Intra-Golgi Transport Inhibition by Megalomicin*

(Received for publication, June 29, 1995, and in revised form, November 1, 1995)

Pedro Bonay, Sean Munroš, Manuel Fresnòt, and Balbino Alarcoñ†

From the †Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid 28049, Spain and the $MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, United Kingdom

Megalomicin (MGM) is a macrolide antibiotic which has been demonstrated previously to cause an anomalous glycosylation of viral proteins. Here we show that MGM produces profound alterations on Golgi morphology and function. The addition of MGM at 50 μM for 1 h caused a dilation of the Golgi detected by immunofluorescence staining for medial- and trans-Golgi markers. The effect of MGM was clearly more intense on the trans-side of Golgi, as evidenced in electron microscope preparations. The effect on Golgi morphology was reversible and correlated with an impairment of glycoprotein processing in the trans-Golgi. Thus, although the vesicular stomatitis virus G protein was processed in the presence of MGM to an endoglycosidase H-resistant form, it was poorly sialylated. The sialylation of cellular proteins was also inhibited, resulting in cells with low level of sialylation on the cell surface. However MGM did not inhibit the activities of the galactosyl- or sialyltransferase as measured in vitro. MGM inhibited cis- to medial-, and more strongly, medial- to trans-Golgi transport of vesicular stomatitis virus G protein in an in vitro system, suggesting that the impairment in glycoprotein maturation observed in vivo is the result of intra-Golgi transport inhibition.

Proteins which enter the central vacuolar system are synthesized in the endoplasmic reticulum (ER)1 where they acquire N-linked glycans which are then processed and matured as they are sequentially transported through the cis-, medial-, and trans-cisternae of the Golgi complex. From here they move to an array of tubulo-vesicular structures, the trans-Golgi network (TGN) from where sorting occurs to lysosomes, regulated secretory vesicles, and to the plasma membrane.

The use of specific inhibitors has been highly valuable for cellular biologists, allowing the study of intracellular transport processes in intact cells in a convenient way. Nocodazole and other agents that depolymerize microtubules have been widely used to study the role of the cytoskeleton in intracellular transport (Kelly, 1990). In the last years, brefeldin A (BFA) has attracted the attention of many researchers for its effects on intracellular transport (Pelham, 1991). It was first documented that BFA inhibited the secretion of proteins at an early step. In the presence of BFA, secretory proteins were retained in the endoplasmic reticulum (Lippincott-Schwartz et al., 1989, 1990, 1991). It was found that even Golgi enzyme markers were located in the ER after a few minutes of treatment with BFA (Misumi et al., 1986; Fujikawa et al., 1988; Doms et al., 1989).

In addition, no recognizable Golgi stacks were seen in BFA-treated cells. These data suggested that BFA promoted the redistribution of Golgi stacks into the ER. Markers of the TGN did not redistribute into the ER and were, however, found to fuse with endosomes. BFA did not affect the cycling between the plasma membrane and endosomes, although the traffic between endosomes and lysosomes was impaired (Lippincott-Schwartz et al., 1991). BFA produced the tubulation of Golgi stacks, endosomes, and lysosomes, and it has been suggested that these tubules may target membrane fusions (Pelham, 1991). The redistribution of Golgi markers to the ER produced by BFA is microtubule-mediated, requires ATP, and is suppressed by the addition of nonhydrolyzable GTP analogs.

Other compounds that have been lately used as probes of intracellular processes are the macroline antibiotics bafilomycin C (Bowman et al., 1988; Yoshimori et al., 1991; Johnson et al., 1993; Pakolansas et al., 1994) and concanamycin (Ylla et al., 1993). These antibiotics are highly selective and specific inhibitors of vesicular proton ATPases (V-ATPases) (Nelson and Taiz, 1989) identified in organelles belonging to the central vacuolar system such as lysosomes (Moriyama and Nelson, 1989a) and the Golgi complex (Moriyama and Nelson, 1989b), as well as in coated vesicles (Xie and Stone, 1986; Arat et al., 1987). Those ATPases are likely responsible for the generation and maintenance of the acidity in those organelles. The acidic luminal environment has been suggested to be important to assure the fidelity of vesicular transport. In this sense, Yilla et al. (1993), showed that concanamycin B significantly impaired intra-Golgi trafficking and plasma membrane delivery in HepG2 cells without affecting the endoplasmic reticulum to Golgi transport. Bafilomycin A1 (Yoshimori et al., 1991) and concanamycin B (Woo et al., 1992) have been found to inhibit in vivo lysosomal protein degradation through inhibition of the ATP-dependent acidification of endosomes and lysosomes, without an apparent inhibition of the intracellular protein transport, including the endocytic pathway. However other authors have shown an inhibition of protein transport in the endocytic pathway (Oda et al., 1991; Pakolansas et al., 1994; Johnson et al., 1993). In addition, Xu and Shields (1994) provided evidence that prosomatostatin processing in the TGN is inhibited by bafilomycin A1.

