A Monoclonal Antibody that Recognizes Golgi-associated Protein of Cultured Fibroblast Cells

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ABSTRACT   We have obtained a hybridoma clone, JLJ5a, which secretes monospecific antibody directed against a 110-kdalton protein of gerbil fibroma cells, Rat-1 fibroblasts, and L6 myoblasts. It appears to be localized in the Golgi apparatus by the following criteria: (a) In double-staining experiments the localization of the 110-kdalton protein by the JLJ5a monoclonal antibody was coincident with the reaction products of thiamine pyrophosphatase (one of the enzyme markers of the Golgi apparatus; Novikoff and Goldfischer, 1961, *Proc. Natl. Acad. Sci. U. S. A.* 47:802–810) in the same cells. (b) The staining pattern of the JLJ5a monoclonal antibody became fragmented and dispersed into vacuoles after pretreatment of the cells with Colcemid or monensin.

The Golgi apparatus is a unique, cytoplasmic organelle of many eucaryotic cells. It appears to play important roles in a variety of cellular activities such as assembly of secretory proteins, formation of lysosomes, differentiation of membranous organelles, phospholipid synthesis, glycosylation, and sulfation (2, 25). However, at the present time, little is known about the molecular organization and mechanisms responsible for the postulated functions of the Goli apparatus.

The lymphocyte hybridoma technique developed by Köhler and Milstein (8) provides an approach to study the Golgi apparatus at the molecular level. An advantage of this technique is the ability to raise the monospecific antibody without purifying the protein. One can then raise antibodies against previously unidentified components of a cell, such as proteins in trace amounts and proteins that are difficult to obtain in pure form. In this communication, we report a hybridoma clone that secretes a monospecific antibody directed against a Golgi-associated protein of 110-kdaltons.

MATERIALS AND METHODS

Cell Culture

Cultured cells used in the present study were gerbil fibroma cells (American Type Culture Collection, CCL-146), Rat-1 fibroblasts, L6 myoblasts, and the hybridoma clone JLJ5a. The culture conditions were carried out as described previously (11).

Preparation of Monoclonal Antibody

The antigen used for immunization was prepared as follows: gerbil fibroma cells grown on 60-mm culture dishes were washed and briefly treated with 0.5% Triton X-100 in phosphate-buffered saline (PBS). The residues scraped off the dishes were collected by low-speed centrifugation. This fraction mixed with complete Freund's adjuvant was then used as antigen to immunize the mouse. The immunization schedule and the cell fusion procedure were carried out as described previously (7, 11). Indirect immunofluorescence on gerbil fibroma cells was used for screening positive hybrids, which were cloned three times on agarose as described previously (11, 19). Monoclonal antibodies were obtained either from mass-cultured medium of the hybridoma or from ascites fluids of hybridoma-bearing mice.

Thiamine Pyrophosphatase and Indirect Immunofluorescence

For detection of thiamine pyrophosphatase (TPPase) activity and for immunofluorescence localization, gerbil fibroma cells were grown on 12-mm round glass cover slips for 1–2 d before use. In the case of drug treatment, gerbil fibroma cells grown on cover slips were exposed to either Colcemid (1×10^{-6} M) for 16 h or monensin (5×10^{-6} M) for 4 h. The procedure for TPPase localization was carried out as described by Novikoff and Goldfischer (15). Indirect immunofluorescence was performed as described previously (11). For double-staining experiments, gerbil fibroma cells were cytochemically stained for TPPase and then counterstained with JLJ5a monoclonal antibody by indirect immunofluorescence.

Immunoprecipitation

The [^{35}S]methionine-labeled lysates were prepared from gerbil fibroma cells, Rat-1 fibroblasts, and L6 myoblasts according to the method described previously (11). Then, 4 μl of JLJ5a or JLA20 monoclonal antibody was added to an equivalent amount of radioactivity in the initial lysates. Immunoprecipitation with JLA20 monoclonal antibody against actin was used here as a control for the background, since JLA20 monoclonal antibody, like JLJ5a, is IgM. After a 2-h incubation at 4°C, 7 μl of rabbit anti–mouse IgM antibody (Litton Bionetics Inc., Kensington, Md.) was added to the mixture; this mixture was then incubated for another hour at 4°C. Finally, the immune complexes were incubated with 100 μl of formalin-fixed *Staphylococcus aureus* for 30 min and pelleted in an Eppendorf centrifuge (Brinkmann Instruments, Inc., Westbury, N. Y.). The washed

immunoprecipitates were analyzed by SDS PAGE (9). Radioactive proteins in the gel were detected by fluorography (3) on Kodak XR-1 film.

