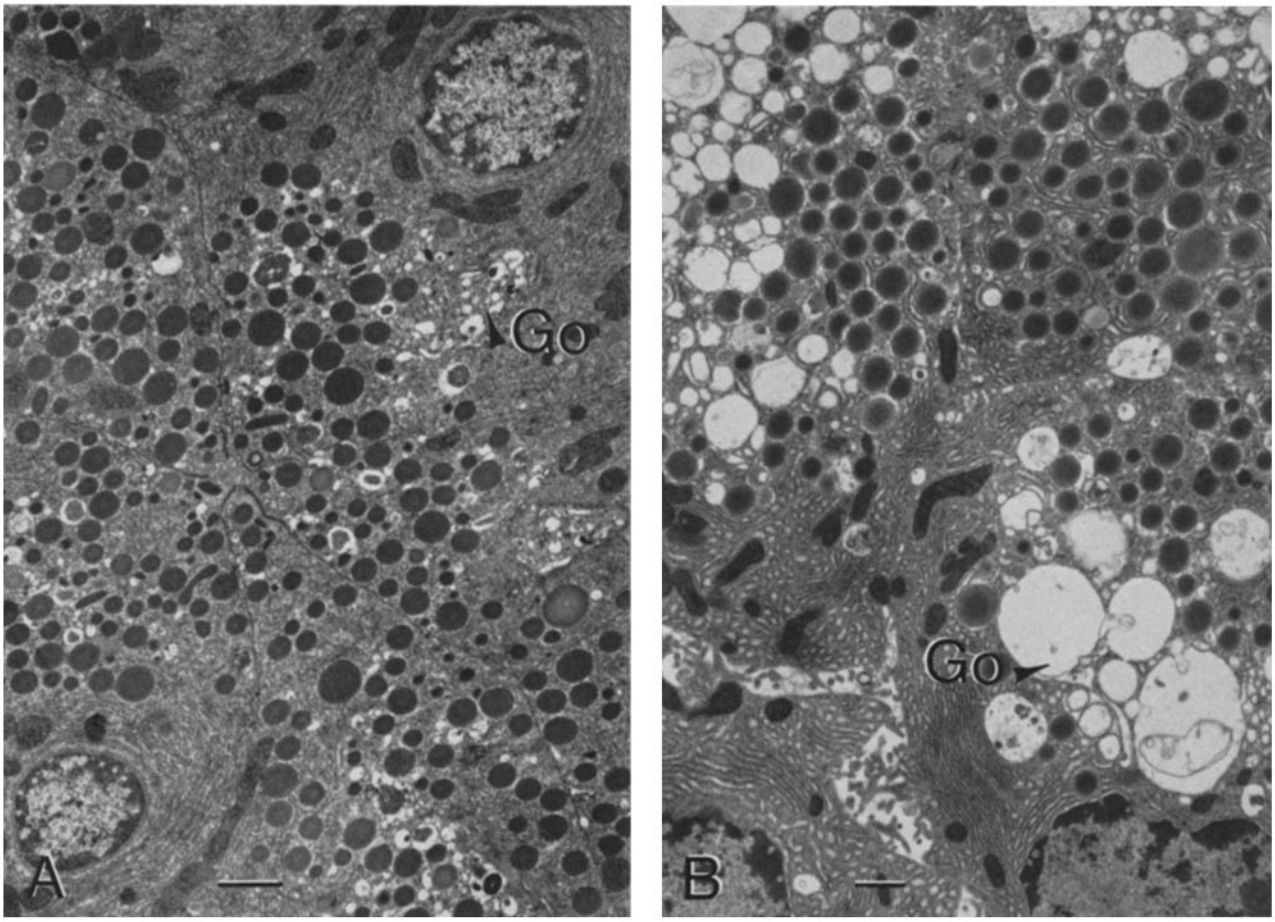


FIGURE 2 Effects of monensin treatment on dissociated pancreatic acinar cells. (*A*) Epon-embedded section of control acinus treated with ethanol alone. (*B*) Epon section of monensin-treated acinus, showing large vacuoles corresponding to the Golgi complexes (*Go*). (*A*) Bar, 2 $\mu$m. × 4,000. (*B*) Bar, 1 $\mu$m. × 6,000.