Two other types of compound have been added to the list of inhibitors of vesicular transport. The sponge metabolite ilimaquinone (IQ) caused the breakdown of the Golgi into small vesicles, allowing the transport of secretory vesicle proteins to...
the cis-Golgi, but not further (Takizawa et al., 1993). Interestingly, IQ inhibited the association of β-COP to transport vesicles but, unlike BFA, did not produce the fusion of the Golgi into the ER. In addition, a product isolated from the culture broth of Streptomyces sp. originally described as an inhibitor of inflammation has been recently reported as an inhibitor of the intracellular transport of VSV G glycoprotein (Seog et al., 1994), although there is no indication on the mechanism or site of action.

Here we describe that megalomicin (MGM), a macrolide antibiotic with wide antibacterial spectrum (Weinstein et al., 1972) and antiviral activity (Alarcon et al., 1988), produces profound morphological and functional effects on the Golgi complex of cultured cells, causing the inhibition of the last steps of glycoprotein processing in the Golgi without an apparent effect on the processing enzymes (glycosyltransferases) themselves, but more probably affecting the intra-Golgi transport.

EXPERIMENTAL PROCEDURES

Materials

Drugs—MGM was obtained from cultures of Micromonospora megalomica (ATCC 27598) by a procedure described (Weinstein, 1972). Briefly, 50 ml of medium 172 were inoculated with the spores of the actinomycete and incubated at 27°C with high aeration for 5 days and was then used as an inoculum for a 400-ml culture. After 3 days this culture was used to inoculate 4 liters of medium 172 in the same conditions. 5 days later, the pH of the culture was raised to 9.5 with sodium hydroxide, and the culture was extracted with an equal volume of ethyl acetate. The ethyl acetate extract was evaporated to 0.01 of the initial volume and was then extracted twice with 0.14 M hydrochloric acid. The acid extract was brought to pH 9.5 and extracted twice with equal volumes of ethyl acetate. The ethyl acetate extracts were pooled and evaporated completely, dissolved in aceton, and precipitated by rapid addition to 1 liter of distilled water brought to pH 9.5 with sodium hydroxide. The precipitate was collected by filtration through Whatman paper.

Bafilomycin A1 was a kind gift of Dr. L. Carrasco (Centro de Biologia Molecular, Madrid, Spain) and BFA was from Epicentre Technologies (Madison, WI). Erythromycins A, B, and C were a generous gift of Dr. J. Corbalan, J. (Lilly).

Cells—COS-7 CHO 15B cells, and normal rat kidney cells (NRK), wild type and ricin-resistant mutant 17 (Ric 17), were grown in DMEM supplemented with 5% fetal bovine serum.

Plasmids—A vector encoding human sialyltransferase tagged with a c-Myc epitope (SSST) was constructed as described (Munro, 1991). Plasmid HE22M encoding human ERD2 tagged with the c-Myc epitope was generously given by Dr. H. Pelham (MRC, Cambridge).

Antibodies and Lectins—The mouse monoclonal antibody against the c-Myc epitope was kindly given by Dr. G. E. Evans (Evans et al., 1985). The mouse monoclonal anti-cis-Golgi antibody MG-160 was kindly given by Dr. N. K. Gonatas (Mourelatos et al., 1990). The mouse anti-β-COP monoclonal M3A5 was kindly given by Drs. V. A. Allen (MRC, Cambridge) and T. Kreis (EMBL, Heidelberg, Federal Republic of Germany). The rabbit antiserum anti-mannosidase II was obtained from Dr. K. W. Morgan (Morgan et al., 1991). The anti-VSV G protein monoclonal antibody P5D4 was purchased from Sigma. Fluorescein and rhodamine-labeled goat antibodies specific for mouse and for rabbit immunoglobulins were purchased from Southern Biotechnology (Birmingham, AL).