RESULTS

Production and Characterization of JLJ5a Monoclonal Antibody

The cell fusion was performed with myeloma cells (NS1) and spleen cells of a mouse immunized with Triton X-100 insoluble fraction of gerbil fibroma cells. This Triton X-100 insoluble fraction contained intermediate-filament components (mainly vimentin), nuclear components, and other unidentified proteins. Therefore, in the initial screening, many positive hybrids produce antibodies for the intermediate filament staining pattern or the nuclear staining pattern. In addition, one clone, JLJ5a, was found that secreted a monoclonal antibody decorating what appeared to be the Golgi apparatus of gerbil
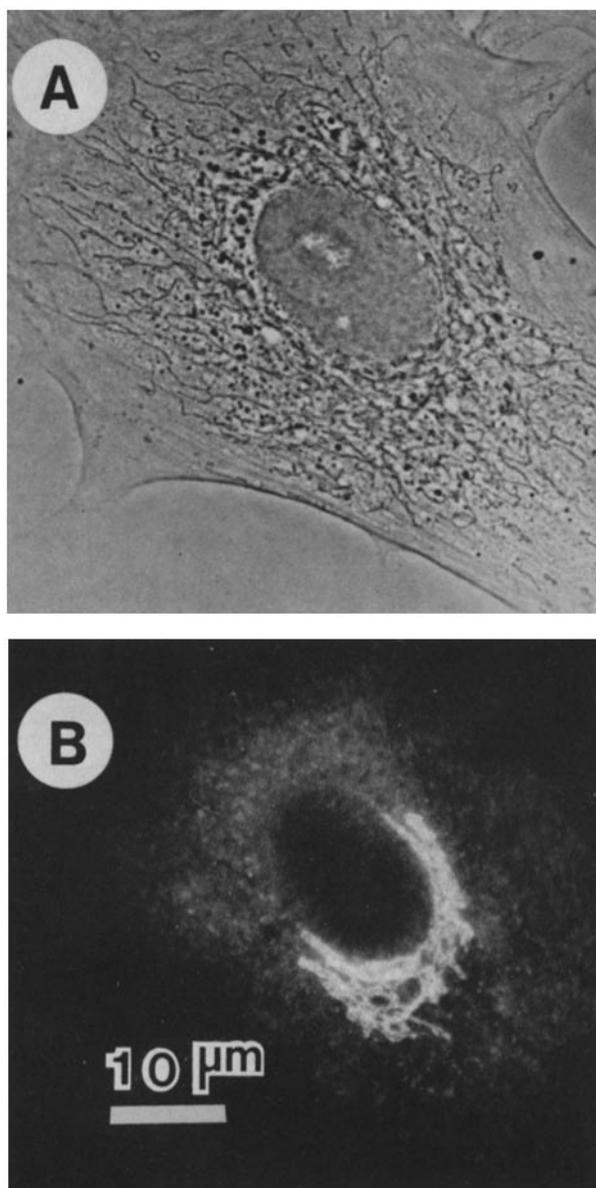
FIGURE 1 Indirect immunofluorescence of gerbil fibroma cells stained with JLJ5a monoclonal antibody. (A) Phase-contrast micrograph; (B) immunofluorescence micrograph.

fibroma cells, Rat-1 fibroblasts, and L6 myoblasts. The experiments described below confirmed this specificity.

The JLJ5a monoclonal antibody is IgM as determined by the Ouchterlony double-diffusion test, using commercial rabbit anti-mouse class-specific immunoglobulin antiserum, and by 10% SDS PAGE. By using indirect immunofluorescence, the antigen recognized by this antibody appeared to be located at the perinuclear region of gerbil fibroma cells (Fig. 1). The immunofluorescence micrographs often showed lamellar and vacuolar structures. These morphological features are characteristics for the Golgi apparatus (2, 25). In addition, we observed a weak, diffuse staining throughout the entire cell (Fig. 1 B). At present, the significance of this diffuse staining is not clear. However, it may suggest that the antigen is also present in the plasma membrane of cells. This possibility is consistent with the reported fusion occurring between Golgi-derived secretory vesicle membrane and plasma membrane (17). Unfortunately, the antibody did not react with intact cells, suggesting that the antigen is not exposed on the cell surface. Furthermore, the JLJ5a antibody also stained nuclei from a certain population (~10%) of gerbil fibroma cells. The number of cells with positive nuclear staining appeared to be increased when cells were recovered from Colcemid treatment (data not shown). It is possible that the antigen in the nucleus may represent a precursor form of the same antigen in Golgi apparatus. Experiments are in progress to prove this possibility.