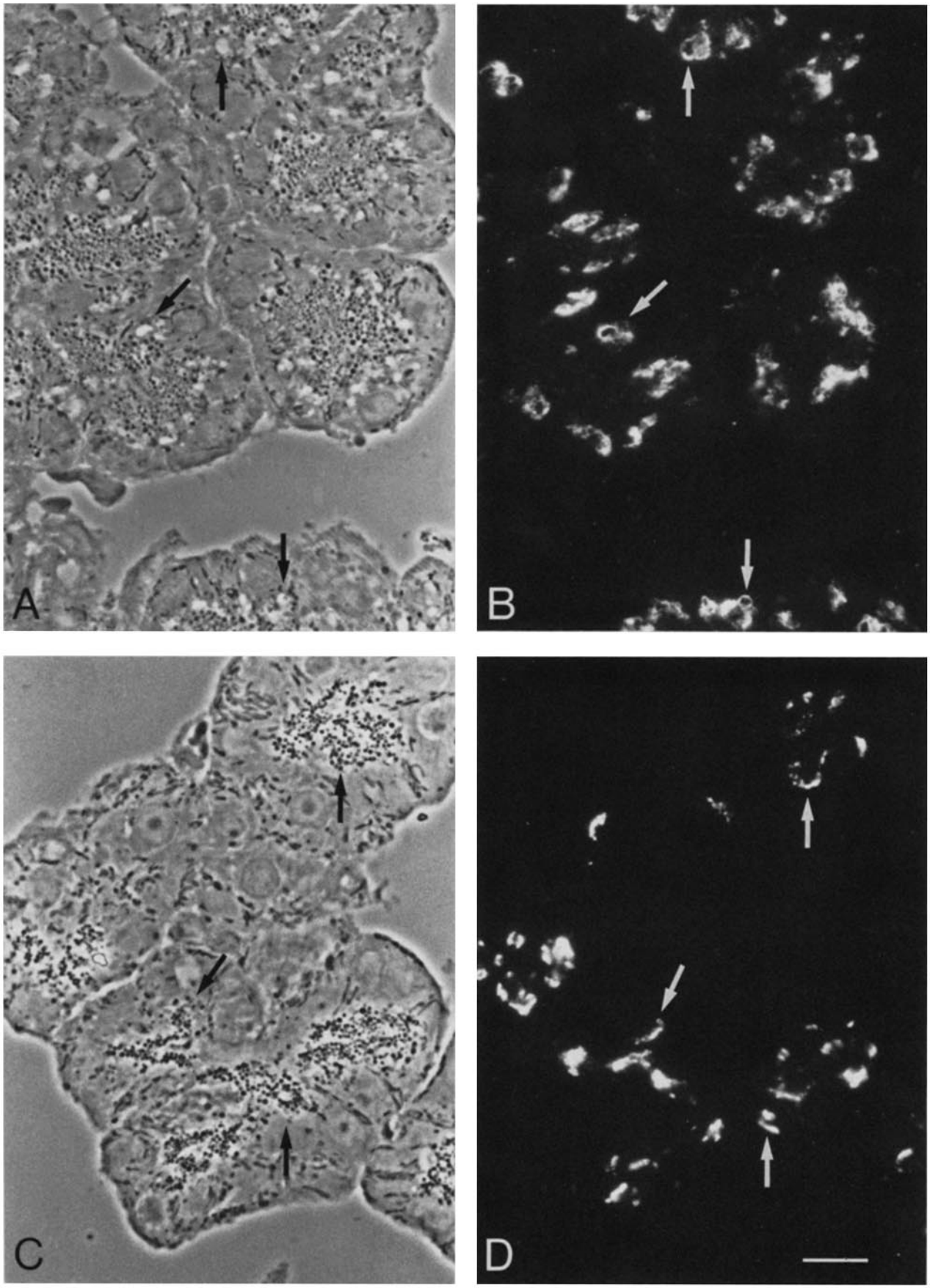


FIGURE 3 Localization of unimmunized mouse serum on pancreatic acini with and without treatment with 5 μM monensin for 2 h on the same tissue shown in Fig. 2. (*A* and *B*) Immunolocalization of normal mouse serum on a frozen section of monensin-treated tissue. Arrows indicate vacuoles corresponding to the Golgi complexes. (C and *D*) Localization of normal mouse serum on control acini. Staining of the Golgi area is flattened and appears normal. Bar, 10 μm. × 1,150.

