

## Effect of Monensin on the Assembly of Uukuniemi Virus in the Golgi Complex

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The effect of the carboxylic ionophore monensin on the maturation of Uukuniemi virus, a bunyavirus, and the transport of its two membrane glycoproteins, G1 and G2, were studied in chicken embryo fibroblasts and baby hamster kidney cells. Virus maturation, which occurs in the Golgi complex (E. Kuismanen, K. Hedman, J. Saraste, and R. F. Pettersson, *Mol. Cell. Biol.* 2:1444-1458, 1982; E. Kuismanen, B. Bång, M. Hurme, and R. F. Pettersson, *J. Virol.* 51:137-146, 1984), was effectively inhibited by the drug (1 or 10  $\mu$ M) as studied by electron microscopy and by assaying the release of infectious or radiolabeled virus. Immunoelectron microscopy showed that association of viral nucleocapsids with the cytoplasmic surface of glycoprotein-containing Golgi membranes, a prerequisite for virus budding, was unaffected by monensin. In the presence of the drug, the virus glycoproteins assembled into long, tubular structures extending into the lumen of Golgi-derived vacuoles, suggesting that monensin inhibited a terminal step in the assembly of the virus. Intracellular transport and expression of the virus membrane glycoproteins G1 and G2 at the cell surface were not inhibited by monensin as studied by immunocytochemical and radiolabeling techniques. Pulse-chase experiments in the presence of monensin showed that intracellular G1 acquired only partially endo-H-resistant glycans. The sialylation of G1 appearing on the cell surface in the presence of the drug was decreased, whereas sialylation of G2 apparently was inhibited to a lesser extent, as shown by external labeling of the cells with the periodate-borof<sup>3</sup>H]hydride method. Thus, monensin exerted a differential effect on the terminal glycosylation of G1 and G2. Unlike several membrane and secretory glycoproteins, both G1 and G2 could enter a functional transport pathway in the presence of monensin and become expressed at the cell surface.

Many of the events involved in the biosynthesis of integral membrane proteins in eucaryotic cells have been characterized by using viral envelope glycoproteins as models (21, 23, 28, 45). The best-studied glycoproteins include the G protein of vesicular stomatitis virus (a rhabdovirus) (46, 59), the E1 and E2 glycoproteins of the two alphaviruses Sindbis and Semliki Forest virus (11, 22), and the hemagglutinin of influenza virus (orthomyxovirus) (23). Common to these viruses is the site of maturation at the plasma membrane. The viral glycoproteins are synthesized in the endoplasmic reticulum (ER) and are then transported via the Golgi complex to the cell surface, where virus assembly takes place. Attention has lately been drawn also to other viruses which mature exclusively at cellular endomembranes, e.g., at the nuclear membrane (herpesviruses) (8), or at the smooth or rough membranes of the ER (coronaviruses) (32, 48, 53). Members of the large Bunyaviridae family appear to mature exclusively at the membranes of the Golgi complex (5, 26, 29, 33).

To understand the processes which determine the budding site of bunyaviruses, we studied the maturation of Uukuniemi virus, a member of the Bunyaviridae family. The lipoprotein envelope of Uukuniemi virus contains two membrane glycoproteins, G1 ( $M_r$ , about 70,000) and G2 ( $M_r$ , about 65,000) (56), to which three types of *N*-glycosidic oligosaccharide are attached (36). All of the G1-bound glycans are resistant to endo-H, whereas G2 also contains high-mannose oligosaccharides which are sensitive to this enzyme (49). In the viral membrane, the glycoproteins are complexed to form hollow, cylindrical morphological units which are organized in an icosahedral surface lattice ( $T = 12$ ,

$P = 3$ ) (56). The internal helical nucleocapsid consists of three single-stranded RNA segments, the major nucleocapsid protein, N protein ( $M_r$ , 25,000), and a minor component L protein ( $M_r$ , about 200,000), the putative RNA polymerase of the virus (38, 56).

It has been shown by electron microscopy that the final step in the morphogenesis of bunyaviruses takes place at smooth membranes in the perinuclear region (33). Recently, by applying immunocytochemical techniques, we showed that the budding of Uukuniemi virus occurs at the membranes of the Golgi complex (26). One factor determining the site of maturation of the virus is probably the property of the glycoproteins to accumulate in the Golgi membranes. This accumulation apparently results in the dilation and vacuolization of the Golgi cisternae, and in parallel with this process the nucleocapsids also concentrate in the Golgi region (25). At present, it is not known where in the Golgi complex budding occurs, but since the terminal glycosylation of the glycoproteins and the incorporation of newly synthesized glycoproteins into virions occur with similar kinetics, it is possible that the virus matures at a functionally distal location in the Golgi complex (24). The release of the virus particles from the cells is thought to occur through vesicular carriers which fuse with the plasma membrane.

Although the glycoproteins accumulate in the Golgi complex, a small fraction is also transported to the cell surface as judged by immunofluorescence (26). Monensin, a carboxylic ionophore which affects the Golgi complex, has been shown to efficiently block the transport of membrane and secretory glycoproteins from the Golgi complex to the plasma membrane (50-52, 54). We studied the effects of monensin on the maturation of Uukuniemi virus and the transport of virus glycoproteins. The results indicate that the drug affects the

