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Uukuniemi Virus Maturation: Immunofluorescence Microscopy with Monoclonal Glycoprotein-Specific Antibodies

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Monoclonal antibodies directed against Uukuniemi virus glycoproteins G1 and G2 in combination with polyclonal antibodies against the nucleoprotein (N) were used to study the maturation of the virus in Golgi complexes of infected chicken embryo fibroblasts and BHK cells. Of 25 monoclonal antibodies obtained, 10 were shown to be G1 specific and 15 were shown to be G2 specific by immunoblotting and immunoprecipitation. In double-staining experiments, some of the monoclonal antibodies gave similar distributions of fluorescence as compared with the staining obtained from polyclonal rabbit anti-G1-G2 antibodies. Others, however, preferentially stained either the glycoproteins in the Golgi complex or those at the cell surface. This may indicate that the glycoproteins underwent conformational changes during their transport. Uukuniemi virus infection resulted in the vacuolization of the membranes of Golgi complexes where the maturation of the virus was taking place. Double-staining experiments with monoclonal antibodies which preferentially stained the Golgi-associated viral glycoproteins and with anti-N polyclonal rabbit antiserum showed a correlation between the progressive vacuolization of the Golgi complex and the accumulation of viral nucleoprotein in the Golgi region, suggesting that a morphological alteration of the Golgi complex may be a prerequisite for intracellular maturation of the virus. Treatment of Uukuniemi virus-infected cells with tunicamycin, a drug which inhibits N-linked glycosylation, resulted in the accumulation of both glycoproteins at an intracellular location, apparently representing the endoplasmic reticulum. Double-staining experiments showed a parallel accumulation of nucleoprotein at these sites, indicating that local accumulation of glycoproteins is required for nucleoprotein binding to intracellular membranes.

A characteristic feature of the members of the Bunyaviridae family (1) of arthropod-borne viruses is that they mature intracellularly at smooth-surfaced membranes close to the nucleus (21, 26, 40). We have recently shown by immunological, cytochemical, and immunoelectron-microscopic techniques that this region corresponds to the Golgi complex (13). During infection, viral glycoproteins and nucleoprotein accumulate in the Golgi area, which progressively becomes vacuolized. Virus particles mature by budding at the Golgi membranes. The reason for the maturation of the bunyaviruses in the Golgi region and the nature of the route of transport to the cell surface are so far unknown. We have been using Uukuniemi virus, the prototype of the *Uukuvirus* genus of the Bunyaviridae (1), as a model to study these events. This virus has a lipoprotein envelope containing two glycoproteins, G1 (M_r, ca. 70,000) and G2 (M_r, ca. 65,000) (30). The internal nucleocapsid consists of three single-stranded RNA segments (28, 29) of negative polarity (39) to which multiple copies of the N protein (M_r, 25,000) and a few copies of the L protein (M_r, ca. 200,000) (30) are associated. The glycoproteins are synthesized as a 110,000-dalton precursor (p110), which is cotranslationally cleaved roughly in the middle (38). The virion G1 glycoprotein is terminally glycosylated and contains the typical complex type of glycans, whereas the glycans of G2 are mainly of the high-mannose type (endoglycosidase H sensitive). In addition, G1 or G2 or both contain a novel type of small endoglycosidase H-resistant glycans, which probably represent glycosylation intermediates. This unusual type of glycan may reflect the site of virus maturation in the Golgi complex (27).

To be able to study the maturation of Uukuniemi virus and the intracellular transport of both glycoproteins, as well as the mechanism of virus release, we prepared a set of monoclonal antibodies directed against the G1 and G2 glycoproteins. In addition, we have previously reported on the preparation of polyclonal antibodies against G1-G2 and the N protein (13). This paper describes immunofluorescence microscopy studies in which the specificities and staining properties of the different monoclonal antibodies were examined. In addition, double-staining experiments were carried out to study the localization of viral membrane glycoproteins G1 and G2 and cytoplasmic nucleoprotein N in the same cells.

MATERIALS AND METHODS

Cells and virus. The origin and cultivation of the BHK-21 and chicken embryo cells and the preparation of stock virus from the prototype strain S-23 of Uukuniemi virus after several successive plaque purifications have been described previously (29). The titer of the stock virus was 10^8 PFU/ml. A multiplicity of infection of about 10 PFU per cell was used in all experiments.

Purification of viral glycoproteins. Uukuniemi virus (ca. 2 mg of protein), purified as described previously (29), was disrupted with 2% (vol/vol) Triton X-100 at 20°C for 10 min, and the glycoproteins were purified by centrifugation on CsCl and sucrose gradients as described previously (10, 13). The soluble detergent and lipid-free spike protein complexes were used to immunize mice.

Production of hybridomas. Four BALB/c mice were immunized intraperitoneally with 50 μg of purified G1-G2 glycoprotein complex in complete Freund adjuvant. The animals were given intravenous booster immunizations 4 weeks later

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with 20 μg of protein in phosphate-buffered saline, 4 days before fusion. The myeloma cell line used in this hybridization was P3/NS 1/1-Ag 4-1, which was derived from the BALB/c MOPC 21 line. The myeloma cell line does not grow in HAT selective medium (18) (see below). Immune spleen cells were fused with the myeloma cells by the method of Galfre (6) with minor modifications. Briefly, 10^8 spleen cells from immunized mice and 10^7 myeloma cells were washed with serum-free RPMI 1640 (Flow Laboratories, Irvine, U.K.) and collected by centrifugation at 1,000 rpm for 10 min. A 0.5-ml amount of warm, 50% (wt/vol) polyethylene glycol (M_w, 1,500; no. 29575; BDH Chemicals Ltd, Poole, England) in RPMI 1640 was slowly dropped onto the cell pellet with gentle agitation. Warm (37°C) serum-free RPMI 1640 was slowly added, initially dropwise, to dilute the polyethylene glycol. After centrifugation, the cell pellet was resuspended in HAT medium: Dulbecco modified Eagle medium (Gibco Bio-Cult, Glasgow, Scotland) supplemented with (per liter): 20% fetal bovine serum (Flow), L-glutamine (Orion Diagnostica, Helsinki, Finland), antibiotics (50,000 IU of penicillin and 50 mg of streptomycin), 0.1 mmol of hypoxanthine, 0.4 μmol of aminopterin, and 16 μmol of thymidine (Sigma Chemical Co., St. Louis, Mo.). The cells (2×10^5 cells per well) were seeded into 96-well tissue culture plates (Linbro Scientific Inc., Hamden, Conn.). Peritoneal exudate cells from BALB/c mice (2×10^3 cells per well) were used as feeder cells (5). The cells were incubated at 37°C in 5% CO_2. After 4 days, 50% of the cell supernatant in each well was replaced with fresh HAT medium; this procedure was repeated three times per week. Aminopterin was omitted from the medium after 3 to 4 weeks, and hypoxanthine and thymidine were omitted after 5 to 6 weeks. After the cells reached 50% confluency in the majority of the wells showing cell growth, the supernatants were collected and assayed for production of antibodies against G1 and G2 by using an enzyme immunoassay (EIA) method as described below. The cells from the wells in which antibody production against the immunizing antigens was observed were grown in vitro to stabilize the lines. For ascites production, 0.4×10^6 to 2.0×10^6 cells from these hybridoma lines were inoculated intraperitoneally into BALB/14 mice. When ascites formation was observed (in about 3 weeks), the ascites fluids were withdrawn. The ascites cells were stored in liquid nitrogen, and the fluids were stored in small portions at −20°C.