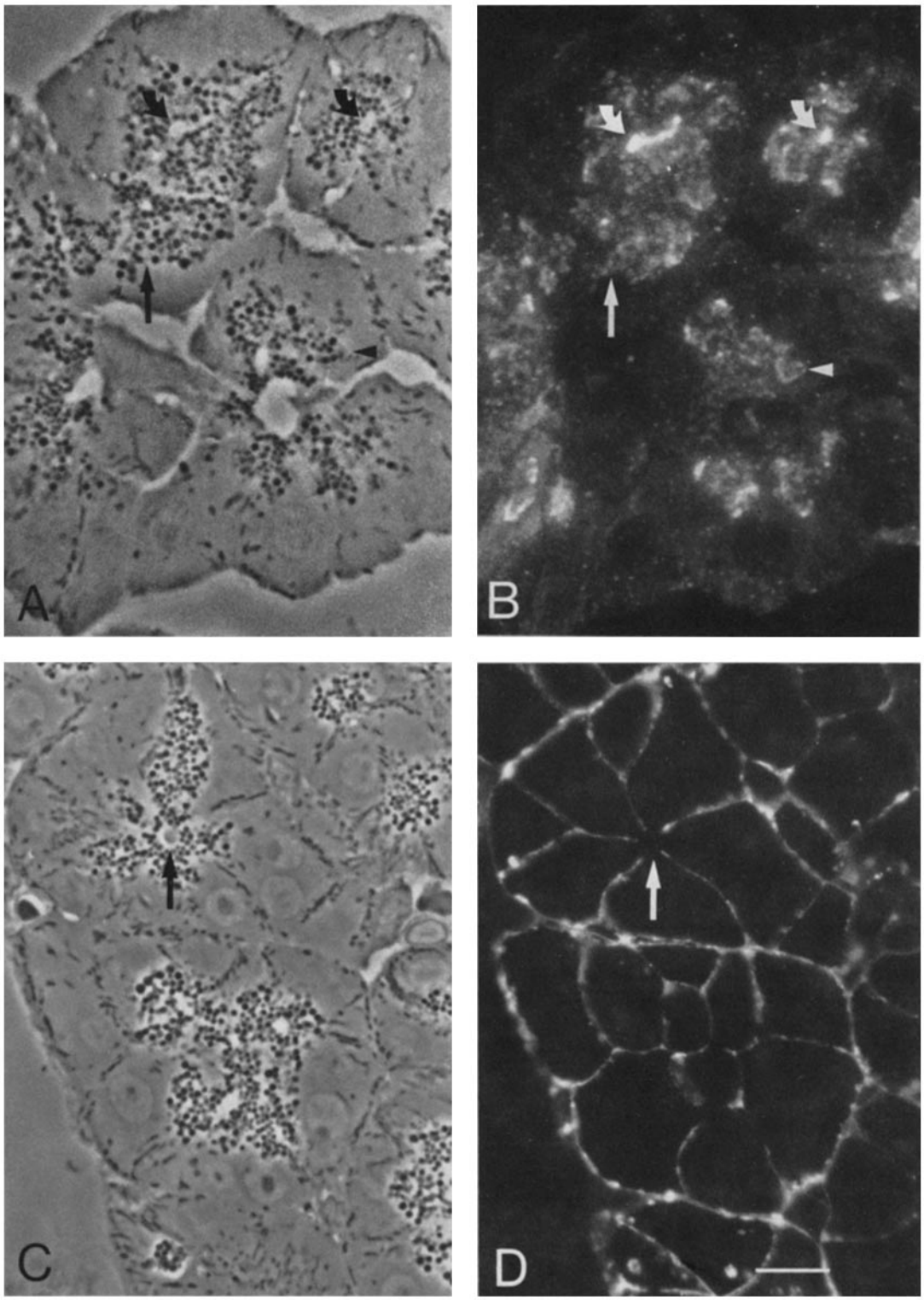


FIGURE 4 Control immunolocalizations for nonspecific staining, on semithin frozen sections. (*A* and *B*) Localization of mouse antiserum raised to secretory proteins. Curved arrows are directed to lumens, which are filled with secretion and react brightly with the antiserum. Long arrow points to an area where punctate staining of secretory granules is observed. Arrowhead denotes Golgi-like staining (*C* and *D*). Localization of monoclonal antibody directed to acinar cell basolateral membranes. Note the absence of staining similar to that seen in Fig. 1. Arrow indicates a lumen, which is not stained. Bar, 10 $\mu$m. × 1,150.

ies are found to be of the IgG 1, 2, and 3 classes, with no detectable activity in the IgM fraction. While it is possible that some component of the rat pancreatic acinar cell Golgi complex recognizes a domain of the mouse antibody proteins that does not bind antigen, analogous to the operation of the Fc-receptor of macrophages (30), this possibility is greatly reduced by the observation that several monoclonal mouse IgGs, raised for other studies in progress, show no localization to the Golgi complex area (Fig. 4, *C* and *D*). These control mouse IgG monoclonal antibodies (including an anti-acetyl-

choline receptor, courtesy Dr. E. Hawrot, Yale University) were derived from tissue-culture supernatants, since monoclonal antibodies raised as ascites fluids contain the endogenous activity, as do as the IgG fractions derived from ascites. In addition, no Golgi complex localization was seen by immunofluorescence of mouse IgG Fc molecules at 5 mg/ml. Competition of serum antibody binding with 0.1 M galactose, or *N*-acetylglucosamine was ineffective in displacing antibody localization.

The annular appearance of the anti–Golgi complex localization following monensin treatment, along with the lack of localization to the secretory granules and lumen content, provides evidence that the antigen recognized in the Golgi complex is not a secretory protein. Instead, the antigen is probably an integral membrane component of the Golgi complex, since antigenicity is retained after 0.2 M sodium carbonate washing of pancreatic smooth microsomal membranes. This fraction is enriched with Golgi membranes, since it is the fraction with the highest antigen activity (with the mouse serum antibody and also with monoclonal antibodies of known specificity for Golgi membranes, *vide infra*), and it shows at least 9- to 10-fold more antibody-binding activity than rough microsomal membranes. Moreover, using ELISA methods, preabsorption of the mouse serum with secretory proteins does not interfere with its ability to bind to carbonate-washed membranes (Fig. 5).

The titer of the mouse serum antibody is quite low in many mice, especially those bled immediately or shortly after receipt from the supplier, being about 4 by immunofluorescence, and about 32 by ELISA with ammonium sulfate-precipitated and concentrated mouse serum. The titer of endogenous anti-Golgi activity in mice that have been housed for some time can be higher, and we have observed titers as high as 3,125 by immunofluorescence and ELISA in mouse serum from mice housed in our facility, and as high as 400 by immunofluorescence of nonspecific ascites fluids from other facilities (courtesy of Dr. P. Kelly, University of Kansas). Partly because of this low or variable level of activity, attempts to determine by biochemical methods the antigen recognized in the rat pancreas have not yet been successful. The stimulus giving rise to this endogenous mouse antibody is also unknown, although it does not seem to be autoimmune since no comparable localization is observed in mouse pancreas by immunofluorescence.