The specific antigen recognized by JLJ5a monoclonal antibody was identified by immunoprecipitation. Total cell extracts from gerbil fibroma cells, Rat-1 fibroblasts, or L6 myoblasts labeled in vivo with [^{35}S]methionine were prepared in 0.5% Triton X-100 soluble fraction as described previously (11). Immunoprecipitation was performed as described in Materials and Methods. The fluorograms of immunoprecipitates analyzed by 12.5% or 10% SDS PAGE are shown in Fig. 2. A specific protein band estimated at 110 kdaltons was precipitated by the JLJ5a monoclonal antibody from total cell extracts of all three cell lines. The control monoclonal antibody (JLA20) specifically precipitated the actin molecules from the same cell extracts. For unknown reasons, the background in experiments using gerbil fibroma cells is always higher.

Double-staining Experiments to Localize TPPase and 110-kdalton Protein

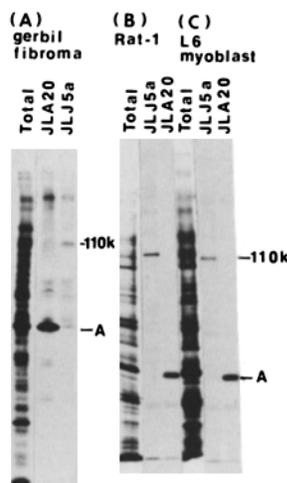It has been shown that both nucleoside diphosphatase and

FIGURE 2 Fluorograms of immunoprecipitates analyzed by 12.5% (for gerbil fibroma cells) or 10% (for Rat-1 fibroblasts and L6 myoblasts) SDS PAGE. Lanes: *Total*, total extract (the high-speed supernatant, 100,000 × g, 2 h) of gerbil fibroma cells, Rat-1 fibroblasts, or L6 myoblasts; *JLJ5a*, immunoprecipitate of total extract by JLJ5a monoclonal antibody against a Golgi-associated protein; *JLA20*, immunoprecipitate of total extract by JLA20 monoclonal antibody against actin. A, actin.

TPPase activities are localized in the Golgi apparatus of a variety of cells (1, 6, 12, 15, 20). To investigate the relationship of a 110-kdalton protein recognized by JLJ5a antibody to TPPase, we first incubated gerbil fibroma cells for TPPase activity. The same cells were then counterstained with JLJ5a monoclonal antibody by indirect immunofluorescence. The result is shown in Fig. 3. In the phase-contrast micrograph (Fig. 3A), reaction products (dark granules or threads) of TPPase form a saccular or lamellar structure located at perinuclear or juxtanuclear regions. The immunofluorescent staining patterns revealed by JLJ5a antibody on the same cells (Fig. 3B) are essentially identical to that of the TPPase, except that the fluorescence seems to occupy broader areas of Golgi apparatus in some cells. Such broadening may be expected since TPPase-positive material is found only in the innermost element of the Golgi stacks in neurons (16), whereas the 110-kdalton protein may be more widely distributed in the Golgi stacks.