Enzyme immunoassay for anti-glycoprotein antibodies. Antibody response in mice and the presence of specific antibodies in culture medium of the hybridoma cells were tested by using a solid-phase EIA, the essential steps of which have been described previously (31). Flat-bottomed polystyrene microtiter plates were from Nunc, Roskilde, Denmark. In coating the microtiter plates, purified glycoprotein G1-G2 was used at a concentration of 0.3 μg/ml. Rabbit anti-mouse antiserum conjugated with alkaline phosphatase (Orion Diagnostica, Espoo, Finland) was used at recommended dilutions (1:100 to 1:200). The absorbance values after the enzyme reaction were recorded with a vertical pathway spectrophotometer (Titertek; Multiscan, Eflab, Finland).

Immunoprecipitation and SDS-polyacrylamide gel electrophoresis. Cell lysates were prepared by disrupting monolayer cells with NET buffer (1% Nonidet P-40, 0.005 M EDTA, 0.05 M Tris-hydrochloride [pH 8.0], 0.4 M NaCl, 100 IU of Trasylol [Bayer, Leverkusen, Federal Republic of Germany] per ml). NET (500 μl) was added to 20-cm^2 dishes. After incubation on ice for 10 min, the lysate was centrifuged for 15 min at 10,000 × *g*, and the supernatant was used for immunoprecipitation. Immunoprecipitation was carried out in 1.5-ml tubes by mixing 10 to 50 μl of lysate, 1.0 ml of NET buffer, and 5 to 10 μl of monoclonal antibody (ascites fluid). After incubation for 60 min at 25°C, 100 to 200 μl of 10% (vol/vol) protein A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) was added, and the mixture was incubated overnight at 4°C in an end-over mixer. The precipitate was washed three times with NET buffer and once with 10 mM Tris-hydrochloride (pH 6.8). Finally, 50 μl of electrophoresis sample buffer was added, and the samples were boiled for 3 min and analyzed on 10% polyacrylamide gels by the method of Laemmli (14). Fluorography was carried out by the method of Bonner and Laskey (2).

Immunofluorescence. Immunofluorescence was carried out as described previously (13, 15). Swine anti-rabbit immunoglobulin G (IgG) conjugated either to fluorescein or rhodamine (DAKO, Copenhagen, Denmark) and goat anti-mouse IgG conjugates (Cappel Laboratories, Cochranville, Pa.) were used. The fluorescence was examined with a Leitz Dialux 20 microscope fitted with ×100 and ×63 oil immersion objectives and filters for fluorescein isothiocyanate and tetramethyl rhodamine isothiocyanate fluorescence.

Immunoblotting. SDS-polyacrylamide gel electrophoresis and electrophoretic transfer of proteins to nitrocellulose sheets were carried out as described previously (13, 37). In the SDS gel, 0.5 μg of viral protein was used in each lane. The proteins were then electrophoretically transferred to 0.45-μm nitrocellulose sheets (BA-85; Schleicher & Schüll Co., Keene, N.H.). In immunological staining, the monoclonal antibodies were used at dilutions of 1:100 to 1:400. Anti-mouse peroxidase conjugate (DAKO) was used at a dilution of 1:200.

RESULTS

Production and specificity of monoclonal antibodies against G1 and G2. After myeloma cells were fused with immune spleen cells, the fused cells were seeded into 480 microtiter wells. Cells grew in 100 wells, and in 72 of the wells, antibody production was observed as determined by a solid-phase EIA method with a 1:5 dilution of the medium. The antibody-producing cell lines were grown in vitro to stabilize them. For ascites production, cells of 31 hybridoma lines were inoculated intraperitoneally into BALB/c mice. Of 26 ascites fluids collected, 25 contained antibodies that reacted with either of the two glycoproteins as judged by immunoprecipitation, immunoblotting, and immunofluorescence (Table 1). The specificity with respect to G1 or G2 was studied by immunoblotting by the immunoperoxidase staining method (37). Some of the antibodies gave weak staining or were negative, but in all positive cases, only G1 or G2 was stained (Fig. 1 and Table 1). The specificity of the antibodies was checked also by immunoprecipitation of cell lysates prepared from Uukuniemi virus-infected cells labeled with [^{35}S]methionine (Fig. 2). Also, in immunoprecipitation, some of the antibodies showed no reaction with either G1 or G2, but the combined results from the immunoblotting and immunoprecipitation gave the specificities of all 25 antibodies (Table 1).

Double-immunofluorescence staining with G1- and G2-specific monoclonal antibodies and polyclonal anti-G1-G2 serum. To characterize the immunofluorescence staining properties of the monoclonal antibodies as compared with the staining properties of polyclonal anti-glycoprotein serum prepared in rabbits, Uukuniemi virus-infected chicken embryo cells were fixed with paraformaldehyde at 9 h after infection and

permeabilized with Triton X-100. The cells were then double stained by indirect immunofluorescence with anti-G1 or -G2 monoclonal antibodies, followed by tetramethyl rhodamine isothiocyanate-conjugated goat anti-mouse IgG, and with polyclonal rabbit anti-G1-G2 antiserum, followed by fluorescein isothiocyanate-conjugated swine anti-rabbit IgG. A positive staining of infected but not uninfected cells was obtained with 23 of the antibodies. Two antibodies (UG2-2 and UG2-12) showed no positive fluorescence. The individual antibodies showed marked differences in their staining patterns. Some (e.g. UG2-10, UG2-17, and UG1-22) gave a staining pattern indistinguishable from that obtained with polyclonal antiserum. Two such examples are shown in Fig. 3. Cells double stained with G1-specific UG1-22 (Fig. 3a) and polyclonal G1-G2 antiserum (Fig. 3b) showed a perinuclear, reticular staining, typical of the endoplasmic reticulum (ER). Staining of the nuclear periphery and a juxtanuclear region corresponding to the Golgi complex were also evident. Double staining with G2-specific monoclonal antibody UG2-17 (Fig. 3c) and polyclonal antiserum (Fig. 3d) gave very similar results.