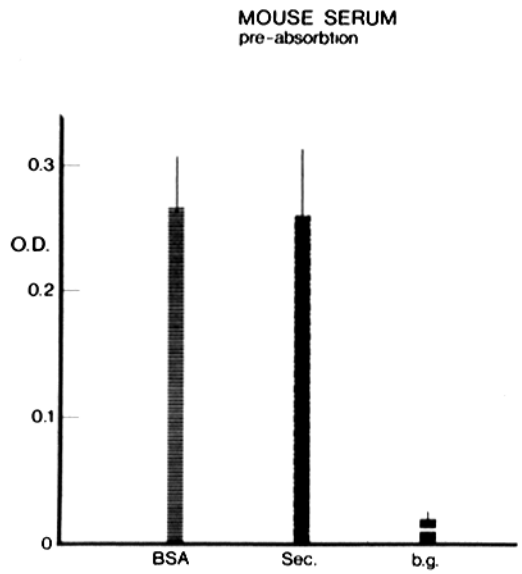


FIGURE 5 ELISA analysis of the binding of normal mouse serum to smooth microsome membranes following absorption with BSA or secretory proteins (*Sec.*), compared with the background in the absence of serum (*b.g.*). Ordinant shows the optical density of the peroxidase-linked color reaction, and is proportional to the amount of antibody bound to the membranes. Bars represent variance ($s^2$).

An indication of the tissue specificities of the mouse serum antibody is shown in Table I, which shows the results of a survey of various rat tissues. As described above, the antibody localizes to the Golgi complexes of the pancreatic acinar cells, but whether this is also true for the duct cells could not be established with certainty. In the parotid, lacrymal, and submandibular glands, the Golgi complex areas of acinar cells of all types are positive, and the striated duct cells are negative. In these tissues, as in the pancreas, the difficulties of unambiguously recognizing the nonstriated duct cells in unstained frozen sections precludes their assessment. However, in the other tissues surveyed, there is no indication of localization of the antiserum to the Golgi complex or other cell organelles. The antiserum thus indicates the existence of tissue-specific specializations in the constitution of the Golgi complex, although we cannot say that the observed specialization is due to the same antigen everywhere.

## Monoclonal Antibodies

The felicitous existence of an endogenous mouse serum antibody to the membranes of the rat pancreatic Golgi complex suggested it would be relatively simple to generate monoclonal antibodies with the same specificity. The necessity for doing so rested on two observations. First, although the antibodies are apparently found in all mice, there is no indication this will always be the case; and second, the titer of the antibody is too low for our purposes. Accordingly, we proceeded to isolate monoclonal antibodies to the Golgi mem-

TABLE I

*Rat Tissue Specificities of Anti-Golgi Antibodies*

| | Mouse serum | Monoclonal antiGolgi 1 | Monoclonal antiGolgi 2 |
|---|---|---|---|
| Pancreas | | | |
| Acinar cells | +* | + | + |
| Islet cells | 0 | 0 | 0 |
| Parotid | | | |
| Acinar cells | + | + | + |
| Duct cells | 0 | 0 | 0 |
| Lacrymal | | | |
| Acinar cells | + | + | + |
| Duct cells | 0 | 0 | 0 |
| Submandibular | | | |
| Acinar cells | + | + | + |
| Duct cells | 0 | 0 | 0 |
| Kidney | 0 | 0 | 0 |
| Liver | 0 | 0 | 0 |
| Brain | 0 | 0 | 0 |
| Smooth muscle | 0 | 0 | 0 |
| Seminiferous tubule | 0 | 0 | 0 |
| Blood vessels | 0 | — | — |
| Epididymis | 0 | — | — |
| Lymph nodes | — | 0 | 0 |
| Thyroid | — | 0 | 0 |

* + denotes presence, 0, absence, and —, not tested. All screenings were done on 500–1000-nm-thick frozen sections, fixed in formalin.

branes. Initial attempts to do so, using in vitro stimulation of lymphocytes with lipopolysaccharide (31), did not result in the detection of any antibodies that were positive by both ELISA and immunofluorescence. Immunization of mice with smooth microsomal membranes led to a suppression of the serum anti-Golgi response. Ultimately, an immunization protocol was worked out whereby a mouse was immunized intraperitoneally with a pancreatic plasmalemmal fraction containing a small amount of Golgi membranes, boosted intravenously some months later with smooth microsomal membranes, and fused 3.5 d later. About 20 positive colonies were thus obtained, and on the basis of ELISA responses on carbonate-washed membranes and immunofluorescence two were chosen for further study, called antiGolgi 1 and antiGolgi 2. Both monoclonal antibodies are IgMs.

Immunofluorescence using tissue culture supernatants suggested that antiGolgi 2 reacted with only the Golgi complex, but that antiGolgi 1 localized to the lumenal border as well as to the Golgi complex. When ascites fluids from both clones were normalized for equal titers, however, the localization patterns were the same (Fig. 6). At the dilutions shown in Fig. 6, the staining of the lumen border is faint, sporadic from lumen to lumen, and it usually encompasses only a portion of the lumen border. At higher concentrations of the antibodies, the outlining of the lumen is brighter and complete. The staining of the Golgi complex appears saturated at the lower concentrations of ascites fluid shown in Fig. 6, and hence the lighter staining at the lumen edge probably reflects a lesser amount of the antigens at this site.