Flow Cytometry

Fluorescein isothiocyanate labeling of Limulus polyphemus agglutinin, Maackia amurensis agglutinin, and Sambucus nigra agglutinin was carried out as described (Kaku et al., 1993). The unlabeled lectins were obtained from Sigma. The molar ratio of fluorescein isothiocyanate to protein was 0.8, 1.2, and 1.1, respectively. Cells (control and MGM treated) detached off the plate with 0.05% EDTA were stained with the fluorescein isothiocyanate-labeled lectins at 4°C for 1 h, washed three times with PBS/BSA, and fixed with 2% paraformaldehyde. The stained cells were analyzed on a Coulter Profile Cytometer.

Glycan Analysis

Cells were metabolically labeled with [3H]mannose (50 μCi/ml, Amersham, Amersham, Iberica, Madrid, Spain) in glucose-free DMEM, 5% dialyzed fetal calf serum for 16 h. The preparation of the glycopeptides and analysis by lectin affinity chromatography on concanavalin A-Sepharose and lentil lectin-Sepharose was carried out as described previously (Monis et al., 1987; Bonay and Hughes, 1991). Analysis of oligosaccharides was carried out essentially as described (Varki and Kornfeld, 1983).

Analysis of Anionic (Sialylated) Oligosaccharides of VSV Protein G

NRK cells were infected with VSV and 4 h after infection were metabolically labeled with [3H]mannose (10 μCi/ml in low glucose DMEM) for 4 h. The medium was removed, the cells were harvested in 1 ml of PBS, lysed in lysis buffer containing 1% Nonidet P-40, and immunoprecipitation with antibody P5D4 was performed as described previously. The immunoprecipitates were resuspended in 75 μl of citrate-phosphate buffer, pH 5.5, containing 0.1% SDS and then heated for 5 min at 100°C. After cooling, samples were treated with 2 ml units of endo-H (Oxford Glycosystems, Oxford) and 5 ml units of N-glycanase (Oxford Glycosystems) at 37°C for 20 h. The digest was boiled for 5 min and diluted to 500 μl with 2 mM Tris-HCl, pH 8.2. The negative charges on the released oligosaccharides were determined by chromatography on a 1-ml Q-Sepharose FF column (Pharmacia Biotech Inc., Upsala, Sweden) equilibrated in 2 mM Tris-HCl, pH 8.2. The bound material was eluted with increasing concentrations of NaCl in the same buffer. Under these conditions, oligosaccharides with one, two, and three negative charges elute at 20, 57, and 120 mM NaCl, respectively. Fractions of 0.75 ml were collected and the radioactivity monitored. To exclude the putative presence of phosphate residues that could be contributing with negative charges, a control sample was treated with alkaline phosphatase prior to Q-Sepharose chromatography. The results obtained indicated that phosphate residues were not mediating binding to the Q-Sepharose column.

Glycosyltransferase Assays

The assays for the Golgi enzyme UDP-galactose:glycoprotein galactosyltransferase and CMP-sialic acid:glycoprotein sialyltransferase activities were performed as described (Vischer and Hughes, 1981).

Intra-Golgi Transport Assay

The in vitro cis- to medial-Golgi transport assay was carried out essentially as described by Rothman (Balch et al., 1984; Beckers and Rothman, 1992) using donor Golgi membranes from VSV-infected CHO 15B cells (defective in N-acetylglucosaminyltransferase I) and acceptor membranes obtained from uninfected CHO wild type cells. For the medial- to trans-Golgi transport the method described by Rothman (1987) was employed with slight modifications. Briefly, addition of
Effect of Megalomicin on the Golgi

RESULTS

Morphological Alterations of the Golgi Apparatus Induced by MGM—We have reported previously that MGM, a macrolide antibiotic, produces anomalous glycosylation of herpes simplex virus (HSV) proteins (Alarcón et al., 1988). This led us to examine if this effect was due to drug-induced alterations of the Golgi apparatus, where maturation to complex carbohydrates takes place. Fig. 1 shows the immunofluorescence patterns of normal and MGM-treated NRK and COS cells stained for specific Golgi subcompartmental markers. The perinuclear, punctuated, and compact pattern, revealed in control NRK cells by the cis- and medial-markers MG-160 (a membrane protein) and Golgi mannosidase II (a luminal Golgi protein), respectively (Fig. 1), was shifted in MGM-treated cells to a more loose and dilated structure that remained in the vicinity of the nucleus. The effect of MGM treatment on Golgi morphology was more evident when the trans-Golgi markers galactosyl- and sialyltransferase were tested. The compact appearance of the trans-Golgi in COS cells was changed upon treatment with MGM to a large vesicular, swollen structure that, sometimes, spread out through a large portion of the cytoplasm (Fig. 1). The Golgi morphology, as surveyed when ERD2, the KDEL receptor (Lewis and Pelham, 1992), was used as a marker, was also modified after drug treatment. No effect was, however, detected on the morphology of the ER upon MGM treatment (data not shown). These data, together with previously described data in which MGM was shown to affect protein glycosylation but not protein synthesis (Alarcón et al., 1988), suggest that MGM specifically acts on the Golgi complex. The specific effect of MGM is further supported, because another macrolide antibiotic, bafilomycin A₁, which inhibits vacuolar H⁺-ATPases (Bowman et al., 1988), did not have any detectable effect on Golgi’s morphology (Fig. 1). Moreover, erythromycins A, B, and C, which are structurally related to MGM, did not produce any alteration on the morphology of the Golgi apparatus (data not shown). The main structural difference between erythromycin and MGM is the presence in the latter of an uncommon sugar, D-rhodamine which seems, therefore, to participate in MGM activity.