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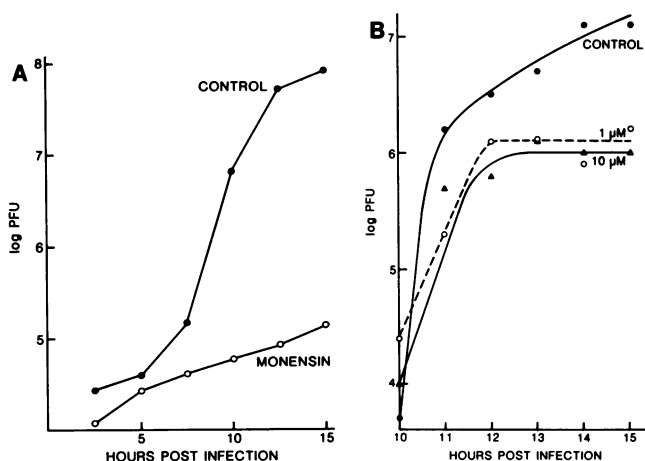


FIG. 1. Production of infectious virus particles in CEF is inhibited by monensin. Samples from control and monensin-treated cultures were taken at various times after infection and assayed for infectivity. (A) Monensin was added to infected cells at 2.5 h after infection. (B) The effect of monensin on virus yield when the drug was added at 10 h after infection.

assembly process of Uukuniemi virus in the Golgi complex. The transport of virus glycoproteins to the plasma membrane is, however, not inhibited by monensin.

#### MATERIALS AND METHODS

**Cells and virus.** The origin and cultivation of BHK-21 cells and chicken embryo fibroblasts (CEF) and the preparation of stock virus from the prototype strain S-23 of Uukuniemi virus after several successive plaque purifications have been described previously (9, 55). 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.

**Surface radioimmunoassay.** Expression of viral membrane proteins at the cell surface was measured by a radiometric method described in detail previously (20). In the assay, a rabbit antiserum specific for Uukuniemi virus membrane proteins (26) was used at a dilution of 1:200.

**Radioactive labeling of cells.** Confluent monolayers of CEF or BHK-21 cells grown on 32-mm petri dishes were infected with Uukuniemi virus. The cells were pulse-labeled at 8 h after infection with 100 to 500  $\mu$ Ci of [ $^{35}$ S]methionine (Amersham Corp., Arlington Heights, Ill.; 1,160 to 1,500 Ci/mmol) per ml and chased in the presence of an excess of unlabeled methionine. Surface labeling of the cells was carried out by the periodate- $\text{NaB}^3\text{H}_4$  technique at 15 h after infection, as described in detail previously (10). After labeling, the cells were lysed 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 apoprotinin per ml [Trasyol; Bayer, Leverkusen, Federal Republic of Germany]), as described previously (24). Immunoprecipitation was carried out in 1.5-ml tubes by mixing 10 to 50  $\mu$ l of lysate, 1.0 ml of NET buffer and 5 to 10  $\mu$ l of monoclonal antibody (mixture of three G1- or three G2-specific antibodies [24, 25]) or polyclonal anti-G1, anti-G2 serum. After incubation for 60 min at 25°C, 100 to 200  $\mu$ l 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). Endo-H (Seikakagu) treatment of the samples was carried out as described previously (24, 44, 49) by an enzyme concentration of 20 mU/ml. Finally, the samples were analyzed on 10% linear or 7.5 to 15% gradient polyacrylamide gels, as described by Laemmli (27). Fluorography was carried out as described by Bonner and Laskey (6).

**Immunocytochemistry.** For immunofluorescence microscopy, infected cells fixed with 3% paraformaldehyde were stained with G1- and G2-specific antibodies and swine anti-rabbit immunoglobulin G conjugated to tetramethylrhodamine isothiocyanate (Dako, Copenhagen, Denmark), as described earlier (26). Photographs were taken with Agfapan 400 professional film. A Reichert-Jung Polyvar microscope equipped with Nomarski optics and filters was used to detect rhodamine fluorescence. The immunoperoxidase method of electron microscopy (16, 26), in which diffusion of the peroxidase reaction product was inhibited by carrying out the enzyme reaction on ice for cells embedded in 2% gelatin, has been described in detail before (43). Horizontally cut thin sections were examined in a JEOL 100CX II electron microscope operated at 60 kV with small objective apertures.

#### RESULTS

**Monensin inhibits virus production.** The growth of Uukuniemi virus is rather slow. Extracellular virus is first detected about 5 h after infection (37), and the exponential growth phase is over by 16 to 18 h. When monensin (10  $\mu$ M) was added to infected CEF at 2.5 h after infection, very little infectious virus was released from the cells (Fig. 1A), although more than 90% of the cells had become infected as judged by immunofluorescence. The difference between the infectivity yields obtained at 15 h from monensin-treated and untreated cultures was about 1,000-fold. In another experiment, infected cells were treated with monensin (1 or 10  $\mu$ M) starting at 10 h after infection. In this case, inhibition of virus release became apparent after about 1.5 h (Fig. 1B). The reduction in infectivity yield was about 10-fold when assayed 5 h after the addition of monensin. Both concentrations of monensin inhibited virus release to a similar extent. These results indicate that the release of infectious virus is inhibited by monensin. In an experiment in which monensin (1  $\mu$ M present from 2.5 to 17 h postinfection) inhibited the release of infectious virus by more than 99%, the production of cell-associated virus was inhibited by only about 90%. This suggests that some infectious virus was formed in the presence of the drug but was not released from the cell. Whether this infectivity corresponds to the aberrant filamentous forms made in the presence of monensin (see below) or to authentic particles is not known.

To analyze whether noninfectious particles were produced in the presence of monensin, we pulse-labeled infected CEF or BHK-21 cells with [ $^{35}$ S]methionine at 8 h after infection for 10 min; this was followed by a 3-h chase in the presence or absence of the drug (1 or 10  $\mu$ M). Extracellular virus was collected, purified, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (24). Production of labeled virus was inhibited by more than 95% with both concentrations of monensin (data not shown).

