Molecular Characterization of Two Human Autoantigens: Unique cDNAs Encoding 95- and 160-kD Proteins of a Putative Family in the Golgi Complex

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Summary

Serum autoantibodies from a patient with autoantibodies directed against the Golgi complex were used to screen clones from a HepG2 λZap cDNA library. Three related clones, designated SY2, SY10, and SY11, encoding two distinct polypeptides were purified for further analysis. Antibodies affinity purified by adsorption to the λZap-cloned recombinant proteins and antibodies from NZW rabbits immunized with purified recombinant proteins reproduced Golgi staining and bound two different proteins, 95 and 160 kD, from whole cell extracts. The SY11 protein was provisionally named golgin-95 and the SY2/SY10 protein was named golgin-160. The deduced amino acid sequence of the cDNA clone of SY2 and SY11 represented 58.7- and 70-kD proteins of 568 and 620 amino acids. The in vitro translation products of SY2 and SY11 cDNAs migrated in SDS-PAGE at 65 and 95 kD, respectively. The in vitro translated proteins were immunoprecipitated by human anti-Golgi serum or immune rabbit serum, but not by normal human serum or preimmune rabbit serum. Features of the cDNA suggested that SY11 was a full-length clone encoding golgin-95 but SY2 and SY10 together encoded a partial sequence of golgin-160. Analysis of the SY11 recombinant protein identified a leucine zipper spanning positions 419-455, a glutamic acid-rich tract spanning positions 322-333, and a proline-rich tract spanning positions 67-73. A search of the SwissProt data bank indicated sequence similarity of SY11 to human restin, the heavy chain of kinesin, and the heavy chain of myosin. SY2 shared sequence similarity with the heavy chain of myosin, the USO1 transport protein from yeast, and the 150-kD cytoplasmic dynein-associated polypeptide. Sequence analysis demonstrated that golgin-95 and golgin-160 share 43% sequence similarity and, therefore, may be functionally related proteins.

Human autoantibodies have proven to be useful reagents in elucidating the structure and function of eucaryotic organelles. For example, naturally occurring autoantibodies have been used to identify and clone chromatin, nucleolar, nuclear envelope, and cytoplasmic proteins (reviewed in references 1-3). Antibodies directed against the Golgi complex have been reported in the sera of patients with SLE (4), Sjögren's syndrome and other systemic rheumatic diseases (4-9), idiopathic cerebellar ataxia (10), paraneoplastic cerebellar degeneration (11), and viral infections, including the EBV (12), and HIV (13). This study reports the identification and cloning of two Golgi autoantigens that react with autoantibodies in the sera of patients with autoimmune diseases. The Golgi apparatus is a complex cytoplasmic organelle that has a prominent function in the processing, transporting, and sorting of intracellular proteins (14, 15). Structurally, the Golgi complex is localized in the perinuclear region of most mammalian cells and is characterized by stacks of membrane-bound cisternae as well as a functionally distinct trans–Golgi network (16, 17). The intracellular transport of newly synthesized and recycled proteins requires directed movement between the endoplasmic reticulum, the intracellular vesicles to the cis, medial, and trans compartments of the Golgi complex, and the plasma membrane (15, 18, 19). The signals and molecular characteristics of the proteins that control this intracellular traffic are poorly understood, but it is known that intracellular microtubules are important structural and functional components (20, 21). Constituent and resident proteins of the Golgi complex believed to play a role in these processes include families of proteins such as the adaptins (22),
the "coatomer" proteins (e.g., α,β,γ,δ COPs)\(^1\) (23–25), GTP-binding proteins (26), including ADP ribosylation factors (ARFs) (27, 28), and resident enzymes (reviewed in reference 14).

Although the Golgi complex has been identified as a target of autoantibodies for almost a decade, little is known about the antigenic targets or the events that lead to the induction of anti–Golgi antibodies. An attractive feature to the study of Golgi antigens is the knowledge that viruses, including HIV (29), coronaviruses (30), rubella (31), and various others (reviewed in reference 32), are processed in, and disrupt (33, 34), the Golgi complex. Observations that certain viruses induce anti–Golgi antibodies in mice (35, 36), and that individuals infected with EBV (12) and the HIV-1 virus (13) bear anti–Golgi antibodies, add incentive to the study of the molecular characteristics of the Golgi autoantigens.

In this study, we have used the antibodies from a patient with SLE to clone and characterize two distinct Golgi autoantigens. Studies of the characteristics of these proteins and the effects of brefeldin A (BFA) on their intracellular distribution suggest that the proteins are related to the coatomer proteins and may belong to a family of myosin-like molecules. The molecular features of these proteins suggest that they have a unique function in the processing and transport of proteins through the Golgi complex.