Confocal laser scanning and electron microscopy were performed to evaluate the effect of MGM on Golgi morphology. Confocal microscopy of COS cell samples stained for the trans-Golgi marker sialyltransferase showed that upon incubation with MGM for periods as short as 1 h, the distribution of the marker was well spread and a dilation and engorging of the compartment became evident as noticed by the larger size of the stained vesicles extending through a reticular-like structure (Fig. 2, compare a with b and c). Fig. 2 also shows that the effect of MGM on the trans-Golgi is reversible, returning, although slowly, to normal morphology after a 1–3 h washout (d and e).

The immunofluorescence data presented above showed that MGM caused a more intense alteration on the trans-Golgi. This was confirmed by electron microscopy of NRK cells treated with MGM and stained with a Golgi mannosidase II-specific antibody by the immunoperoxidase method. As shown in Fig. 3, the stacks of flattened cisternae seen in the control sample (left panel) were dramatically altered in MGM-treated cells (right panel). In these cells, the Golgi apparatus appeared to be formed by stacks of normal size cisternae, mostly located on the cis-side of the Golgi, which were infiltrated by dilated cisternae that were specially abundant on the trans-side.

MGM Disrupts the Normal Processing of Carbohydrates in the Golgi Apparatus—As described above, MGM had dramatic effects on the morphology of the Golgi apparatus causing a dilation of trans-Golgi stacks. To determine whether MGM could be distorting the normal processing of glycoproteins, NRK cells were infected with VSV, and the processing of the viral G glycoprotein was followed by pulse-chase labeling with [35S]methionine. As shown in Fig. 4, VS G protein was processed to an endo-H-resistant form after a 30-min chase in control cells as well as in cells treated with 50 μM MGM. Although MGM caused an expansion of the medial-Golgi in NRK cells (Figs. 1 and 2), the VSV G protein data indicate that, in the presence of MGM, glycoproteins could be almost normally transported to the medial-Golgi, but does not allow to conclude anything about further transport. Somewhat different...
results were obtained when total oligosaccharides from [\textsuperscript{3}H]mannose-labeled, uninfected NRK cells were analyzed by lectin chromatography. A preparation of total oligosaccharides was subjected to chromatography on a concanavalin A column to separate them according to their mannose content. As shown in Fig. 5A, MGM inhibited, to a certain degree, the processing of glycans to complex type in cell lysates (11% of the total pool of oligosaccharides in MGM-treated cells versus 23% in control cells, representing a decrease of about 50%). The inhibition in the conversion to the complex type was paralleled by an increase in the absolute amount of high mannose oligosaccharides (60% in nontreated cells versus 88% upon drug treatment). Interestingly, the fraction corresponding to hybrid-type oligosaccharides, although being a minor component in control cells (6% of the total distribution of oligosaccharides), was completely absent in lysates from MGM-treated cells. It is important to highlight that MGM incubation only had a marginal effect on the total incorporation of [\textsuperscript{3}H]mannose, [\textsuperscript{3}H]galactose, and [\textsuperscript{3}H]-N-acetylglucosamine (8, 13, and 11% inhibition, respectively).

Concomitantly to the inhibition in the formation of complex type oligosaccharides, MGM caused a lower secretion of glycoproteins containing this type of sugars (Fig. 5B). In this figure, it can be clearly seen that the composition of the secreted glycoproteins from MGM-treated cells was richer in the forms with a higher content in mannose (hybrid and mannose-rich). Interestingly, the addition of MGM resulted in the secretion of proteins containing high mannose oligosaccharides, which were not detected in culture supernatants from untreated cells.