We have previously shown that newly synthesized G1 is incorporated into virus particles with faster kinetics than G2 (24). When monensin was added to virus-infected cells 20 min after the 10-min pulse, no labeled glycoproteins were incorporated into extracellular virions during the following 3-h chase period, although labeled N protein, which appears

rapidly in released virions, was present (Fig. 2, lane a). When the drug was added 45 min after the pulse, only labeled G1 was readily incorporated into released virions, whereas labeled G2 was almost undetectable (lane b). Labeled N protein was incorporated readily in both cases. These results suggest that monensin inhibits the formation of virus particles but does not affect the release of those viruses which were formed before addition of the drug.

**Effect of monensin on glycosylation of G1 and G2.** To analyze the effect of monensin on the glycosylation of G1 and G2, we pulse-labeled the viral glycoproteins for 10 min with [<sup>35</sup>S]methionine; this was followed by a 0-, 60-, 120-, or 180-min chase in the presence of monensin. The proteins were immunoprecipitated with G1- or G2-specific monoclonal antibodies, the precipitates were treated with endo-H, and the proteins, with untreated controls, were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. As shown previously (24), the bulk of G1 in untreated cells acquired endo-H-resistant glycans during a 120-min chase (Fig. 3A). Less than 50% of total cellular G1 acquired endo-H-resistant glycans in the presence of monensin (Fig. 3B). The degree of glycosylation of G2 was also affected by monensin (Fig. 3D). However, in contrast to G1, as shown below, a portion of G2 acquired sialylated oligosaccharides even in the presence of monensin (see Fig. 6).

**G1 and G2 transport to the cell surface in the presence of monensin.** By using a polyclonal antiserum specific for the glycoproteins (26), we analyzed the appearance of the viral glycoproteins at the cell surface of infected control and monensin-treated cells by radioimmunoassay. The glycoproteins were transported to the plasma membrane in the presence of the drug with about the same kinetics and efficiency as in untreated cells (Fig. 4). Very little radioactivity was bound to uninfected cells treated with G1- and G2-specific antibodies. By immunofluorescence microscopy, positive staining of the cell surface was observed in both untreated (Fig. 5a) and monensin-treated (Fig. 5c) cells. In untreated cells, typical patchy surface staining and weak, uniform staining of the plasma membrane (26) were seen. In monensin-treated cells, the uniform staining appeared stronger, whereas the patchy staining pattern was not as marked.

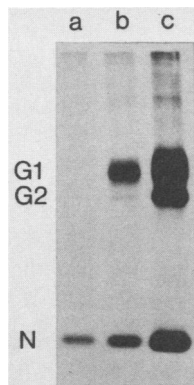


FIG. 2. CEF were pulse-labeled for 5 min, and monensin (10 μM) was added at 20 (lane a) or 45 min (lane b) after the pulse. The untreated control is shown in lane c. The culture medium was harvested at 3 h after the pulse, and secreted virus was purified and analyzed in a polyacrylamide gel (24). The experiment demonstrates clearly the different incorporation kinetics for the N, G1, and G2 virus proteins.

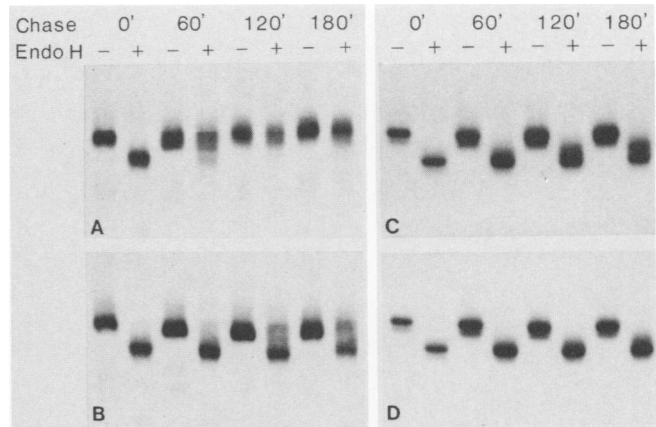


FIG. 3. Effects of monensin on the glycosylation of Uukuniemi virus glycoproteins G1 and G2. After a 10-min pulse of [<sup>35</sup>S]methionine followed by a 0-, 60-, 120-, or 180-min chase in the absence (A, C) or presence (B, D) of monensin (10 μM), the glycosylation stage of G1 and G2 was analyzed by using endo-H sensitivity as a marker. Immunoprecipitation of the glycoproteins was carried out with G1- (A and B) or G2-specific (C and D) monoclonal antibodies. More than 50% of G1 remained sensitive to the enzyme in the presence of monensin (B). G2 also showed a change in glycosylation pattern (compare 180-min lanes in C and D).

Monensin-treated and untreated infected cells were also labeled with borol[<sup>3</sup>H]hydride after periodate oxidation. This procedure labels terminally modified glycans containing sialic acid residues exclusively at the cell surface (10). Glycoproteins were allowed to accumulate at the cell surface for 15 h in the presence of monensin. Both G1 and G2 were readily labeled in control cells (Fig. 6). In monensin-treated cells, only G2 was clearly labeled, whereas labeling of G1 was greatly reduced.