Materials and Methods

Human Sera. Sera from patients with Golgi autoantibodies were obtained from serum banks at the University of Calgary (Calgary, Alberta), The Scripps Research Institute (La Jolla, CA), and Immuno Concepts Inc. (Sacramento, CA). The serum samples were stored at −20 or −70°C. The specificity of the autoantibodies for Golgi antigens was first identified on the basis of indirect immunofluorescence (IIF) microscopy (4). Control sera were randomly selected from a bank of 2,000 female Red Cross blood donors (37) or sera pooled from healthy volunteers.

Cell Lines. HeLa (ATCC CCL 2.2; American Type Culture Collection, Rockville, MD); MOLT4 (human T lymphoblastic leukemia; ATCC CRL 1582); HEP-2 (human epidermoid carcinoma), and HepG2 (human hepatic carcinoma, ATCC HB 8065) cells were maintained in RPMI 1640 supplemented with 10% FCS, 2 mM t-glutamine, and 5 μg/ml gentamicin sulfate.

Indirect Immunofluorescence. IIF was performed on cells grown on teflon-masked slides or commercially prepared HEP-2 cell substrates (Immuno Concepts Inc.) using a fluorescein-conjugated goat anti–human IgG (light and heavy chain) as previously described (38, 39). Double-labeling and colocalization studies used TRITC-conjugated wheat germ agglutinin (Sigma Chemical Co., St. Louis, MO) and rhodamine-conjugated goat anti–rabbit IgG (Pierce, Rockford, IL). Slides were viewed with a microscope fitted with epifluorescence (Carl Zeiss, Inc., Thornwood, NY) or a confocal scanning laser microscope equipped with a krypton-argon light source (MRC600; Bio-Rad Laboratories, Richmond, CA).

Immunoelectron Microscopy. Human HeP-2 cells were grown in DMEM containing 10% FCS in Lux Permanox dishes (Electron Microscopy Sciences, Fort Washington, PA). After 2 d in culture, monolayer cells were rinsed in PBS, fixed 30 min at room tempera-

ture (RT) with 4% electron microscopy–grade formaldehyde (Polysciences, Warrington, PA) buffered with PBS, rinsed with PBS, and then permeabilized with 100% acetone for 1 min at −20°C. After a rinse in PBS, cells were blocked for 30 min at RT with 1% BSA/PBS, incubated 1 h at 37°C with the serum SY diluted 1:200 in 1% BSA/PBS, rinsed three times for 10 min in PBS, blocked 30 min with 1% BSA/PBS, and then incubated 1 h at 37°C with affinity-purified goat anti–human IgG coupled to peroxidase (Cappel Laboratories, Cochranville, PA) diluted 1/100 in 1% BSA/PBS. Cells were then rinsed three times for 10 min in PBS, fixed 30 min at RT with 1% glutaraldehyde buffered with PBS, rinsed in PBS, rinsed in 50 mM Tris-HCl (pH 7.6), and then incubated 5 min at RT in 1 mg/ml diaminobenzidine/0.03% H2O2 dissolved in Tris buffer and distilled water, and then stained for 30 min with 2% OsO4 in distilled water, rinsed in distilled water, dehydrated with ethanol, and then embedded in Polybed 812 (Polysciences). Embedded whole cells were photographed in the light microscope, thin sectioned as monolayers, and then examined unstained in the electron microscope.

SDS-PAGE and Immunoblotting. Proteins or cellular preparations were solubilized in SDS sample buffer and separated by discontinuous SDS-PAGE according to the method of Laemmli (40). The separated proteins were transferred to nitrocellulose as described by Towbin and Gordon (41) and adapted in our laboratory (42). After transfer, nitrocellulose strips were blocked with 3% nonfat milk in PBS containing 0.05% Tween-20 (PBST), and then overlaid with the primary antibody and washed with PBST to remove any unbound antibody. Bound antibody was traced with polyvalent, peroxidase-conjugated goat anti–human Ig (Calbiochem-Behring Corp., La Jolla, CA) or 125I–protein A (2–4 × 106 cpm/ml [ICN Radiochemicals, Irvine, CA]. Bound antibodies were visualized by incubating the washed nitrocellulose strips in substrate solution or by exposing the air-dried nitrocellulose to X-OMAT AR film (Eastman Kodak Co., Rochester, NY).

cDNA Library Screening. Clones from a HepG2 λZap cDNA library were initially screened by an immunological method as modified by Young and Davis (43). All screening was performed on duplicate filters and positive bacteriophages were subsequently purified to 100%. Before screening the cDNA library, the sera were extensively adsorbed against bacteria and wild-type λZap phage to reduce background binding. Antibodies were detected by immunoblotting as described above.

DNA Subcloning and Sequence Determination. Purified λZap clones were subcloned in vivo into pBluescript plasmids using R408 helper phage as recommended in the manufacturer's instructions (Stratagene Inc., La Jolla, CA). The nucleotide sequence was determined using the dye primer cycle sequencing kit and DNA sequence (373A; Applied Biosystems, Inc., [ABI], Foster City, CA). Oligonucleotide primers were synthesized with a DNA synthesizer (380B; ABI). DNA sequences were determined in both strands and compiled using the alignment program SeqEd (ABI).