Interestingly, some monoclonal antibodies gave a more restricted staining. These monoclonal antibodies were categorized according to the staining patterns observed, paying attention to the staining of ER, Golgi complexes, and cell surfaces (Table 1). Some monoclonal antibodies (e.g., UG1-6, UG1-8, UG2-9, UG2-16, UG1-18, UG1-19, and UG1-20) preferentially stained the glycoproteins in the Golgi complex. Two such examples are shown in Fig. 4. G1-specific UG1-19 (Fig. 4a) and G2-specific UG2-16 (Fig. 4c) almost exclusively stained a perinuclear region, which was also stained heavily with the polyclonal anti-G1-G2 serum and can also be labeled with markers such as wheat germ agglutinin and thiamine pyrophosphatase activity (13) (data not shown), indicating that it represents the Golgi complex. When stained with these monoclonal antibodies, vacuolar elements which were frequently seen in the vicinity of the Golgi region and at the cellular periphery were also intensely decorated (Fig. 4).

In general, the majority of the monoclonal antibodies did not stain the glycoproteins at the cell surface, as shown by staining of unpermeabilized cells (Table 1). Of those monoclonal antibodies giving positive surface staining, 9 of 10 were G1-specific. Figure 5 shows the fluorescence patterns obtained with two surface-positive monoclonal antibodies, UG1-24 (b and c) and UG1-26 (e and f), in Triton X-100-permeabilized cells. The reticular pattern of fluorescence characteristic of the ER and also staining of the Golgi complex were absent (Fig. 5e) or weak (Fig. 5b). The surface of the cell facing the glass (Fig. 5c and f) showed the characteristic patchy or granular fluorescence of Uukuniemi virus-infected cells (13). Both monoclonal antibodies also recognized vacuolar structures (Fig. 5b and e), which became prominent, especially at later stages of infection.

Double immunofluorescence with glycoprotein-specific monoclonal antibodies and polyclonal anti-nucleocapsid se-

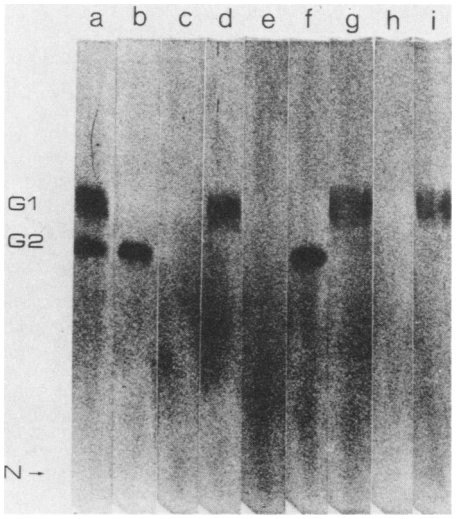

FIG. 1. Analysis of the specificity of anti-G1 and -G2 monoclonal antibodies by immunoblotting. The viral structural proteins (0.5 μg per slot) were separated in a 10% SDS-polyacrylamide gel under nonreducing conditions to separate G1 and G2, and the proteins were transferred electrophoretically to a nitrocellulose sheet. Binding of monoclonal antibodies to G1 or G2 was detected by the immunoperoxidase staining method (39). Only a few examples of the analyses are shown in the figure. Lane a, rabbit polyclonal anti-G1-G2 antiserum (control). Lanes b to i, monoclonal antibodies UG2-5, UG2-7, UG1-8, UG2-9, UG2-14, UG2-16, UG1-19, and UG1-24, respectively. The complete data from the immunoblotting experiments are shown in Table 1.

TABLE 1. Specificity and immunofluorescence properties of monoclonal antibodies against Uukuniemi virus glycoproteins

Antibody or serum	Specificity as determined by:		Immunofluorescence[a]		
	Immunoblotting	Immunoprecipitation	ER	Golgi complex	Cell surface
Monoclonal antibody					
UG2-1		G2	+	++	−
UG2-2		G2	−	−	−
UG2-3	G2	G2	+	−	−
UG2-4	G2		++	+++	−
UG2-5	G2	G2	+	−	−
UG1-6	G1		+	+++	±
UG2-7	G2	G2	+	++	−
UG1-8	G1	G1	±	+++	+
UG2-9	G2	G2	−	+++	−
UG2-10	G2	G2	++	++	−
UG2-11	G2	G2	+	+	−
UG2-12		G2	−	−	−
UG2-13	G2		+	−	−
UG2-14	G2		+	−	−
UG2-15	G2		±	++	−
UG2-16	G2	G2	±	+++	+
UG2-17	G2	G2	++	++	−
UG1-18	G1	G1	+	+++	+
UG1-19	G1	G1	±	+++	+
UG1-20	G1	G1	±	+++	+
X-21			−	−	−
UG1-22	G1	G1	++	++	+
UG1-23	G1	G1	+	++	+
UG1-24	G1	G1	−	−	+
UG1-25	G1	G1	+	++	+
UG1-26	G1	G1	−	−	+
Polyclonal anti-G1-G2 serum (control)			++	+++	++

[a] Relative intensity of fluorescence: −, negative; ±, barely detectable; +, weak; ++, moderately strong; +++, strong as compared with the fluorescence obtained with polyclonal anti-G1-G2 serum. Antibody dilutions used: monoclonal antibodies 1:100; polyclonal anti-G1-G2 serum 1:400.