To study whether both glycoproteins reach the cell surface, we pulse-labeled virus-infected cells for 30 min and then subjected them to a 2-h chase in the presence or absence of monensin. Immunoprecipitation of cell surface-associated glycoproteins with polyclonal G1- and G2-specific

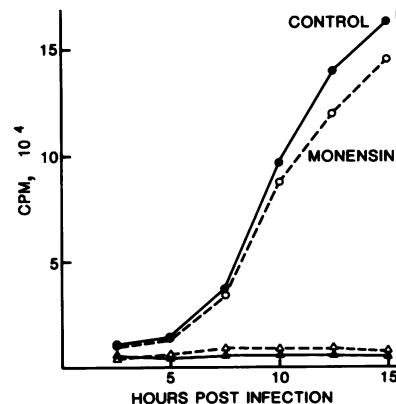


FIG. 4. Expression of Uukuniemi virus glycoproteins G1 and G2 at the surface of untreated (control) and monensin-treated BHK-21 cells as studied by surface radioimmunoassay. Monensin (10 μM) added at 2.5 h after infection did not inhibit transport of the glycoproteins to the cell surface. Negligible radioactivity was bound to monensin-treated or untreated mock-infected cells (▲ and △, respectively). The background binding observed was not subtracted from the recorded values.

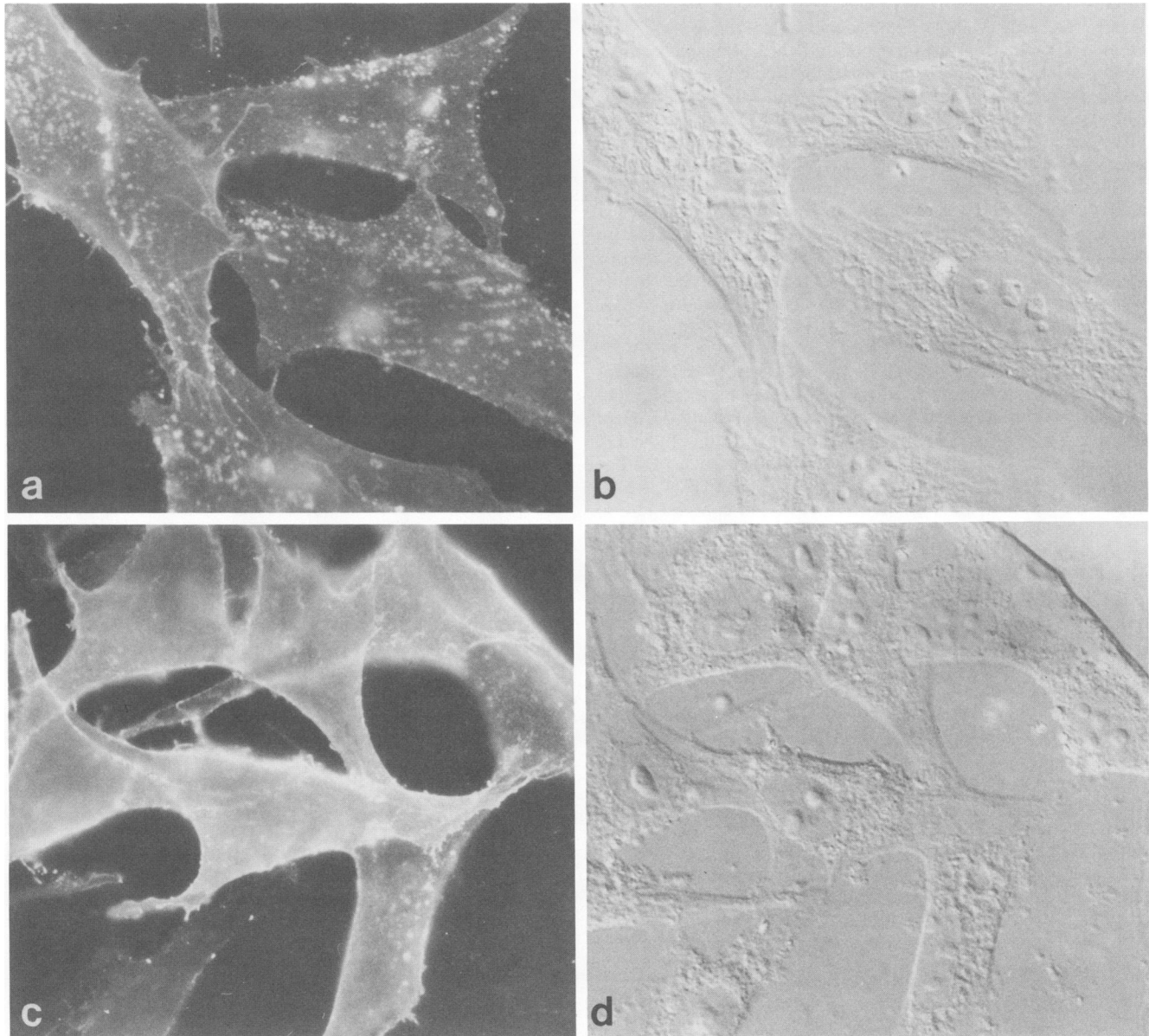


FIG. 5. Surface expression of Uukuniemi virus G1 and G2 glycoproteins in control (a) and monensin-treated (c) BHK-21 cells as studied by indirect immunofluorescence. Virus-infected cells were fixed with 3% paraformaldehyde and stained with G1- and G2-specific antiserum followed by anti-rabbit immunoglobulin G conjugated with tetramethylrhodamine isothiocyanate. Monensin (10  $\mu$ M) was added at 2.5 h after infection. Interference contrast micrographs of the cells are shown in panels b and d. Note the difference in the surface staining of monensin-treated (c) and control (a) cells.

antiserum showed that both G1 and G2 were expressed at the plasma membrane in both control and monensin-treated cells (Fig. 7). The G1 band was sharper in monensin-treated cells compared with control cells, suggesting a difference in glycosylation (24). In contrast to untreated cells, in which the bulk of the surface-associated G1 was resistant to endo-H treatment, in monensin-treated cells more than half of the G1 at the plasma membrane remained sensitive to the enzyme (Fig. 7, arrow). Immunofluorescence of non-permeabilized cells with monoclonal antibodies also confirmed that both glycoproteins were expressed on the plasma membrane in the absence and presence of the drug (data not shown).