PCR. PCR was used to determine the size and orientation of cDNA inserts using methods essentially as described in the GeneAmp DNA Amplification Reagent Kit (Perkin Elmer Corp., Norwalk, CT). 50- or 100-μl reaction volumes were used and were composed of PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.0 mM MgCl2, 0.01% gelatin), dATP, dCTP, dGTP, and dTTP to a final concentration of 0.2 μM. Sample DNA was added and solutions were heated to 95°C for 5 min, followed by addition of 2.5 U Taq DNA polymerase (Perkin Elmer Corp.), and one drop of paraffin oil. DNA amplification was performed using a microcycler (Eppendorf Inc., Freemont, CA) with up to 35 cycles of 94°C for 30 s; 55°C for 30 s; 72°C for 90 s; and a final 7-min elongation.

1 Abbreviations used in this paper: BFA, brefeldin A; COP, coatomer protein; IIF, indirect immunofluorescence.
Affinity Purification of Autoantibodies. Affinity purification of antibody from XZap clones used confluent plates that were induced to produce recombinant protein with isopropyl-thiogalacto-pyranoside (IPTG; Fisher Scientific, Springfield, NJ)–impregnated nitrocellulose filters. After incubating the plates overnight, the filters were blocked and probed with primary antibody and washed as described above for immunoblotting. Bound antibody was eluted from the filters with 3 ml of elution buffer (50 mM KH2PO4, pH 2.5, 150 mM NaCl, 1.0% BSA) by rocking at room temperature for 5 min. The filter was quickly rinsed with an additional 1-ml elution buffer and the eluates were collected and neutralized with 225 μl 1 M Tris, pH 8.8. The eluted antibodies were concentrated with a microconcentrator (Centricon 100; Amicon Corp., Danvers, MA) and used for IIF and immunoblotting analysis.

In Vitro RNA Transcription and Translation. Appropriate clones identified in the screening outlined above were used for in vitro transcription and translation experiments. 1 μg of linearized plasmid DNA was used as template for in vitro transcription with T7 or T3 RNA polymerase. RNA transcripts were analyzed in 0.8% agarose gel containing 2.2 M formaldehyde. 1 μg of the transcribed RNA was added in a 50-μl translation reaction containing rabbit reticulocyte lysate, [35S]methionine (Trans-35S label, 70% methionine, 15% cysteine; ICN Biochemicals, Irvine, CA), and RNase block II (Stratagene Inc.) as suggested by the manufacturer (Promega Biotec, Madison, WI). Translation was carried out at 30°C for 1 h followed by SDS-PAGE of a 2-5-μl aliquot to confirm the presence of translation products. Samples were stored at −80°C until required.

Immunoprecipitation. Immunoprecipitation of 35S-labeled in vitro translation products was performed using protein A–Sepharose beads (45). Briefly, 10 μl of human serum and 2-5 μl of in vitro translation product were incubated with protein A–Sepharose beads 1–2 h at 4°C. After incubation, the Sepharose beads were washed five times with buffer and resuspended in SDS sample buffer. Samples were then analyzed by SDS-PAGE and autoradiography as described above.

Recombinant Protein Production. Plasmids were transformed in Escherichia coli strain XL1Blue (Stratagene Inc.). The recombinant protein was prepared from 200-ml cultures of the recombinant cells grown to OD600 = 0.6 at 37°C and induced with IPTG added to a final concentration of 10 mM. After overnight incubation, the bacteria were harvested by centrifugation. The recombinant protein was enriched by the method of Adams et al. (46). The final pellet of inclusion bodies was extracted with 1 M urea in 0.1 M Tris (pH 7.3) on ice for 1 min to remove residual bacterial proteins. The inclusion bodies were then pelleted in an Eppendorf microfuge at 13,000 rpm and extracted with 8 M urea in 0.1 M Tris buffer for 15 min on ice. In some experiments, the SY11 inclusion granules were extracted with Triton buffer containing 8 M urea, 0.2% β-mercaptoethanol. The supernatants from the extractions were stored at −70°C. Proteins were separated by SDS-PAGE and stained with Coomassie blue or transferred to nitrocellulose for immunoblotting.

Rabbit Immunization. Three New Zealand white rabbits were separately immunized by subcutaneous and intramuscular injection of 1.5 mg of extracted recombinant proteins in an equal volume of CFA. 2 wk later, the animals were boosted with 2.0 mg of the protein inIFA. The appearance and titer of Golgi antibodies was monitored by indirect immunofluorescence using goat anti-rabbit IgG (H + L chain) antibody (Calbiochem-Behring Corp., La Jolla, CA) as described above.