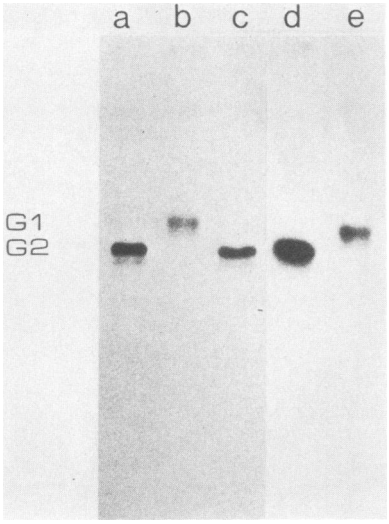

FIG. 2. Analysis of the specificity of anti-G1 and -G2 monoclonal antibodies by immunoprecipitation. Uukuniemi virus-infected cells were labeled with [^{35}S]methionine, and cell extracts were prepared at 10 h postinfection. The viral glycoproteins were immunoprecipitated with different monoclonal antibodies and protein A-Sepharose. A 5- to 10-μl volume of ascites fluid was used per precipitation. The proteins were analyzed in a 10% SDS-polyacrylamide gel. Only a few examples are shown in the figure. The complete immunoprecipitation data are shown in Table 1. Lanes a to e, immunoprecipitation with monoclonal antibodies UG2-7, UG1-8, UG2-9, UG2-16, and UG1-19, respectively.

rum. A typical feature of Uukuniemi virus-infected cells is the progressive vacuolization of the Golgi region, which, as examined at electron-microscopic level, at later stages of infection results in the disappearance of the typical stacked organization of the Golgi cisternae (13, 40). In immunofluorescence microscopy, after labeling with anti-glycoprotein antibodies, the staining of the Golgi complex is typically reticular in appearance at early stages of infection. This pattern is converted to vacuolar type of staining at later stages of infection (13). To be able to study the distribution of the two glycoproteins and the N protein in the Golgi region of single, infected cells, the double-staining technique described above was used with monoclonal antibodies against G1 and G2, and polyclonal rabbit anti-N serum. The results are shown in Fig. 6. At 9 h postinfection, some of the cells still displayed the reticular type of the Golgi, whereas the morphology of the Golgi in other cells was already vacuolar (Fig. 6a). Staining of the same cells with nucleocapsid antiserum (Fig. 6b) shows that the nucleocapsid protein accumulated in the Golgi region only in those cells in which the Golgi was vacuolized. Apparently, the vacuolization of the Golgi correlated with the intensity of glycoprotein staining, reflecting an interdependence between the stage of infection and the vacuolization in individual cells. Accordingly, at later stages of infection (15 to 20 h), practically all cells show both the change in the Golgi morphology and the nucleocapsid accumulation.

The localization of virus glycoproteins and nucleoprotein at different stages of Golgi vacuolization is shown in Fig. 6, c to h. Figure 6c shows the Golgi region of a cell stained with G1-specific monoclonal antibody. In this cell, partial vacuolization of the Golgi is visible. In the same cell the concentration of the N protein (Fig. 6d) is restricted to the vacuolized regions. More extensive vacuolization of the Golgi region has occurred in the cell shown in Fig. 6e, and also in this cell, the concentration of the N protein was restricted to the vacuolized regions. In cells in which the infection progressed further, complete vacuolization of the Golgi region is seen (Fig. 6g), and the accumulation of the N protein occurs throughout the vacuolized Golgi (Fig. 6h). Thus, it appears that accumulation of N protein in the Golgi region, an event likely to correlate with virus maturation, is intimately connected with the progressive vacuolization of the Golgi region.

Double-immunofluorescence microscopy of tunicamycin-treated cells. We have previously shown that all glycans on G1 and G2 are asparagine linked (27). To study the effect of inhibition of glycosylation of G1 and G2 on protein transport and virus maturation, Uukuniemi virus-infected chicken embryo cells were treated with tunicamycin, a known inhibitor of N-linked glycosylation (35, 36). The drug was added at 3 h after infection, and the cells were fixed and permeabilized at 9 h. When the cells were stained for G1 (Fig. 7a) or G2 (Fig. 7c) with monoclonal antibodies which recognize the glycoproteins apparently in all cellular locations or with polyclonal anti-G1-G2, no labeling of the Golgi region was observed. Instead, G1 and G2 were found to aggregate intracellularly throughout the perinuclear region (Fig. 7a and c). Double-staining with polyclonal anti-N serum showed that the nucleoprotein accumulated in the same regions. The nuclear membrane was also clearly decorated by the anti-N antibodies (Fig. 7b and d), a situation not seen in the absence of tunicamycin (see, e.g., Fig. 6). In addition, double staining of tunicamycin-treated Uukuniemi virus-infected BHK-21 cells with a monoclonal antibody that specifically stains the Golgi complex (3) (Fig. 7f) and with anti-G1-G2 polyclonal antiserum (Fig. 7e) also showed that the glycoproteins were not localized in the Golgi area. We therefore conclude that the glycoproteins aggregated and apparently remained in the ER in the absence of glycosylation and that the nucleoprotein could recognize the aggregated glycoproteins at this intracellular site.

DISCUSSION

Here we have studied the maturation of Uukuniemi virus by using monoclonal antibodies against the glycoproteins G1 and G2 together with polyclonal antibodies against the membrane glycoproteins and the cytoplasmic nucleoprotein in a double-immunofluorescence technique. Our previous studies have shown that the intracellular maturation of Uukuniemi virus involves the accumulation of virus glycoproteins and nucleoprotein in the Golgi membranes of infected cells (13). The present approach enabled us to localize the glycoproteins and the N protein in the Golgi region of individual infected cells.

To prepare monoclonal antibodies, a highly purified glycoprotein preparation (rosettes) (10, 13) was used to immunize the mice. This gave a high frequency of hybridomas producing glycoprotein-specific antibodies. Out of the final 26 monoclonal antibodies tested, 1 was negative, 10 were G1-specific, and 15 were G2-specific. Cross-reactivity with cellular antigens was observed with two of the antibodies: UG1-18 also immunoprecipitated some cellular proteins, and UG1-20 gave a staining of small vesicles in mock-infected cells in indirect immunofluorescence. That at least the majority of the monoclonal antibodies were directed against the protein moiety of G1 or G2 was supported by immunoprecipitation results showing that several of them reacted with nonglycosylated G1 and G2 which had been synthe-