Taken together, our results indicate that Uukuniemi virus glycoproteins are transported to the cell surface in the presence of monensin, but their glycosylation is differentially affected by the drug.

**Immunoelectron microscopy of control and monensin-treated cells.** The intracellular localization of Uukuniemi virus membrane glycoproteins G1 and G2 and cytoplasmic nucleocapsid protein N in BHK-21 cells fixed at midcycle (10 h) of infection is demonstrated in Fig. 8. To improve the resolution of the immunoperoxidase method used previously in electron microscopy (16, 26), we embedded saponin-permeabilized cells in gelatin after treating them with specific antibodies and protein A-peroxidase conjugate and

carried out the enzyme reaction at 0°C (43). At 10 h after infection, the Golgi complex displayed a vacuolized structure in most of the infected cells (25, 26; Fig. 8A and D). Only in a minority of the cells could the stacked organization of the Golgi complex still be seen (Fig. 8B and E). In addition to the network of ER membranes, G1- and G2-specific antibodies also labeled numerous (about 100 nm) virus particles present in the lumen of stacked or dilated Golgi membranes and Golgi-derived vacuoles (Fig. 8A and B). Immunoperoxidase labeling also indicated the presence of large amounts of glycoproteins in the limiting membranes of these virus-containing Golgi elements. Stained, virus-containing vacuoles were also located peripherally in close proximity to the plasma membrane (Fig. 8C). Interestingly, the improved immunocytochemical method allowed the detection of the virus membrane proteins in numerous small (60 to 80 nm) vesicles, which were typically found in the vicinity of virus-containing Golgi elements (Fig. 8A and B, arrows).

According to immunofluorescence data, most infected cells show a strong concentration of N protein in the juxtannuclear Golgi region at midcycle of infection (25, 26). Figure 8D and E demonstrate immunoperoxidase labeling of the Golgi complex in cells harvested at 10 h after infection, after labeling of the cells with N protein-specific antibodies. In the extensively vacuolized Golgi complex (Fig. 8D), the peroxidase reaction product delineates the cytoplasmic surface of virus-containing large vacuoles. Strong labeling is also seen in the cytoplasm close to the Golgi membranes, especially at areas containing numerous small vesicles of the Golgi region (Fig. 8D). According to immunoperoxidase

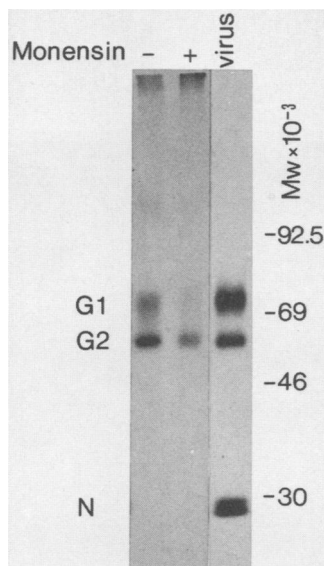


FIG. 6. External radioactive labeling of surface-associated virus glycoproteins in untreated and monensin-treated BHK-21 cells. Uukuniemi virus-infected control cells and cells treated with monensin (10  $\mu$ M) starting at 2.5 h after infection were surface labeled at 15 h postinfection by the periodate-boro[ $^3$ H]hydride method. The glycoproteins were immunoprecipitated with G1- and G2-specific antibodies and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Purified [ $^{35}$ S]methionine-labeled virus was used as a marker. In control cells, both G1 and G2 were clearly labeled, whereas in monensin-treated cells the labeling of G1 was almost completely inhibited. N, N protein. Symbols: +, monensin treated; -, control.

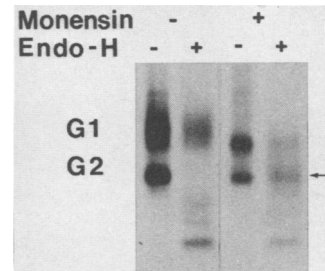


FIG. 7. Expression of G1 and G2 on the cell surface. Cell surface-associated glycoproteins pulse-labeled for 30 min with [ $^{35}$ S]methionine and chased for 2 h were isolated by antibody adsorption with G1- and G2-specific antiserum. In the presence of monensin, both G1 and G2 could be chased to the plasma membrane. Note that in the presence of the drug, most plasma membrane-associated G1 was sensitive to endo-H (arrow), but it was resistant to endo-H in untreated cells. Symbols: +, treated cells; -, control cells.

results, N protein also associated with virus-containing vacuoles located in the cell periphery. Figure 8E shows the distribution of the nucleocapsid protein in the Golgi region in a cell in which the Golgi membranes, although dilated, have partially retained their stacked structure. In such cases, the strongest peroxidase labeling was seen at the cytoplasmic surface of virus-containing Golgi cisternae and vacuoles at one aspect of the Golgi complex. Occasional labeling of the ER and the nuclear membrane was observed with the N protein-specific antibodies. No label was observed in association with the other endomembranes or the plasma membrane.