The titer of the ascites fluid containing the monoclonal antibodies is sufficiently high (78,000 for antiGolgi 1 and 16,000 for antiGolgi 2, by ELISA) to allow direct confirmation of the Golgi complex localization at the electron microscopic level (Fig. 7). Immunolocalization of the antiGolgi 1 and antiGolgi 2 on sections of Lowicryl K4M-embedded pancreas reveals the same pattern as that seen by immunofluorescence of both monoclonals as well as of the mouse antiserum. However, the finer resolution afforded at the election microscopic level reveals that both antiGolgi 1 and antiGolgi 2 localize to the lengths of the *trans*-most cisternae of the Golgi complex, which are recognizable in tannic acid–treated Lowicryl sections by their darker staining (not shown). Both antibodies often show patchy localization to lumen microvillar membrane, and occasionally to entire lumen borders, but the staining of the lumen content not adjacent to microvillar membranes is no higher than background. On occasion, localization was detected to what appeared to be small vesicles apparently located beyond the *trans*-most boundry of the Golgi complex. These were seen irrespective of the concentration of Protein A-gold used for localization, and may correspond to the hazy punctate appearance of the supra-Golgi region of acinar cells seen at the light level when higher concentrations of antibodies or fluorescent secondary are employed (not shown). The antigen for antiGolgi 2 is quite sensitive to 0.1% glutaraldehyde fixation, and it was necessary to localize it in tissues fixed solely with paraformaldehyde. Only background gold is seen over other cell organelles, and no localization was observed in the absence of the primary antibody, nor with a nonspecific mouse IgM fraction instead of the monoclonal antibody.

The antigens for antiGolgi 1 and antiGolgi 2 were identified by antibody overlays of filter-transferred smooth microsomal membranes. AntiGolgi 1 revealed a diffuse band of from 103 to 108 kilodaltons (kd). AntiGolgi 2 bound to two bands, of 180 kd and 103–108 kd (Fig. 8). The bands detected are very likely proteins, since chloroform-methanol extracts of total lipids from smooth microsomal membranes have little antigenic activity by ELISA, whereas the nonextractable fraction has much more activity. Neuraminidase treatment of the membranes in vitro did not interfere with the subsequent binding of either antibody. Both monoclonal antibodies share the same tissue distribution of Golgi complex localization as the mouse serum (Table I).

## DISCUSSION

By immunofluorescence of tissue sections reacted with normal mouse serum, we observed an antibody that localized to the Golgi complex area of the rat pancreatic acinar cell. Further investigation revealed the antibody to be of IgG classes, and the antigen to be an integral membrane component of the Golgi complex, based on immunolocalization after monensin treatment and the maintenance of antigenicity after sodium carbonate washing of the membranes. The anti–Golgi complex activity of normal serum is also found to be directed against other rat tissues, including the parotid, submandibular, and lacrymal glands. This antibody is present in a wide variety of mice and appears to be endogenous, though probably not autoimmune. The variability of endogenous antibody titers with time and place suggest the recognition of the rat pancreatic Golgi complex may be a cross-reaction with a common viral or bacterial organism found in the murine environment.

Because of the potential usefulness of such an antibody for our laboratory's investigations of membrane biogenesis, the endogenous anti-Golgi activity of normal mouse serum was exploited for the isolation of monoclonal antibodies of the same specificity. Two monoclonals have been described, called antiGolgi 1 and antiGolgi 2. Both antibodies are IgMs which recognize integral membrane proteins of the rat pancreatic acinar cell Golgi complex, and which share the same tissue distribution of anti-Golgi activity as the normal mouse serum. Electron microscopic immunolocalization on thin sections shows both monoclonal antibodies to consistently localize to the *trans*-most cisternae of the rat pancreas Golgi complexes, with patchy localization to the lumen membrane as well. Despite these commonalities, the two antibodies are not entirely directed to the same antigens. AntiGolgi 1 recognized a band on an SDS gel centered at ~105 kd, and AntiGolgi 2 is directed to two protein moieties, at ~180 and 103–108 kd.

Although no anti–Golgi complex activity was observed in the IgM fraction of normal mouse serum, both monoclonal antibodies described herein are IgMs. This may be coincidental, since only four of 20 positive hybridoma cultures were cloned and typed (all were IgMs); or it may result from the particular immunization protocol used in this study. If the latter is the case, it suggests the possibility that mice find some proteins in the rat pancreatic Golgi complex to be highly immunogenic, and that a primary immune response was captured by the fusion. In light of the apparently identical specificities by immunolocalization of the mouse serum IgGs and the monoclonal IgMs, it might be surprising that "re-immunization" of the normal mice with antigens that are already demonstrably recognized does not result in the proliferation of IgG-specific memory cells, and their capture by fusion. However, we have observed that the mouse serum