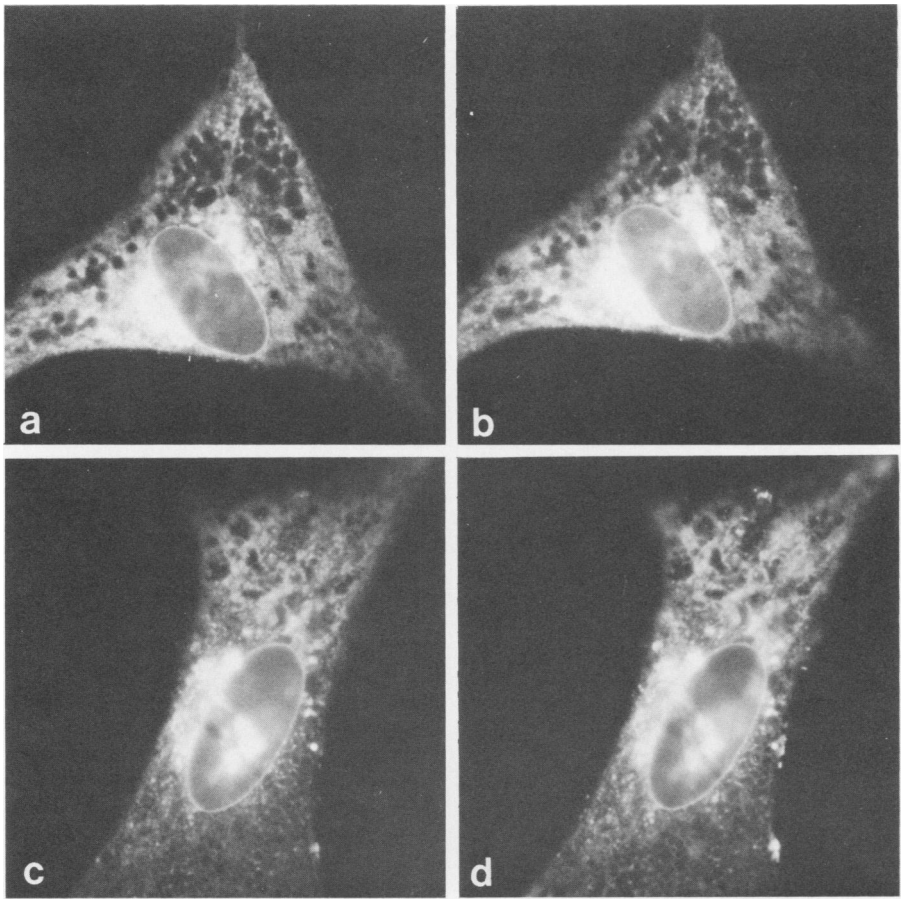

FIG. 3. Double-immunofluorescence staining of Uukuniemi virus-infected cells with monoclonal antibodies UG1-22 and UG2-17 and with anti-glycoprotein serum. Infected chicken embryo cells were indirectly stained at 10 h postinfection with monoclonal antibodies UG1-22 (a) or UG2-17 (c) by using tetramethyl rhodamine isothiocyanate-conjugated goat anti-mouse IgG. The cells were double stained with rabbit polyclonal anti-G1-G2 serum (b and d) by using fluorescein isothiocyanate-conjugated swine anti-rabbit IgG. Both monoclonal antibodies gave a distribution of fluorescence indistinguishable from that obtained with the polyclonal antiserum.

sized in the presence of tunicamycin (data not shown). UG2-16 and UG1-19, which preferentially stained the glycoproteins in the Golgi complex, and UG1-24 and UG1-26, which stained G1 at the cell surface, did not detect the nonglycosylated forms of the proteins in immunofluorescence, whereas all four antibodies precipitated the nonglycosylated forms from cell lysates (data not shown). Apparently, the proteins have different conformations under the immunofluorescence and immunoprecipitation conditions used, resulting in exposure of different epitopes and thus different reactivities with the monoclonal antibodies, as has been shown for rhodopsin (24).

Interestingly, the immunofluorescence studies showed that in the case of different monoclonal antibodies the staining pattern obtained was markedly dissimilar and in most cases, as shown by double-staining experiments, also differed from the staining seen with the polyclonal anti-G1-G2 antibodies. When the staining of the Golgi complex and the cell surface was examined, the fluorescence in one or two of the compartments was weak or lacking with most of the monoclonal antibodies. Monoclonal antibodies UG1-8, UG2-9, UG2-16, and UG1-19 showed preferential intracellular staining of the Golgi complex. In addition, UG2-16 and UG1-19 but not UG2-9 also stained the glycoproteins at the plasma membrane. Surface labeling was observed with 10 of the monoclonal antibodies. Interestingly, two of the surface-positive, G1-specific monoclonal antibodies, UG1-24 and UG1-26, which intracellularly gave negligible staining of the ER and the Golgi complex, heavily stained a population of large vacuoles located near the Golgi region or at the cell periphery. The surface labeling obtained with these antibodies was patchy or dotted in appearance (13). It is possible that these monoclonal antibodies specifically recognize mature virus particles in intracellular vacuoles and at the cell surface.

Recent studies with membrane and cytoplasmic proteins have indicated that the reactivity of a monoclonal antibody with the protein may depend on change in the functional state (24), covalent modification (33), or complex formation (20) of the protein. Our observation that different monoclonal antibodies preferentially stained the Uukuniemi virus G1 or G2 in different cellular locations must be an indication of structural changes or conformational changes or both in the glycoproteins, which occur during intracellular transport and virus maturation (11). Different antigenic determinants may be exposed and thus be accessible to different monoclonal antibodies. Membrane and secretory proteins have been described to undergo a number of maturation steps during their transport from the site of synthesis to the final destination (8, 12, 19, 34). These include covalent modifications