Distribution of 110-kdalton Protein in the Monensin- or Colcemid-treated Cells

The ionophore monensin has been thought to alter the ultrastructure of the Golgi apparatus in a variety of mammalian cells such as human fibroblasts, macrophages, muscle cells, plasma cells, and pancreatic cells (10, 21–23). In all cases, monensin causes the Golgi apparatus to become swollen; the Golgi region appears to be filled with a number of large vacuoles. To further confirm that the 10-kdalton protein recognized by JLJ5a antibody was located at the Golgi apparatus, we treated gerbil fibroma cells with 5×10^{-6} M monensin for 4 h and then reacted them with JLJ5a antibody. As can be
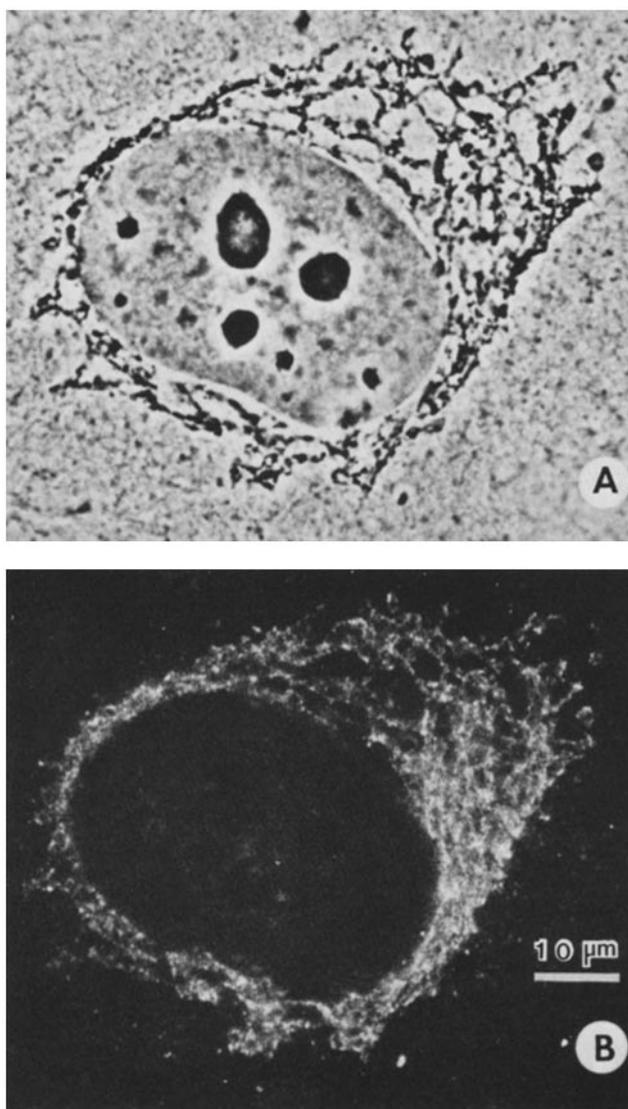

FIGURE 3   Double-staining experiment. (A) Phase-contrast micrograph of gerbil fibroma cell cytochemically stained for TPPase activity. The TPPase positive Golgi apparatus appears to be localized at the perinuclear region (B) Immunofluorescence micrograph of the same cell stained with JLJ5a monoclonal antibody by indirect immunofluorescence shows the fluorescence to be coincident with the TPPase stain.
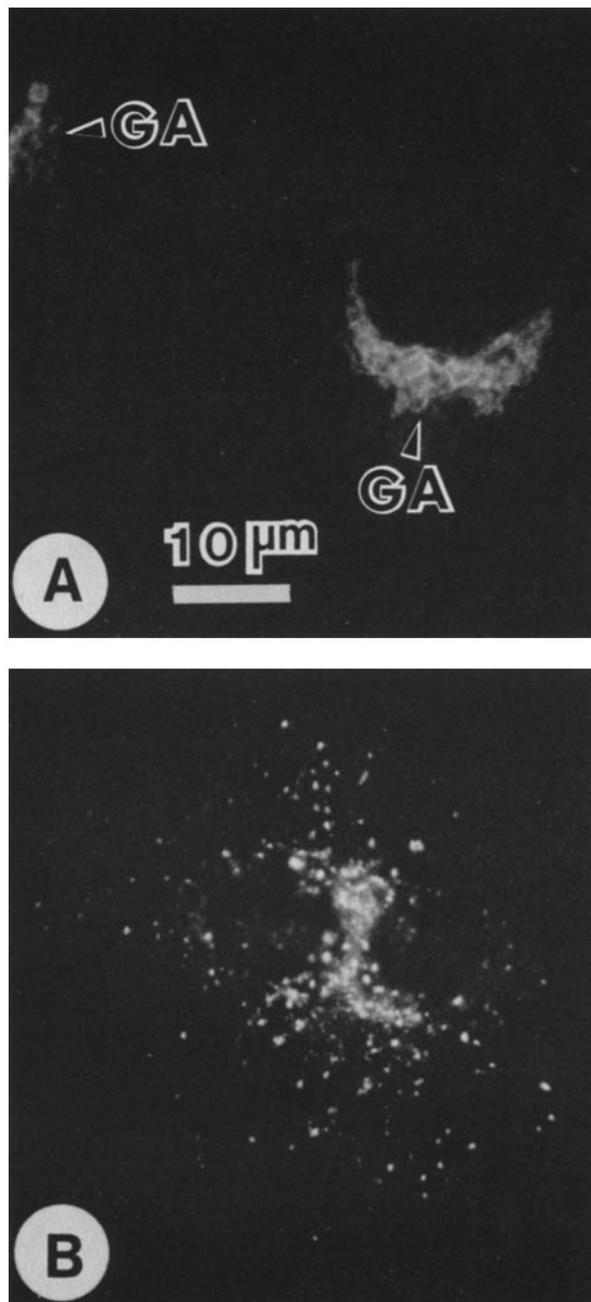