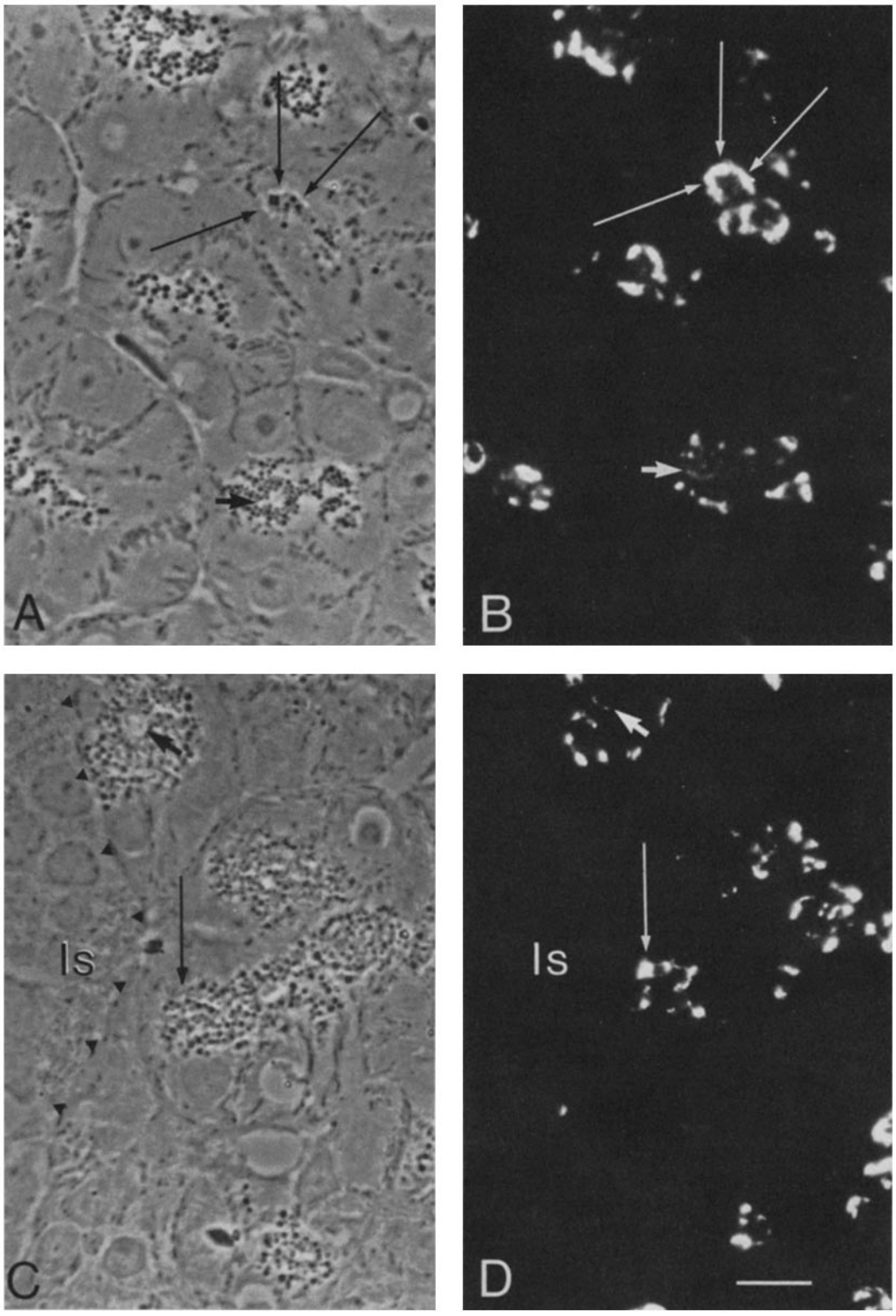


FIGURE 6 Immunolocalization of monoclonal antibodies directed to the Golgi complex. (*A* and *B*) AntiGolgi 1 at 1:250 dilution. Long arrows indicate a Golgi complex and the short arrow points to a lumen, the border of which is partially stained. (*C* and *D*) AntiGolgi 2 at 1:50 dilution. Arrowheads denote the boundary between an islet of Langerhans (*Is*) and acinar tissue. The long arrow is directed to a Golgi complex and the short arrow indicates a lumen, which is partially stained. Bar, 10 μm. × 1,150.

antibody is easily suppressable, suggesting a tight regulation of the IgG response. Alternatively, possibly IgM-specific blast cells were predominant in the spleen at the time of fusion, and had the fusion been done earlier or later than 3.5 d, IgG monoclonal antibodies may have been found. Since antiGolgi 1 and antiGolgi 2 both proved to be quite workable, no attempt was made to capture monoclonal IgGs. The failure to capture the endogenous-antibody secreting B cells following lipopolysaccharide stimulation in vitro may be attributable to the failure of lipopolysaccharide to interact with more than about ⅓ of murine B cells (32, 33).

There have been prior reports of antibodies directed specifically to the Golgi complex. From immunizations with brain tissue, Lin and Queally (34) reported a monoclonal antibody to a heat-shock protein which localizes to the Golgi complex area of the rat pancreas. The antigen recognized by this antibody may not be an integral membrane component, since staining in the nucleus is also seen in some cases (35), and we