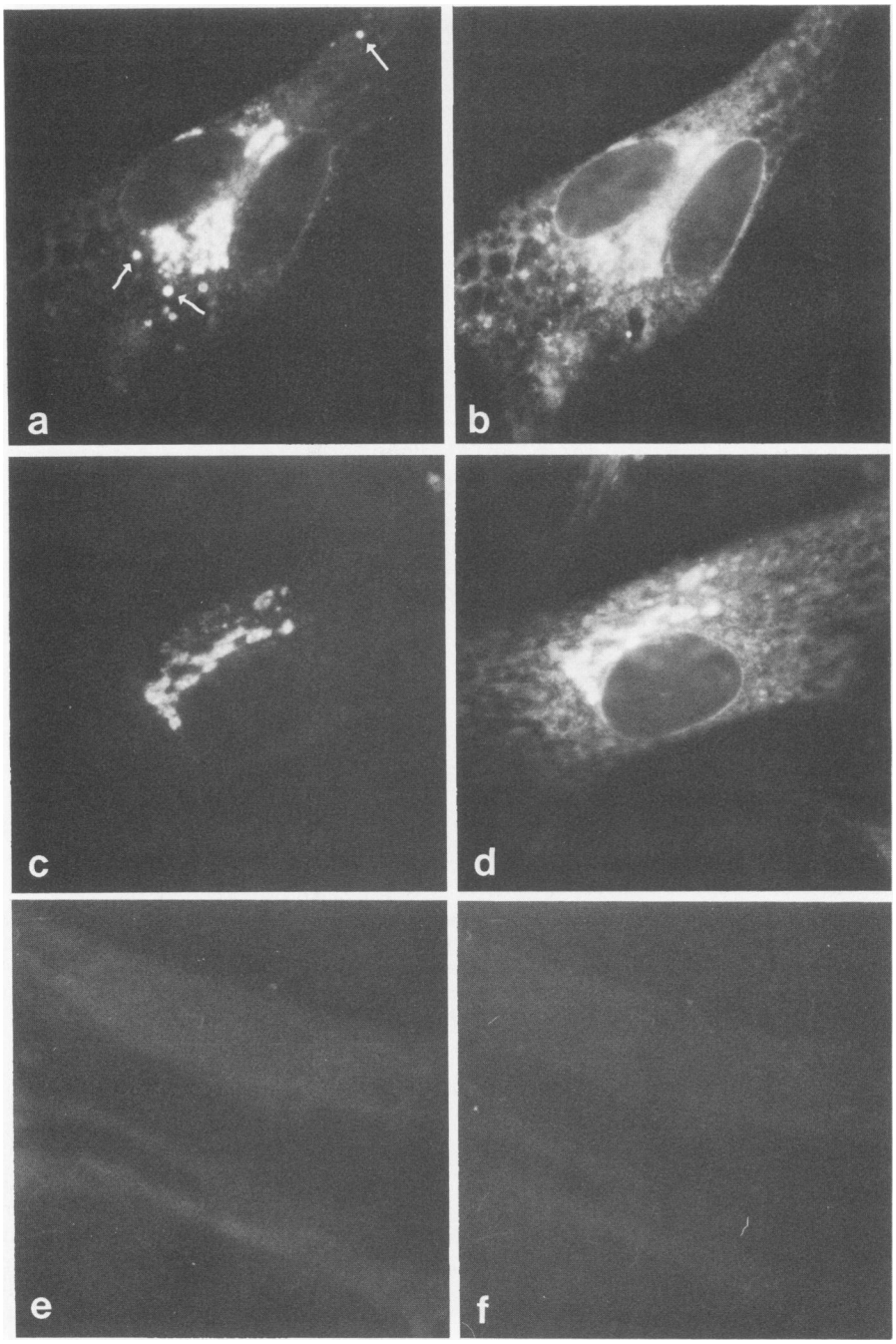

FIG. 4. Double immunofluorescence of Uukuniemi virus-infected cells demonstrating preferential staining of G1 and G2 in the Golgi region. Infected cells were stained as in Fig. 3 with monoclonal antibodies UG1-19 (a) or UG2-16 (c) and polyclonal anti-glycoprotein serum (b and d). As controls, mock-infected cells were similarly stained with monoclonal and polyclonal antibodies. Shown in (e) and (f) are examples of such stainings, in this case, with UG2-16 and anti-G1-G2 polyclonal antiserum, respectively. Arrows indicate intensely stained vesicles.

(e.g., glycosylation, acylation, and sulfation) (12, 39), as well as noncovalent interactions such as complex formation (42). All of these could result in changes in the tertiary structure and thus result in the exposure of different epitopes. In addition, changes in the physicochemical milieu (e.g., pH and ionic conditions) may result in conformational changes. Low-pH-induced conformational changes of influenza virus hemagglutinin have, for example, recently been studied by using differential binding of monoclonal antibodies (41).

Since tens of structurally and serologically different bunyaviruses grown in a variety of cells have been studied by electron microscopy and all have been found to mature in the Golgi region (1, 26), this mode of maturation is a unique property of the bunyaviruses. The site of maturation of other viruses is different. Coronaviruses appear to mature in some region of the ER (23), whereas herpesviruses mature at the nuclear membrane (4). Most enveloped viruses, however, mature at the plasma membrane where a sufficient concen-