FIGURE 4   Immunofluorescence micrographs of gerbil fibroma cells treated with (B) or without (A) ionophore monensin before reaction with JLJ5a monoclonal antibody. GA, Golgi apparatus.
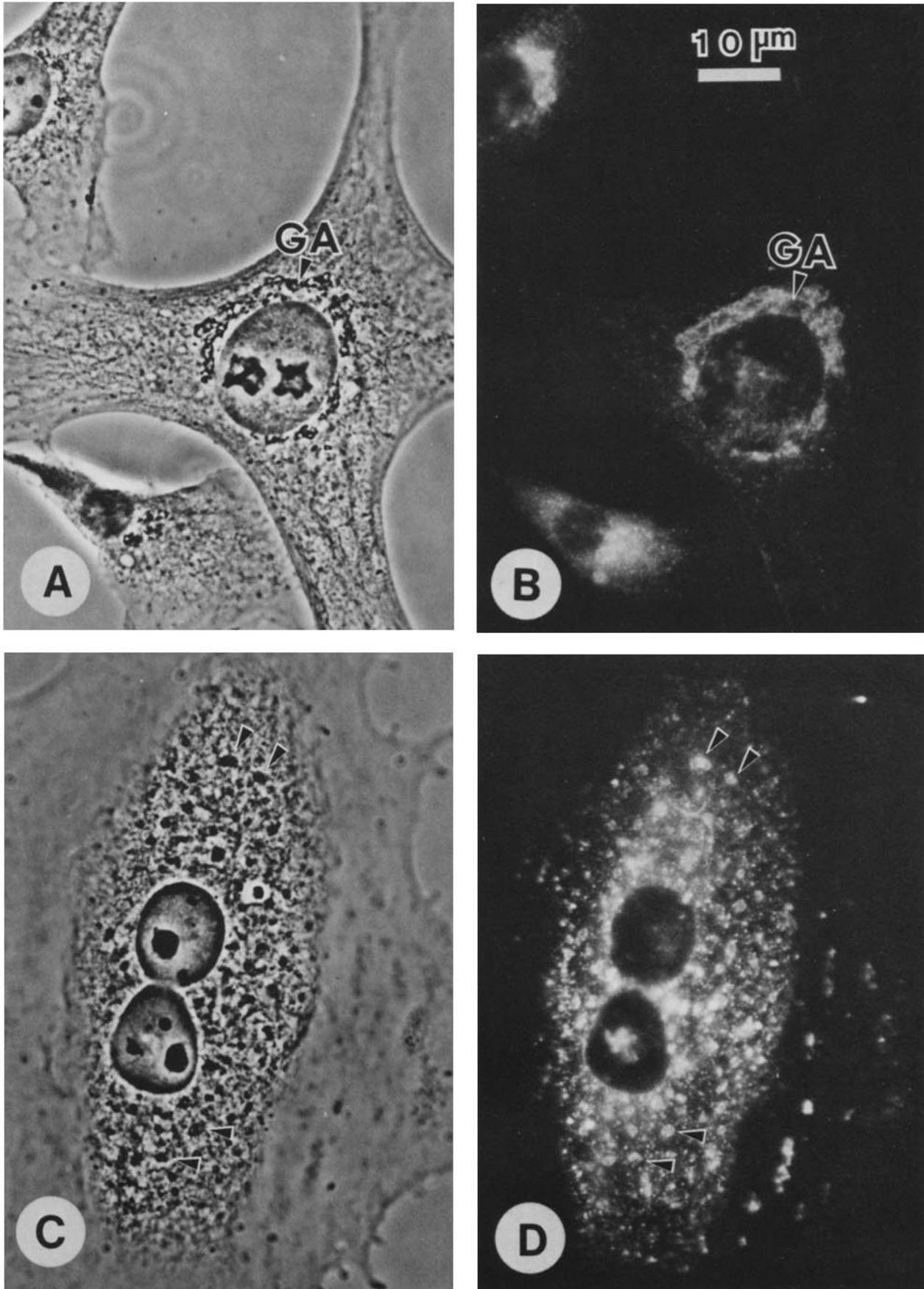
FIGURE 5   Double-staining experiment. (*A* and *C*) Phase-contrast micrographs of gerbil fiboma cells cytochemically stained for TPPase activity. (*B* and *D*) immunofluorescence micrographs of the same cells as in *A* and C, respectively, stained with JLJ5a monoclonal antibody by indirect immunofluorescence. (*A* and *B*) Control cells. (*C* and *D*) Colcemid-treated cells. *GA*, Golgi apparatus. Arrowheads indicate that some of TPPase products have localization identical to that revealed by JLJ5a monoclonal antibody.