Results

Immunofluorescence and Immunoelectron Microscopy. Nine sera that demonstrated the typical IIF pattern for Golgi autoantibodies (4) on HEp-2 cells were identified. A serum from a SLE patient with pericarditis and central nervous system involvement (SY) was selected for the cloning studies because it demonstrated high titer (>1:2,560) and relatively monospecific staining of the Golgi complex on methanol-fixed HEp-2 cells (Fig. 1 a). Analysis of a variety of cell lines, including HepG2 and HeLa cells, revealed similar patterns of staining. Fixation of cells in acetone or 1% paraformaldehyde did not alter the staining pattern.

Immunoelectron microscopy of HEp-2 cells demonstrated that antibodies from the serum SY bound to juxtapanel cisternae of the Golgi complex (Fig. 2, a and b). Further confirmation that the antigens were localized to the Golgi complex was demonstrated by colocalization of TRITC-conjugated wheat germ agglutinin to the corresponding structures stained by the prototype serum (Fig. 2, c and d).

Immunoblotting. Immunoblotting of the prototype serum (SY) using MOLT-4 cell extracts revealed reactivity with a number of antigens, including 30-, 95-, 110-, 150-, and 160-kD proteins (Fig. 3, lane 1). When additional anti–Golgi sera were screened by immunoblotting a heterogeneous number of proteins were recognized. For example, one serum (lane 2) bound to the 30-, 150-, and 160-kD proteins, and five (lanes 4–8) demonstrated reactivity with the 95-kD and other proteins. One serum (lane 4) bound 140- and 150-kD proteins and another (lane 9) strongly reacted with proteins <60 kD. Other bands of reactivity with the anti–Golgi sera included proteins of 40, 50, 60, 110, and 190 kD.

Cloning and Sequencing of Golgi cDNA. The prototype human serum (SY) reactive with the 160-, 150-, 110-, and 95-kD Golgi antigens was chosen to screen a HepG2 XZap expression library. From screening 460,000 plaques, 11 positive signals were obtained, 3 of which (SY2, SY10, SY11)
Figure 1. (a) Indirect immunofluorescence of anti-Golgi serum SY on HEp-2 cells. The Golgi antigen is identified as lamellar and vesicular structures located outside the nuclear membrane. The single cell staining more intensely is in metaphase. (b) Indirect immunofluorescence of antibody elicited from SY11 ~78 recombinant protein. Stereo reconstruction from optical sections of HEp-2 cells recorded on the confocal microscope at 0.4-μm intervals. The antibody is primarily localized to the lamellar stacks of the Golgi complex. HEp-2 cells were fixed in methanol.

retained specificity after secondary and tertiary screening to 100% purity. When antibodies from the prototype serum were affinity purified on nitrocellulose filters containing 5 x 10^4 phage-expressing SY2 and SY11 recombinant proteins, all reproduced the Golgi pattern of immunofluorescence on HEp-2 cells (Fig. 1 b). The stereo reconstruction of the staining derived from confocal scanning laser microscopy showed that the antibodies affinity purified from SY11 clones (Fig. 1 b) bound primarily to the lamellar Golgi stacks of HEp2 cells. Some reactivity with vesicular structures was also noted. No reactivity with microtubules, intranuclear components, or the plasma membrane was observed with the affinity-purified antibodies. A similar pattern of reactivity was observed with affinity-purified anti-SY2 (not shown).

The cDNA inserts were subcloned in vivo into pBluescript plasmid, and both strands were sequenced across the polylinker arms. The nucleotide sequences of the DNA and the deduced amino acid sequences of SY2 and SY11 are shown in Fig. 4. Our data suggested that SY11 encoded a complete protein sequence because it contained a 3' poly(A) tail and an open reading frame of 620 amino acids (Fig. 4 a). The deduced amino acid sequence of the cDNA clone of SY2 and SY11 represented 58.7- and 70-kD proteins of 568 and 620 amino acids, and calculated isoelectric points (pI) of 6.09 and 4.57, respectively (Table 1). The three clones were found to represent two groups of unique sequences with SY2 and SY10 having an ~95% overlap (Figs. 4 b and 5 a).

A comparison of the length of the cDNA clones and the overlap of SY2 and SY10 is illustrated in Fig. 5 a. Analysis of the deduced amino acid sequence of SY2 predicted that this polypeptide was exclusively an α-helical structure with an absence of β sheets (Fig. 5 b). Similarly, SY11 had primarily an α-helical structure with some β sheet regions identified (Fig. 5 c). Other special features of SY11 included a leucine zipper (coiled-coil motif) spanning positions 419–455, a glutamic acid–rich tract spanning positions 322–333, a proline-rich tract spanning positions 67–73, and a single putative glycosylation site (Fig. 5 c). Analysis of the sequences of SY2 or SY11 did not disclose any transmembrane domains or signal sequences. Of interest, the amino acid sequence of SY2 demonstrated 43% sequence similarity and 22% sequence identity with SY11 (Fig. 5 d).