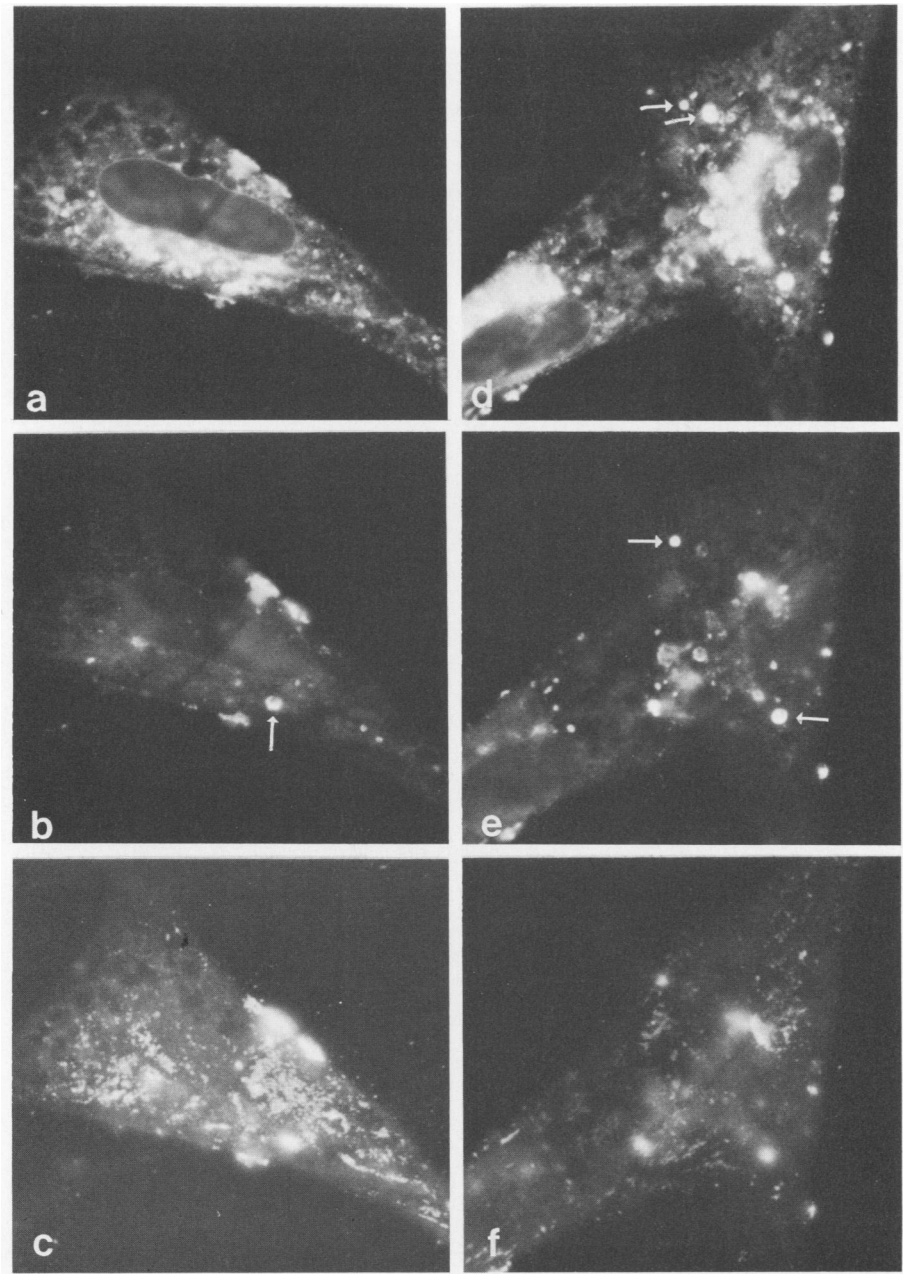

FIG. 5. Double immunofluorescence of Uukuniemi virus-infected cells with monoclonal antibodies UG1-24 and UG1-26. Infected cells were stained as in Fig. 3 with monoclonal antibodies UG1-24 (b and c) or UG1-26 (e and f) and polyclonal anti-glycoprotein serum (a and d). The cells were focused to show intracellular fluorescence (b and e), or fluorescence on the ventral cell surface (c and f). Arrows in (b) and (e) indicate intensely stained vesicles.

tration of the glycoproteins is probably a prerequisite for virus budding (32). The reason bunyaviruses bud through membranes in the Golgi region is still unknown. An important question is whether merely a critical concentration of the glycoproteins determines the site of maturation. For the Uukuniemi virus nucleocapsid to be able to bind to Golgi membranes, the concentration of the glycoproteins must apparently first reach a critical level. In the presence of tunicamycin, when N-linked glycosylation is inhibited (35, 36), the viral glycoproteins are apparently retained and become aggregated in the rough ER, similarly to the G protein in tunicamycin-treated vesicular stomatitis virus-infected and Sindbis virus-infected cells (7, 16, 17). Under these conditions, the Uukuniemi virus nucleoprotein was associated with the aggregated glycoproteins.

A progressive expansion and vacuolization of the Golgi is observed during Uukuniemi virus infection. The reason for the vacuolization of the Golgi is not known, but it is possible that the accumulation of the glycoproteins (13, 22) in the Golgi is related to the changed morphology. It is also possible that virus budding itself causes the morphological changes. The monoclonal antibodies which preferentially stained the virus glycoproteins in the Golgi region were found to be useful for studying morphological changes of the

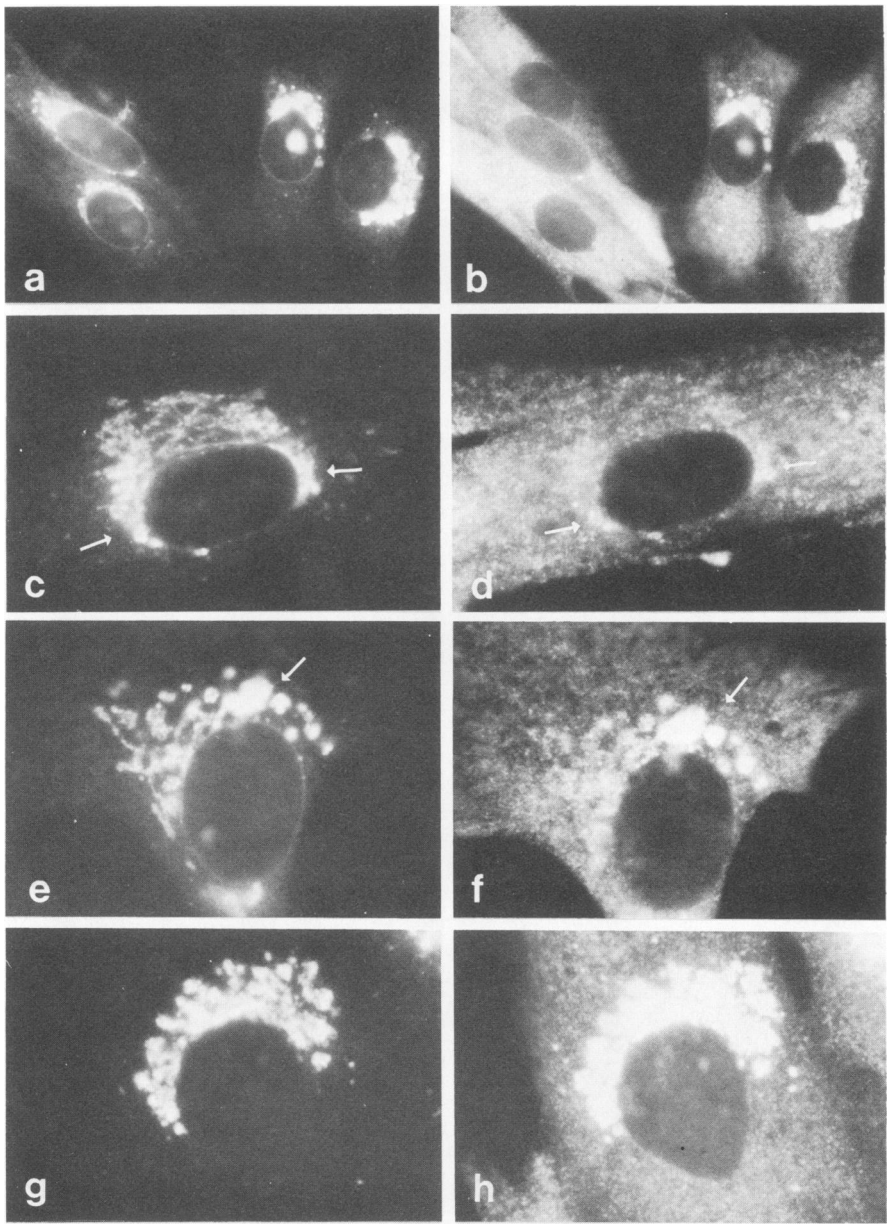

FIG. 6. Correlation between changes in the morphology of the Golgi complex and accumulation of the nucleoprotein into the Golgi. Uukuniemi virus-infected BHK-21 cells were stained with monoclonal antibodies UG2-16 (a) or UG1-19 (c, e, and g) and double-stained with rabbit polyclonal antibodies against the nucleoprotein (b, d, f, and h). Different stages of nucleoprotein association with the Golgi complex are shown. The nucleoprotein accumulated only in Golgi regions which have vacuolized (arrows).