In monensin-treated cells, typical morphological effects, including extensive vacuolization of Golgi membranes (50), were observed. Figure 9 shows the intracellular distribution of the virus glycoproteins and the cytoplasmic N protein in cells fixed at 10 h after infection, 8 h after addition of the drug. Monensin resulted in the formation of aberrant tubular structures which could be stained with anti-glycoprotein antibodies (Fig. 9A). Only a few virus particles could be seen in the lumen of large monensin-induced Golgi vacuoles (Fig. 9C and D). It is likely that many of these viruslike structures may in fact represent cross sections of the tubular forms. Antiglycoprotein antibodies labeled the limiting membranes of a number of the large vacuoles but, interestingly, unreactive vacuoles were also typically seen (Fig. 9B and C). In addition to the dilated Golgi membranes, label was detected in the ER, nuclear membrane, and plasma membrane (Fig. 9B). Labeled small vesicles in the Golgi region, typically seen in untreated cells (Fig. 8A and B), were not seen in monensin-treated cells.

Staining with N protein-specific antibodies produced intensive labeling at the cytoplasmic surface of most of the monensin-induced vacuoles (Fig. 9D). The tubular structures did not react with N protein-specific antibodies (Fig. 9E). As in control cells, no labeling was seen at the cytoplasmic aspect of the plasma membrane (Fig. 9D).

## DISCUSSION

In a previous study, we concluded that Uukuniemi virus matures by budding at the membranes of the Golgi complex (26). By using immunocytochemical double-labeling techniques, we showed that in virus-infected cells both glycoproteins G1 and G2, as well as nucleocapsid protein N, accumulate in the Golgi region (25). In the present study, we



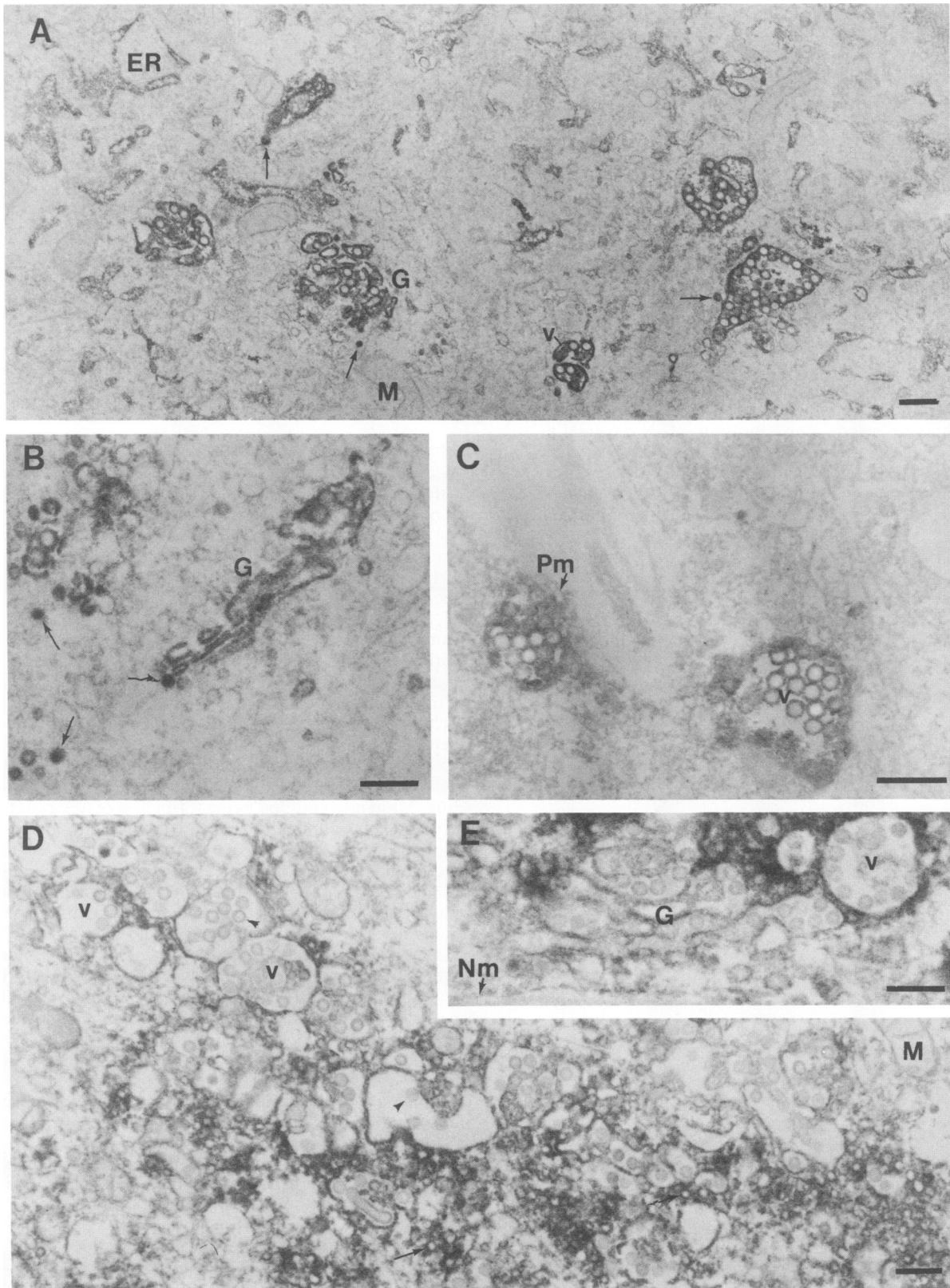


FIG. 8. Immunoelectron microscopy of Uukuniemi virus-infected cells with G1- and G2-specific (A, B, and C) and protein-specific (D and E) antibodies. (A and B) Virus glycoproteins localized in the reticular membranes of the ER, in the stacked and dilated membranes of the Golgi complex (G), and in membranes of vacuoles in the Golgi region (v). Numerous labeled virus particles are present in the lumen of these Golgi elements. The arrows indicate glycoprotein-containing 60- to 80-nm vesicles present in large numbers in the Golgi region of Uukuniemi virus-infected cells. (C) Large virus- and glycoprotein-carrying vacuoles (v) located close to the plasma membrane (Pm). (D and E)