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seen in Fig. 4 B, the normal, cup-shaped Golgi apparatus no longer existed in the monensin-treated cells. Instead, the 110-kdalton protein was distributed as a dotted or granular material throughout the cytoplasm. This result further suggests that the 110-kdalton protein is a Golgi-associated protein. It remains to be determined whether the 110-kdalton protein represents a protein in the membranes or in the cavities of Golgi cisternae.

Another feature of Golgi apparatus is that it fragments in cells treated with antimitotic drugs such as colchicine, Colcemid and vinblastine. This drug effect on the Golgi structure has been reported for a variety of cells such as mouse embryonic cells, chondrocytes, pancreatic cells, embryonic spinal ganglion cells, and HeLa cells (4, 5, 13, 14, 18, 24). We have examined the Colcemid effect on both the 110-kdalton protein and TPP enzyme distribution in gerbil fibroma cells. The normal Golgi apparatus (GA) revealed by TPPase product (Fig. 5 A) is identical to that stained by JLJ5a antibody (Fig. 5 B) in control cells. In Colcemid-treated cells, it became fragmented and randomly distributed around the nucleus, as seen in Fig. 5 C and D. This result provides further support that the 110-kdalton protein recognized by JLJ5a monoclonal antibody is a Golgi-associated protein.

DISCUSSION

The JLJ5a monoclonal antibody described here reacts with a 110-kdalton protein from gerbil fibroma cells, Rat-1 fibroblasts, and L6 myoblasts. This protein in all three cell types is distributed at the perinuclear and juxtanuclear region, which we have identified as a Golgi zone by cytochemically staining for TPPase (one of enzyme markers for Golgi apparatus) (1, 6, 12, 15, 20). The Golgi apparatus revealed by JLJ5a antibody may represent the whole stacks of the Golgi, since the area detected by the antibody appears to be broader than that occupied by the reaction products of TPPase. Immunoelectron microscopy currently in progress shall provide definitive proof for this possibility. In monensin- and Colcemid-treated cells, the cup-shaped Golgi stacks no longer exist and become fragmented, as revealed by JLJ5a monoclonal antibody and TPPase localization, throughout the cytoplasm. This is consistent with previous reports by other investigators (4, 5, 10, 13, 14, 18, 21–24). The results from these drug-treatment experiments also suggest that 110-kdalton protein is located at the Golgi apparatus. Thus, we can conclude that JLJ5a monoclonal antibody recognizes a Golgi-associated, 110-kdalton protein. Purified Golgi fraction but not endoplasmic reticulum fraction from guinea pig liver can block the Golgi staining of JLJ5a antibody (data not shown), suggesting that the 110-kdalton protein is also present in Golgi apparatus of guinea pig liver.

The reader may have noticed an apparent contradiction. Although the antigen used for the initial immunization of the mouse was the Triton X-100 insoluble fraction of cells, the 110-kdalton protein was immunoprecipitated from the Triton X-100 soluble fraction of cultured cells. We convinced ourselves (data not shown) that the 110-kdalton protein is, indeed, a Triton X-100 soluble component. The reason for our finding of a hybridoma clone against this protein after injection of the

insoluble cell residues into mice lies in the extreme sensitivity of the hybridoma technique (8). Even small contaminants can give rise to antibody-secreting clones.

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