The nucleotide and deduced amino acid sequences were used to search the Genebank, EMBL, and NBRF data banks for homologous sequences (Table 1). Up to 21% similarity of amino acid sequences was found with several other reported sequences. A search of the SwissProt data bank indicated that SY2 and SY11 had ~20% sequence similarity with the heavy chain of myosin from a number of species, including humans. The sequence similarity with the heavy chain of myosin is distributed over a 371–amino acid segment (amino acids 163–593 of SY11 and 9–373 of the human heavy chain of myosin). SY11 also shared ~20% sequence similarity with the human protein restin and the yeast protein NUM1. SY2 shared ~20% sequence similarity with the yeast intracellular protein transport protein USO1 and the 150-kD dynein-associated polypeptide. Neither SY2 nor SY11 shared significant sequence similarity with β-adaptin, β-COP, or other known mammalian Golgi components.

Rabbit Antibody to Recombinant Protein. Further support for the authenticity of the cloned cDNA as coding for Golgi complex antigens was obtained by analyzing rabbit antisera raised against SY11, SY10, and SY2 recombinant proteins. The resulting antibodies were shown to react in a similar manner to the prototype sera by indirect immunofluorescence (Fig. 6) and immunoblotting (Fig. 7). Fig. 6 a illustrates the immunofluorescence staining pattern on HEp-2 cells of the prototype Golgi serum using a FITC-conjugated anti-human IgG reagent. Fig. 6 b shows the anti-Golgi reactivity of rabbit sera after immunization with recombinant protein SY11. Double staining with fluorescein anti–human and rhodamine anti–rabbit IgG showed overlap of the staining patterns. Similarly, rabbits immunized with the SY2 or SY10 recombinant proteins demonstrated high titer (>1:3,000) anti–Golgi re-
activity by IIF (Fig. 6 d) in a pattern that overlapped completely with the human antibody (Fig. 6 c).

Immunoblotting of HepG2 cellular extract with rabbit antisera showed reactivities similar to that produced by human serum (Fig. 7). The antibodies from the rabbit immunized with SY10 reacted specifically with the 160-kD antigen in HepG2 extract (Fig. 7, lane 1). This band has the same molecular mass as the one identified by the prototype serum in MOLT4 cell extracts (Fig. 3, lane 1) and in HepG2 extracts (Fig. 7, lane 2). Of interest, the serum from the rabbit immunized with the SY10 recombinant protein also reacted with the 160-kD protein (Fig. 7, lane 5) and several other proteins of 110, 90, 50, and 36 kD. The rabbits immunized with recombinant SY11 protein produced strong reactivity with a 95-kD protein but also weaker reactivity with >200-, 85-, and 75-kD proteins (Fig. 7, lane 3). The >200-, 95-, and 88-kD proteins in Hep-G2 cell extracts were also identified by the prototype serum (Fig. 7, lane 2). Rabbit sera 2 wk after immunization with SY10 (Fig. 7, lane 4) showed weak reactivity with some of the same proteins, as did the rabbit-immune serum (Fig. 7, lane 5). Preimmune rabbit serum (Fig. 7, lane 6) and normal human serum (Fig. 7, lane 7) did not react with any proteins.

**In Vitro Translation and Immunoprecipitation.** The $^{35}$S-labeled
in vitro translation product of SY11 cDNA migrated in SDS-PAGE at 95 kD (Fig. 8 b, lane 1) and was immunoprecipitated by the original human anti–Golgi serum (Fig. 8 b, lane 3). Another anti–Golgi serum (Fig. 3, lane 2) that reacted with the 160- and 95-kD proteins (Fig. 8 b, lane 5) also reacted with the recombinant protein. Normal human serum (Fig. 8, c and d, lane 5) and preimmune rabbit serum (data not shown) did not react with the purified recombinant proteins. The data suggest that the epitopes critical for human antibody binding are present on the recombinant protein derived from the original X phage and the subcloned pBluescript plasmid but that they are not present on the in vitro translated and translated protein.

BFA. Our attention turned to possible relationships of the cloned proteins to the coatomer protein β-COP. BFA, an isoprenoid fungal antibiotic, is known to have an early and characteristic effect on β-COP and other coatomer proteins of the Golgi complex (15, 51, 52). Intermediate concentrations (7.5 μM) of the drug were used to study the effects on the Golgi antigens identified by the prototype serum and the rabbit antisera raised against recombinant proteins. At 0 and 2 min, the Golgi staining remained typical of untreated cells (Fig. 9, a and b). However, 5 and 10 min after exposure of Hep2 cells to the drug, there is a remarkable reduction in the perinuclear Golgi complex and the appearance of vesicular and elongated microtubular structures (Fig. 9, c and d). At 30 min, there is complete loss of Golgi staining (e) and 90 min after removal of the drug (f).