obtained further support for this conclusion by showing that monensin, a drug which specifically affects the morphology and function of the Golgi apparatus (50), effectively inhibits the budding of Uukuniemi virus. In a previous study by Cash (7), monensin was also shown to inhibit the production of extracellular La Crosse virus, another bunyavirus. This situation is clearly different from the results obtained with herpes simplex virus (18, 19) or murine hepatitis virus A59 (a coronavirus) (34), which have intracellular maturation sites proximal to the Golgi complex (8, 32, 48, 53). However, the budding of infectious bronchitis virus, an avian coronavirus, appears to be affected by monensin (3). We show here by immunoperoxidase electron microscopy that the association of Uukuniemi virus nucleocapsids with the cytoplasmic face of dilated Golgi membranes was not affected by monensin. Immunofluorescence microscopy with monoclonal antibodies (25) indicated that both G1 and G2 glycoproteins were present in large amounts in perinuclear vacuoles in monensin-treated cells (data not shown). Although these prerequisites for virus budding were present, assembly of the virus was severely impaired. The observation that, instead of normal budding, the glycoproteins were assembled into abnormal tubular structures with a regular diameter (100 nm) extending into the lumen of monensin-induced vacuoles suggested that a terminal step in the budding process was inhibited. It is possible that formation of the hexagonal lattice of the virus spike complexes (56) was not inhibited, but interactions determining the precise icosahedral structure of the viral envelope were affected (15, 56).

The mechanism of action of monensin is based on its ability to exchange electroneutrally monovalent cations and protons across membranes (39). Thus, the drug affects ionic and pH conditions in the lumen of membrane compartments (12, 50). Many recent studies imply that monensin affects the molecular properties of proteins in endocytic membrane compartments (4, 14, 30, 31, 42). The inhibition of Uukuniemi virus budding by monensin could be due to a direct effect of a changed ionic milieu on the assembly process of the virion (57, 58), or it could result from altered glycosylation of virus glycoproteins. Our recent experiments indicate that a similar effect on the glycosylation of G1 and G2, observed here in monensin-treated cells (see below), can also be achieved by using 20 mM ammonium chloride (unpublished data). However, ammonium chloride, which is known to raise pH in acidic membrane compartments, only partially inhibits the budding of Uukuniemi virus. So far, we have not been able to differentiate the roles of terminal glycosylation of G1 and G2 and those of milieu factors, such as pH, in virus assembly. The study of the assembly of bunyaviruses in the Golgi complex may provide a way to analyze the characteristics of this organelle.

During recent years, monensin has been utilized in a number of studies to examine the exocytic transport pathway of secretory and membrane glycoproteins. The drug exerts a general inhibition on protein transport at the level of the Golgi complex (50). Since, in most cases, monensin also affects terminal glycosylation, it has been suggested that

monensin blocks transport in the Golgi complex at a stage preceding the site where these modification reactions occur (1, 13, 17, 35, 40, 50). However, recent results by Strous and co-workers (47) argue against the conclusion that in the presence of monensin the stage of glycosylation of a protein directly reflects its location in the transport pathway. The terminal glycosylation of transferrin, but not of vesicular stomatitis virus G protein, was inhibited in the presence of monensin, although both proteins accumulated in the same Golgi complex-derived vacuoles. Although Uukuniemi virus glycoproteins accumulate intracellularly, a small fraction is transported to the plasma membrane, as shown here by several methods. We present evidence that this transport occurs even in the presence of monensin. The glycosylation of G1 was clearly affected by the drug. Pulse-chase experiments and endo-H analysis showed that, in the presence of monensin, terminal glycosylation of G1 was partially inhibited, whereas glycosylation of G2 was apparently affected to a lesser extent. External labeling of control and monensin-treated cells with the periodate-boro<sup>3</sup>H]hybride method indicated that the sialylation of cell surface-associated G1, but not G2, was inhibited in the presence of monensin. Since it is likely that both G1 and G2 are transported to the cell surface through the same intracellular compartments, the observed differential effect of monensin on terminal glycosylation suggests that the drug may affect the properties of individual glycoproteins as substrates for glycosylation. This conclusion agrees with the results obtained by Strous and co-workers (47) on vesicular stomatitis virus G protein and transferrin. That monensin may directly affect terminal glycosylation is also supported by results obtained with influenza virus hemagglutinin expressed on the cell surface in polarized MDCK cells in the presence of the drug (2, 41).

Immunoperoxidase electron microscopy suggested that one mechanism of transport of Uukuniemi virus glycoproteins is mediated by the same vacuoles which release the virus from the cell. This was also supported by results showing that the kinetics of expression of the glycoproteins at the cell surface followed the growth curve of the virus (Fig. 1 and 4). Whether there are other transport routes for G1 and G2 is not known. Interestingly, pulse-chase experiments demonstrating the different time courses of incorporation of the virus structural proteins into released virions suggested that monensin did not affect the transport of those virus particles which had been formed before addition of the drug. Thus, it is possible that surface expression of the glycoproteins in the presence and absence of monensin occurs by a similar mechanism.

Since the mechanisms of exocytic transport are at present poorly known, it is difficult to say which properties of Uukuniemi virus glycoproteins enable them to overcome or circumvent the monensin block. The viral glycoproteins appear to be able by themselves to induce a morphological change in the Golgi complex, i.e., dilation of the cisternal elements (9, 25, 26; N. Gahmberg, unpublished results). This change resembles the effect caused by monensin on this organelle. It is important to note that transport and terminal

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Immunoperoxidase localization of the sites of N protein accumulated in the Golgi region. The peroxidase reaction product covers the cytoplasmic surface of dilated Golgi complex membranes (G), which contain numerous unlabeled virus particles (arrowheads) in their lumen. Some viruses, apparently those in the process of budding, are labeled with N protein-specific antibodies. Cytoplasmic labeling also surrounds the many small transport vesicles of the Golgi region (arrows). (E) Accumulation of N protein at one aspect of the structurally preserved Golgi complex. Nm, Nuclear membrane; M, mitochondria. Bars, 0.4  $\mu$ m.