Golgi complex, as the ER background was absent. The nucleoprotein was localized in the same cells by using a polyclonal anti-N serum. A constant finding was that the nucleoprotein associated with the Golgi membranes only in the vacuolized regions of the Golgi.

It is clear that the nucleoprotein, synthesized in the soluble fraction of the cytoplasm, must have access to the glycoproteins accumulated in intracellular membranes. In the case of tunicamycin treatment-induced accumulation, the nucleoprotein appears to have free access to the ER membranes, whereas the situation with Golgi membranes may be different. Based on electron microscopy observations, it has been suggested that the Golgi region has a tighter cytoplasmic organization, as compared with other regions of the cytoplasm, and that it contains dense granular-fibrillar material (25). This may explain why certain cytoplasmic entities, such as ribosomes, are scarce or absent in this zone of exclusion (25). Recently, it has been shown that if cells infected with Semliki Forest virus, a virus which matures at the plasma membrane, are treated with monensin, the Golgi cisternae are vacuolized and the viral glycoproteins accumulate in these Golgi-derived membranes (9, 13). Under these conditions, viral nucleocapsids abnormally bind to the intracellular membranes where the glycoproteins accumulate.

Thus, inherent in the strategy of maturation of Uukuniemi virus and bunyaviruses in general may be, in addition to accumulation of glycoproteins in the Golgi complex, the opening up of Golgi structures to give nucleocapsids free

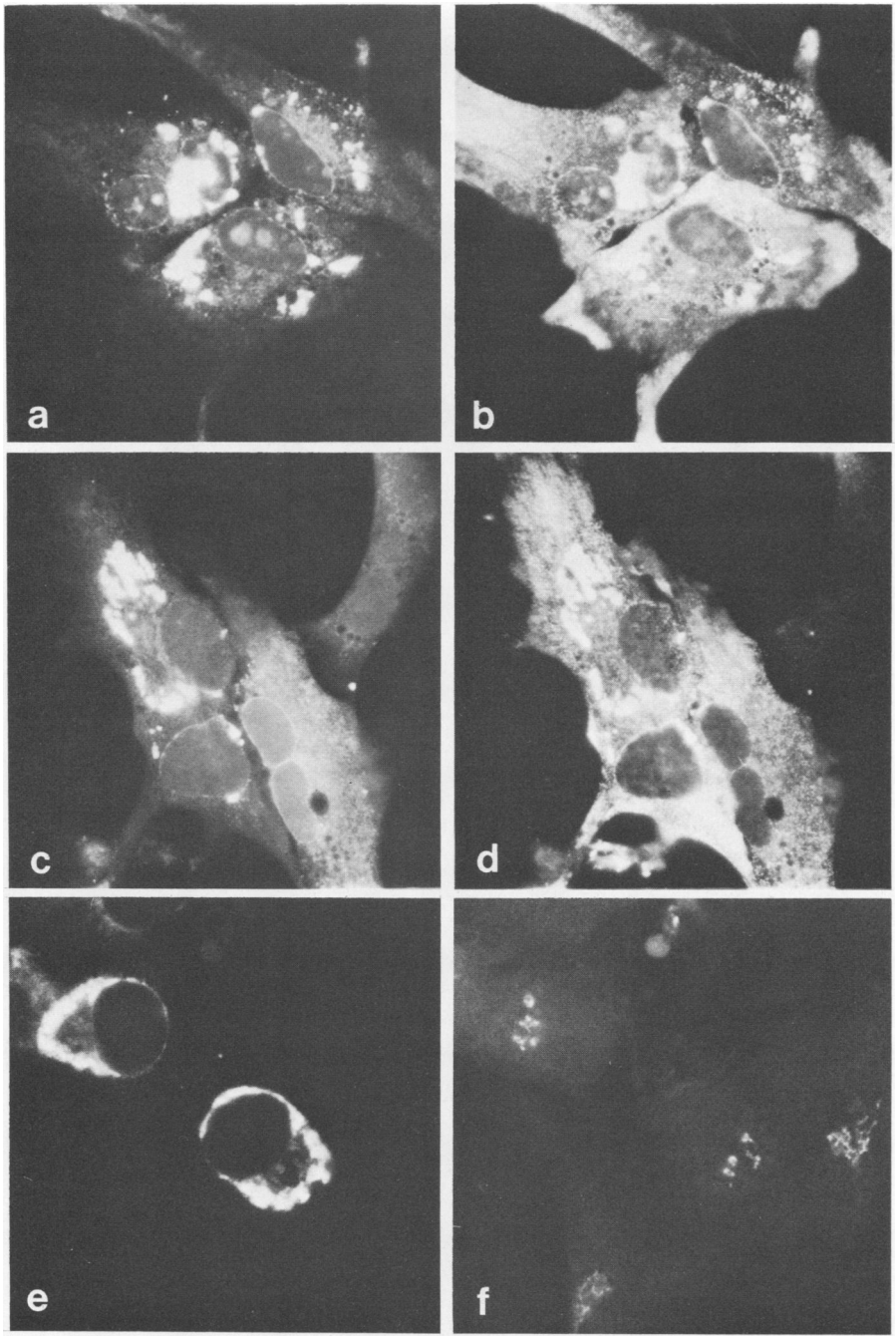

FIG. 7. Uukuniemi virus-infected cells grown in the presence of tunicamycin and double stained for G1 or G2 and for nucleoprotein. Infected chicken embryo cells were grown in the presence of tunicamycin (2 μg/ml). Cells were stained at 10 h postinfection with UG1-22 (a) or UG2-17 (c) and double stained with rabbit polyclonal anti-N antiserum (b and d). Staining of tunicamycin-treated, infected BHK-21 cells with anti-G1-G2 antiserum is shown in (e) and with a monoclonal antiserum directed against a Golgi-specific protein (3) is shown in (f).

access to the site of virus maturation. This still leaves us with the important question: what causes the glycoproteins to accumulate in the Golgi complex instead of at the plasma membrane?

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