Discussion

This work is aimed at characterizing and identifying the Golgi complex autoantigens that are the targets of autoantibodies in the sera of patients with autoimmune diseases. To begin analysis of the Golgi complex, we have cloned the cDNA encoding the complete protein sequence of a 95-kD autoantigen identified by a SLE serum, and have named this golgin-95. We also obtained a cDNA encoding a portion of a second
velop central nervous system disease (53, 54) may be clues to the clinical importance and pathogenic role of Golgi antibodies. We considered the possibility that the Golgi antigens that we have cloned bear resemblance to Golgi autoantigens described by others. The best characterized Golgi autoantigen was recently reported by Kooy et al. (8) as a 230-kD protein that was found in both soluble and insoluble fractions of HeLa cells. Although this 230-kD Golgi complex antigen was cloned and shown to react with a serum from a Sjögren's syndrome patient, the sequence has yet to be published. A 65- and a

Golgi complex antigen, named golgin-160, that reacted with the same sera and has a molecular mass of 160 kD. The clinical relevance of anti-Golgi antibodies is uncertain. It is interesting that two of the sera with antibodies to golgin-160, including the serum used to clone the protein, were from patients who had cerebellar disease. Likewise, the report of anti-Golgi antibodies in patients with cerebellar ataxia (10), paraneoplastic cerebellar degeneration (11), and patients with Sjögren's syndrome (6) who can develop central nervous system disease (53, 54) may be clues to the clinical importance and pathogenic role of Golgi antibodies.

Figure 4. Nucleotide and deduced amino acid sequence of cDNA clones SY2, SY10, and SY11. Sequencing of both DNA strands of each clone was performed with custom synthetic oligonucleotide primers. (a) Sequence of SY11. Underlined nucleotides are the first upstream in-frame stop codons, consensus sequences for the methionine start site, and stop codons, respectively. Amino acids at 67–73 represent a proline-rich region, and at 322–333 a glutamic acid-rich region. Six hepad repeats of leucine (boldface) begin at amino acid 419. This sequence is accessible from EMBL/GenBank under accession number L06147. (b) Combined sequence of SY2 and SY10. The underlined nucleotides are the putative methionine start sites that are functional in the in vitro translation system. The sequences of SY2 and SY10 begin at nucleotides 54 and 1 and terminate at nucleotides 1738 and 1585, respectively. This sequence is accessible from EMBL/GenBank under accession number L06148.
ences between the antigens described in these reports and rheumatoid arthritis, respectively (7, 9). There are some differences with an overlap syndrome of SLE and polymyositis and rheumatoid arthritis, respectively (7, 9). There are some differences with an overlap syndrome of SLE and polymyositis and rheumatoid arthritis, respectively (7, 9). There are some differences in their reported DNA inserts. SY2 (1.7 kb) and SY10 (1.6 kb) represent DNA inserts with ~95% overlap. Neither SY2 nor SY10 was flanked by an untranslatable stretch of DNA. (b and c) Secondary structure predictions for SY2 (Golgin-160) and SY11 (Golgin-95) are illustrated in b and c, respectively. The secondary structure was predicted using the program Peptide Structure (47). The structure predicted for a domain is denoted by the position of the line (turns, \( \alpha \)-helices, \( \beta \)-sheets).

Figure 5. (a) Comparison of the recombinant SY2, SY10, and SY11 cDNA inserts. SY11 is a 2.1-kb insert and it included 5' and 3' untranslated regions (small arrow). SY2 (1.7 kb) and SY10 (1.6 kb) represent DNA inserts with ~95% overlap. Neither SY2 nor SY10 was flanked by an untranslatable stretch of DNA. (b and c) Secondary structure predictions for SY2 (Golgin-160) and SY11 (Golgin-95) are illustrated in b and c, respectively. The secondary structure was predicted using the program Peptide Structure (47). The structure predicted for a domain is denoted by the position of the line (turns, \( \alpha \)-helices, \( \beta \)-sheets).

In addition, although our antigens can be detected in the soluble fraction of cells, the majority of our proteins are found in the insoluble fraction (data not shown). Similarities between our antigens and the 65-kD protein include resistance to detergent extraction, sensitivity to trypsin treatment, and preservation of staining after paraformaldehyde fixation.

An interesting feature of golgin-95 is the presence of a coiled-coil motif (leucine zipper) characterized by six repeats of leucine residues at every seventh position (55). This motif is formed by two parallel amphipathic \( \alpha \)-helices, which structurally resembles a coiled-coil (56). Coiled-coil motifs have been noted in other autoantigens, including 52-kD SS-A/Ro (57) and the 80/86-kD Ku (58) autoantigens that react with autoantibodies present in SLE and Sjögren's syndrome sera (reviewed in reference 3). The leucine zipper motif has also been described in a number of other prokaryotic and eukaryotic proteins, most notably products of nuclear oncogenes (c-fos and c-jun (59)), viral fusogenic proteins (60, 61), and the envelope glycoproteins of HIV1 and other viruses.
Table 1. Characteristics of Golgin-95 and Golgin-160

|                     | Golgin-95 (SY11) | Golgin-160 (SY2/SY10) |
|---------------------|------------------|-----------------------|
| Sequence data:      |                  |                       |
| Polypeptide molecular mass (kD) | 70               | 58.7                  |
| No. of amino acids  | 620              | 579                   |
| Physical Measurements: |                |                       |
| In vitro translated product molecular mass (kD) | 95               | 60                    |
| Protein band: MOLT4 + Hep-G2 cells | 95               | 160                   |
| Sequence Similarities (~20%): |                  |                       |
| EMBL/GenBank accession no. | LO6147          | LO6148                |