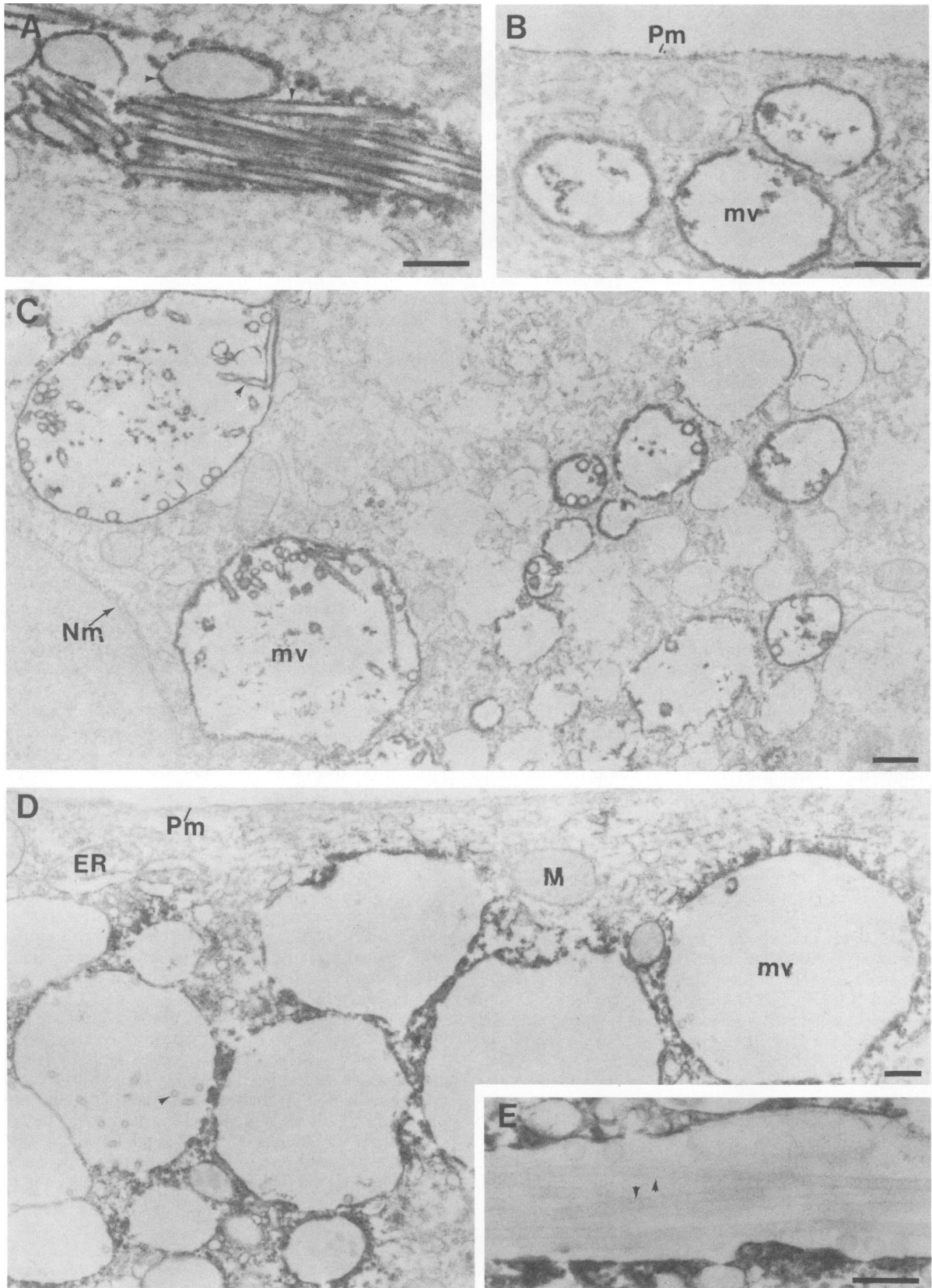


FIG. 9. Immunoelectron microscopy of Uukuniemi virus-infected cells fixed at 10 h after infection. Monensin ( $10 \mu\text{M}$ ) was added to the cells at 2.5 h after infection. Glycoprotein-specific antibodies label the limiting membranes of many of the large monensin-induced vacuoles (mv) in the perinuclear region (C) or at the cell periphery (B). A few viruslike structures are present in the lumen of the vacuoles; in some vacuoles, large numbers of aberrant tubular structures are present (A and C; arrowheads). These react with glycoprotein-specific antibodies. The plasma membrane of monensin-treated cells shows positive labeling (B). No labeled 60- to 80-nm vesicles are seen in the Golgi region. N protein-specific antibodies result in the labeling of the cytoplasmic surface of monensin-induced vacuoles (D and E). The internal tubular and viruslike structures are nonreactive to N protein-specific antibodies (arrowheads). No label is seen at the cytoplasmic aspect of the plasma membrane (Pm). Nm, Nuclear membrane; M, mitochondria. Bars,  $0.4 \mu\text{m}$ .



glycosylation of these membrane glycoproteins can also occur in the absence of monensin (24, 25) under conditions in which the cisternal organization of the Golgi complex is lost.

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