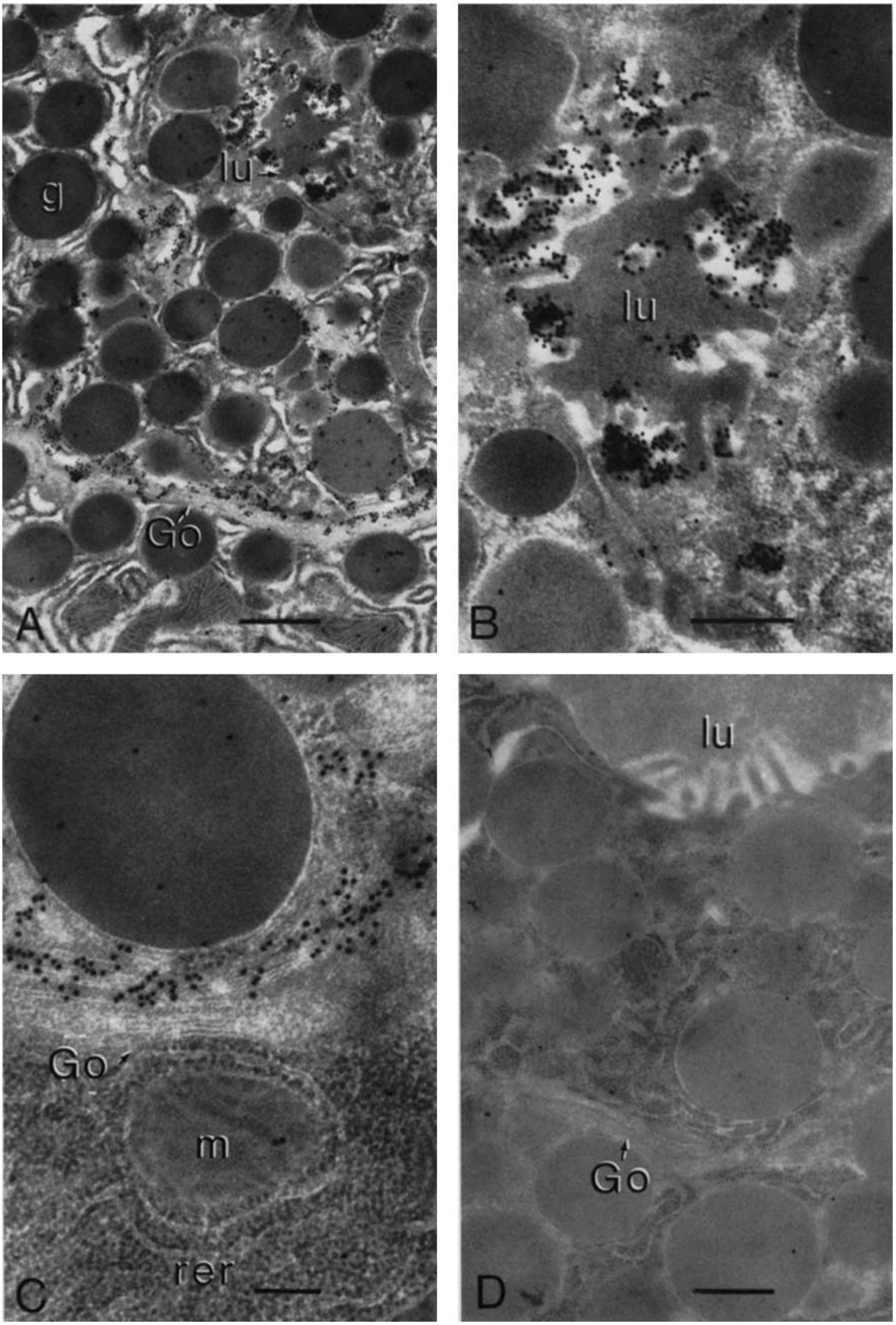


FIGURE 7 Immunolocalization of antiGolgi 1 on Lowicryl thin sections, which were stained postlocalization with gallic acid, uranyl acetate, and lead citrate. (*A*) Low-power field demonstrating heavy Protein A-gold localization of antiGolgi 1 on the *trans* face of the Golgi complex (*Go*) and on the membrane and microvilli of a lumen membrane (*lu*). Little staining is apparent over the secretory granules (*g*), and occasional aggregates of gold are present. Bar, 1 μm. × 13,500. (*B*) A higher-power view of the acinar lumen (*lu*) shown in *A*. The antibody localization is to the microvilli of the lumen membrane, rather than to the content. Bar, 0.5 μm. × 34,500. (C) Higher-power view of a Golgi complex (*Go*), showing the concentration of label on the *trans* cisternae. Background gold labeling can be seen over the large secretory granule, a mitochondrion (*m*), and the rough endoplasmic reticulum (*rer*). Bar, 0.2 μm. × 56,000. (*D*) Control localization, using nonspecific IgM instead of the monoclonal antibodies, and the same concentrations of rabbit anti-mouse and Protein A-gold as in the previous figures. No localization is seen to the lumen (*lu*) or to the Golgi complex (*Go*). Bar, 0.5 μm. × 27,000.

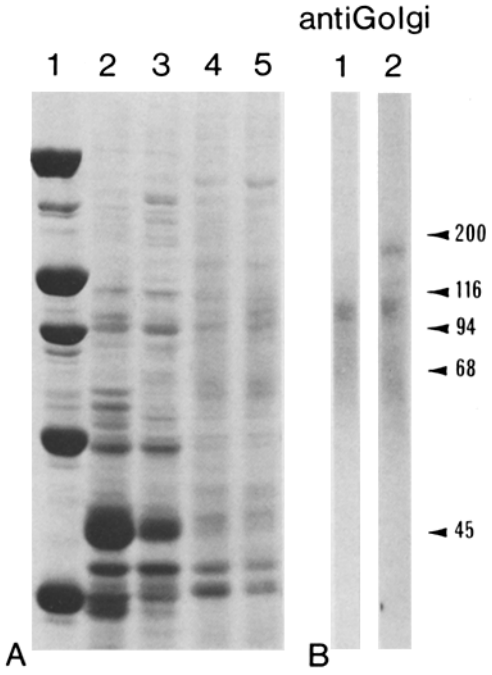


FIGURE 8 (*A*) SDS polyacrylamide gel (7.5%) demonstrating the membrane preparations used for immune overlay. Lane *1* has molecular weight markers of 200, 116, 92.5, 66, and 45 kd, in descending order. Lane *2* is rat pancreatic homogenate. Lane *3* shows smooth microsomes (1.2–0.3 M sucrose interface). Lane *4* is the 100 $kg_{av}$ pellet after $Na_2CO_3$ washing. Lane *5* shows the interface between the $Na_2CO_3$-sucrose solution and distilled water, after centrifugation at 100 $kg_{av}$. As no difference was found between the membranes that pelleted and those that floated after the $Na_2CO_3$ wash, both fractions were used. (*B*) Immune overlays of antiGolgi 1 and antiGolgi 2 on smooth microsomal membranes resolved by SDS PAGE (5–20% gradient) and transferred to nitrocellulose. AntiGolgi 1 shows a major band at ~103–108 kd, while antiGolgi 2 has two major bands, at ~180 kd and 103–108 kd. Background staining due to the second antibody may be seen elsewhere.