Figure 6. Immunofluorescence localization of golgin-95 and golgin-160. Anti-Golgi antibodies from the prototype serum (SY) affinity purified with λZAP recombinant protein SY2 (a) or SY11 (c) colocalized with rabbit antibodies raised against the golgin-160 (b) or golgin-95 recombinant proteins (d), respectively. The binding of affinity-purified anti-SY11 was traced with fluorescein-conjugated goat anti-human antibodies and the binding of rabbit antibodies was traced with rhodamine-conjugated goat anti-rabbit antibodies; ×400.

(62). Although proteins with leucine zippers are able to bind DNA and are thought to be predominantly regulatory in their function (63), this motif also mediates the dimerization of certain transactivators (53, 64), and is essential for recombination of certain viral proteins (65) and oligomerization of viral epitope proteins (61, 66, 67). Unlike golgin-95, some proteins with leucine zipper motifs contain a domain of 30–35 amino acids with a high ratio of the basic amino acids arginine and lysine, which are immediately adjacent to the putative zipper structure (55).

Golgin-95 has a stretch of acidic residues from amino acids 322 to 333. Consecutive stretches of acidic amino acid residues have been described in other autoantigens, including NOR-90 (human upstream binding factor [hUBF]) (68), high mobility group protein 1 (HMG-1), centromere protein B (CENP-B), and nucleolin (reference 69 and references therein). Although the role of these sequences is not clear, it is likely that these stretches are responsible for binding to cationic molecules.

The Golgi complex, localized in the perinuclear region of most mammalian cells, consists of stacks of membranous cisternae with functional and topological polarity (for reviews see references 14 and 15). The processing, maturation, and sorting of secretory and membranous proteins take place in this organelle. Many of the specific enzymes responsible for the sequential modification of proteins passing through the Golgi complex have been characterized. Although considerable detail about these enzymes is known, the mechanisms by which proteins are transported and sorted in the Golgi complex remain unclear.
Recent evidence suggests that important molecules in this process are four high molecular mass coat proteins (COPs): α-COP, 160 kD; β-COP, 110 kD; γ-COP, 98 kD; and δ-COP, 61 kD) that are major constituents of the nonclathrin-coated vesicles (24). These proteins are associated with several other lower molecular mass (20–29-kD) proteins in the cytosol (23), bind reversibly to membranes of the Golgi complex, and exist as a high molecular mass complex in the cytosol referred to as the "coatomer" (23, 24, 70). β-COP displays homology with the human clathrin-coated vesicle protein β-adaptin (24, 25). β-COP does not bear sequence similarity to kinesin or other microtubule-associated proteins (MAPs), nor does it have a transmembrane or other obvious target sequences (25).

BFA, a drug that blocks intracellular transport, blocks the binding of β-COP to Golgi membranes producing a characteristic pattern of immunofluorescence (71). In our study, we have noted similar effects of BFA on the intracellular distribution of golgin-95 and golgin-160. Taken together, these observations suggest that golgin-95 and golgin-160 may be structurally and functionally related to the COPs. However, they do not have sequence similarity to β-COP or the adaptins, and do not bear the nonapeptide SLGEIPIVE described by Serafini et al. (24) or the putative microtubule-binding motif KKEX motif in β-COP suggested by Duden et al. (25). A notable feature of golgin-95 is the sequence similarity to microtubule proteins and, like β-COP, the absence of a transmembrane or signal sequence.

It is possible that golgin-95 and golgin-160 are functionally and structurally related because they share >43% sequence similarity and 22.5% sequence identity over a stretch of 618 amino acids. In this regard, it is interesting that golgin-95 and golgin-160 showed significant sequence similarity to several cytoskeleton-related proteins, including kinesin (72), the 150-kD dynein-associated protein (73), the myosin family proteins (heavy chain myosin, tropomyosin), and desmin. Kinesin is a microtubule-stimulated ATPase (74, 75) and a likely motor protein for organelle transport along microtubules. The sequence similarity of the golgins with these proteins is only ~21%, but the aligned sequences span >300 amino acids in α-helical domains of these proteins. This suggests that the Golgi complex has unique motor proteins that are part of the myosin family. Another observation that supports this conclusion is that golgin-160 has sequence similarity to the yeast cytoskeleton-related protein USO1. It has been demonstrated that USO1 is required for protein transport from the endoplasmic reticulum to the Golgi apparatus (76).

In summary, autoantibodies from a patient with SLE have been used to identify, clone, and sequence two apparently related Golgi-associated proteins having molecular masses of 95 and 160 kD. We have designated these proteins golgin-95 and golgin-160. The function(s) of these two proteins is unknown. Based on the analysis of the sequences of these proteins and the effects of BFA on their distribution, several possibilities can be considered. First, these proteins may be kinesin-like motor proteins that have a role in the transport of vesicles from the endoplasmic reticulum to the Golgi complex or within the Golgi stack. Second, both proteins may form a cytoskeletal structure that is the framework for transport of Golgi vesicles. Sequence similarity of the cloned proteins to cytoskeleton-related proteins supports this possibility. The deduced secondary structure suggests a predominantly coiled-coil structure. Third, golgin-95 may be a structural protein within the Golgi complex responsible for protein transport. The presence of a leucine zipper, and stretches of acidic residues, is consistent with features of a coat protein. This is further substantiated by observations that the behavior of both proteins is similar to β-COP after BFA exposure. Studies using a number of cell biology and molecular tools can help determine the more precise roles of these proteins in the future. In the meantime, sera from patients with SLE continue to provide valuable reagents in the study of immune responses and the structure and function of key cellular organelles.

Figure 7. Immunoblot analysis of HepG2 whole cell extracts separated on 10% gel SDS-PAGE and probed using rabbit antisera raised against golgin-160 and golgin-95. The rabbit antisera diluted 1:100 show reactivity with proteins of similar molecular mass as the prototype serum (SY) diluted 1:50. Rabbit antibodies raised against SY2 (lane 1) react with a 160-kD protein that has the same molecular mass as the fainter upper band reacting with the prototype serum (lane 2). Rabbit antibodies raised against SY11 (lane 3) react strongly with a 95-kD protein that has the same molecular mass as a band recognized by the prototype serum (lane 2). Lane 4 represents the reactivity of a rabbit serum 2 wk after immunization with SY10. Lane 5 was probed with an immune rabbit serum 2 wk after a boost with the recombinant SY10 protein. This serum reacted with the 160-kD protein and several other lower molecular mass proteins. Lanes 6 and 7 are preimmune rabbit serum and normal human serum, respectively.
Figure 8. Immunoprecipitation of in vitro translation and transcription products of SYll and SY2. In vitro translation products derived from the SYll or SY2 plasmid templates linearized with ScaI restriction enzyme and transcribed with T3 or T7 RNA polymerase. The [35S]methionine-labeled in vitro translated products were incubated with rabbit or human serum and the antigen–antibody complexes absorbed to protein A-Sepharose. The complexes were separated by SDS-PAGE and the dried gel was processed and exposed to x-ray film to trace the bound antigen. (a) The translation product [35S]methionine-labeled SY2 (lane 1) migrated at 65 kD with smaller translation products also observed. The 65-kD [35S]methionine-labeled in vitro translated product was not immunoprecipitated by preimmune rabbit serum (lanes 2 and 5) but it was by antibodies from rabbits immunized with the SY2 recombinant protein (lanes 3 and 4) and rabbits immunized with SY10 (lanes 6 and 7). The prototype serum SY (lane 8) and another human anti-Golgi serum (lane 9) did not immunoprecipitate the in vitro translated SY2 protein. (b) SYll (lane 1) migrated at 95 kD with smaller translation products migrating at 68–94 kD also observed. The 95-kD in vitro translation product was not immunoprecipitated by preimmune rabbit serum (lane 2), but was immunoprecipitated by immune rabbit serum (lane 3), the prototype serum SY (lane 4), and another anti-Golgi serum (lane 5). (c and d) Immunoblot analysis of purified SY2 and SYll recombinant proteins extracted from inclusion bodies with 8 M urea and β-mercaptoethanol and separated on a 10% SDS-polyacrylamide gels. Human antibodies to golgin-160 (c, lane 1) and golgin-95 (d, lane 1) and the prototype serum (c and d, lane 2) showed reactivity with an ~70-kD recombinant SY2 (c) and an ~100-kD recombinant SYll (d). Similar reactivity was observed with affinity-purified anti-SY2 (c, lane 3) and anti-SYll (d, lane 3), and sera from rabbits immunized with SY2 (c, lane 4) and SYll (d, lane 4). Normal human serum (c and d, lane 5) did not react with the recombinant proteins.

Figure 9. Effects of BFA on the Golgi apparatus. BFA at 7.5 μM was added to log-phase growing HEP-2 cells in culture. At selected time intervals, slides were removed, fixed, and processed for indirect immunofluorescence using affinity-purified anti-SYll. (a) Time 0, demonstrating clearly defined Golgi organization; (b) 2 min after BFA, the Golgi antigen shows early signs of redistribution; (c) at 5 min, there is loss of staining in the Golgi region; (d) at 15 min, there is more loss of the lamellar Golgi antigen with apparent redistribution to the cytoplasm; (e) at 30 min, there is virtual loss of Golgi staining; (f) 90 min after BFA media was removed and replaced with fresh media, there is a restoration of the staining in vesicular and lamellar structures consistent with the Golgi staining.
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