have seen cross-reactivity in peripheral axons with this antibody as well. Louvard et al. (36) have reported the induction of a rabbit polyclonal antibody to a 135-kd integral membrane protein of the rat Golgi complex. In this latter study, great care was taken *a priori* to insure the universality of the antigen among rodent tissues, and no tissue-specific recognitions of the antiserum were reported. These and other reports (reviewed in reference 36) have established that the Golgi complex, like other cell organelles, has specific protein components. However, observations of tissue-specific variations in the proteins composing the Golgi complex apparently have not been reported. In the present case, tissue specificity of the anti-Golgi antibodies has resulted from the opaque musings of the mouse immune system, rather than any attempt to demonstrate it *ab initio*. The differential organ localizations of anti-Golgi complex activities revealed by the two different monoclonal antibodies suggests that a number of tissue-specific membrane antigens exist in the Golgi complex.

We have not determined that the same antigens are recognized by the mouse serum and monoclonal antibodies in each tissue recognized (Table I). Nonetheless, the precise epitope specificity of the two monoclonal antibodies does establish that the Golgi complexes of all these tissues have at least two specific markers distinguishing them from those of all other tissues. In essence, the antigenic markers appear in acinar cells of tissues that are embryologically derived from the endodermal epithelium giving rise to glands communicating secretions to the digestive tract, with the singular exception of the liver. This antigenic specialization across tissues of Golgi complex membrane proteins suggests a possible common functional specialization as well.

To localize the antigens recognized by the monoclonal antibodies, we employed post-embedding immunolocalization with protein A-colloidal gold on Lowicryl thin sections. This technique allows localization to all intracellular compartments, without the potential artifacts introduced by incomplete access of antibodies to organelles or the trapping or washing away of reaction products (38). Both antiGolgi 1 and 2 localize to the same cellular domains, i.e., the *trans*-most cisternae of the Golgi complex, and patches of microvillar membrane. Although the staining of the lumen membrane is not consistent from lumen to lumen at the antibody concentrations we typically use, it is seen consistently with both antibodies at the light and electron microscopic levels. The patchy staining at the lumen doubtless represents real variation in the amount of antigen present, since equal access to all of the lumen cross-section is afforded by the thin sections used herein, especially at the electron microscopic level. The high degree of access also argues against a nonspecific sticking of antibody to the lumen surface, which would be expected to be more uniform, and also would be seen with other monoclonal antibodies. The staining at the lumen is probably not due to the insertion or incomplete recycling of the secretory granule membranes, since the antigens are not localized to zymogen granule membranes (cf. reference 39). This is so even when tissues are very lightly fixed with 3% paraformaldehyde, which results in the loss by extraction of the secretory proteins bordering the granule cell membrane, and may afford greater access to the inner face of this membrane. Since in general the lumen labeling appeared on the external side of the membrane, it is likely that the antigen in located on the cisternal face of the Golgi membranes.

On immune overlays of filter-transferred SDS gels, both antiGolgi 1 and antiGolgi 2 both seem to recognize the same protein(s) at ~105 kd, and antiGolgi 2 also binds to a band of 180 kd. While efforts were made to limit proteolysis during the preparation of the smooth microsomal membranes (see Materials and Methods), it cannot be ruled out *a priori* in any specific instance. Thus, for either antibody the binding pattern may represent the proteolytic fragments of a single polypeptide, or a common epitope shared among different polypeptides. Alternatively, the data from both monoclonal antibodies suggest that at least two different proteins are being localized to the *trans*-Golgi cisternal membranes, since the epitopes of each monoclonal antiGolgi must be different.

The previous observations showing a limited topography of other Golgi complex enzymes raise the question of how Golgi domain specificity is created and maintained. Distributions of domain specificities similar to that described in this paper have been seen for the other integral *trans*-Golgi membrane proteins thiamine pyrophosphatase (40) and galactosyl transferase (41). While little plasmalemmal localization was seen for the 135-kd rodent Golgi complex antigen except in coated pits (42), the immunoperoxidase technique employed may not have allowed complete detection of an externally directed membrane antigen (M. Farquhar, personal communication). From these examples, and that of the Golgi proteins described in this paper, it seems that the factors responsible for the

placement of membrane proteins into the *trans*-Golgi cisternae are not completely capable of distinguishing that membrane compartment from the plasmalemma, especially the apical or canulicular membrane domain (43–46).

The easy availability of mice "preimmunized" to integral membrane proteins of the Golgi complex, and the concomitant ease of preparing monoclonal antibodies with the same specificities, can be expected to be most useful for identifying and analyzing a family of membrane proteins sharing the same topological domain. The presence of this antibody in normal mouse serum should also be noted, as it may also offer the potential for complicating pre- or nonimmune controls for other monoclonal antibodies (47).

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