The apparent discrepancy between the endo-H sensitivity data of VSV G protein and the concanavalin A chromatography data of total NRK glycoprotein glycans could be due to different times of exposure to the drug or to the different sensitivity of the labeling techniques. Furthermore, VSV G protein could be converted to an endo-H-resistant form in the medial-Golgi, but still be defectively matured in the presence of MGM as their transport to the trans-cisternae of the Golgi is impaired.

The addition of sialic acid is the last step in the maturation of glycoproteins taking place in the trans-Golgi. To determine how MGM affected this process, oligosaccharide preparations of MGM-treated VSV-infected NRK cells were subjected to chromatography on Q-Sepharose. This system allows to distinguish between oligosaccharides that have incorporated different number of sialic acid residues. As shown in Fig. 5C, the fractions corresponding to oligosaccharides containing a low number of sialic acids (0–1) were higher in MGM-treated cells than in control samples, and conversely, MGM inhibited the formation of oligosaccharides with a higher level of sialylation. These data suggest that MGM is acting preferentially on the trans-side of the Golgi or just before it.

The MGM effect on the sialylation of surface proteins was also evaluated by staining NRK cells with sialic acid-specific lectins, such as M. amurensis, L. poliphemus, and S. nigra agglutinins. After an overnight treatment with MGM, the lectin staining of the cell surface was reduced to a 13–24% of the staining in control cells (Fig. 6), further supporting the idea that MGM reduces the sialylation of glycoproteins.

MGM Inhibits Intra-Golgi Transport—Up to this point, the presented evidence indicated that the morphological alterations induced by MGM were paralleled by structural changes in the glycan moiety of the newly synthesized glycoproteins, particularly, by a decreased sialylation. These alterations are consistent with an impairment in the transport of glycoproteins from the medial-Golgi to the trans-Golgi. The results are also consistent with an inhibition of the trans-Golgi located glycosyltransferases. However, MGM did not inhibit the activities of the enzymes galactosyl- and sialyltransferases in in vitro assays (Table I). In order to gain further insight into the mechanisms of action of MGM, in vitro-intra-Golgi transport assays of protein G in VSV infected cells (cis- to medial-Golgi and medial- to trans-Golgi) were carried out. The specificities of the transport systems were assessed by the dependence on the presence of ATP and added cytosol and by the sensitivity to N-ethylmaleimide (NEM) (Fig. 7). The results showed that while MGM inhibited the transport of VSV G protein on both systems, the inhibition was stronger on the medial to trans-Golgi transport assay than in the cis- to medial-Golgi assay (50 versus 25% inhibition at 5 \textgreek{M}). Because MGM did not have a direct effect on the activity of the galactosyl- or sialyltransferase in an in vitro assay (Table I), we can exclude the possibility that the reduced transport observed were the result of lower enzymatic activity.

Effect of MGM on Golgi Acidification—Although MGM did not have a direct inhibitory effect on galactosyl and sialyltransferases activity in an in vitro assay, it is possible that MGM could inhibit them indirectly by altering the milieu optimal for these enzymes via the disruption of the pH gradient in the trans-Golgi stacks. To test this hypothesis, the effect of MGM on the H\textsuperscript{+} pumping activity of vacuolar ATPases was assayed in Golgi-enriched membrane preparations. As shown in Fig. 8, MGM had only a marginal effect (23%) on vesicular H\textsuperscript{+} pumping activity at the active concentration of 50 \textgreek{M}. By contrast, bafilomycin A\textsubscript{1}, which has been shown to be an inhibitor of vacuolar H\textsuperscript{+}-ATPases (Bowman et al., 1988; Crider et al., 1994; Xu and Shields, 1994; Zhang et al., 1994), completely inhibited the acidification of Golgi-enriched vesicles at a concentration of 5 \textgreek{nM}. The results shown here suggest that the inhibitory effects
of MGM detected as a blockade in the final steps of glycoprotein processing are due to a direct inhibition of intra-Golgi transport and not indirectly to an inhibition of the Golgi enzymes or proton gradient-forming ATPases.

**DISCUSSION**

The results presented in this paper show that MGM causes profound effects on the Golgi apparatus morphology and function. Standard fluorescent and confocal laser scanning microscopy showed that MGM produced a general dilatation of the Golgi cisternae, although the effect was more dramatic on the trans-Golgi. Electron micrographs showed the presence of swollen cisternae of the Golgi mostly located on the trans-side, coexisting with cis-located cisternae of normal appearance. The morphological alteration of MGM on the Golgi was reflected in an impaired function, producing an anomalous glycosylation of VSV G protein and cellular glycoproteins. Interestingly, the MGM effects on glycosylation correlated well with its morphological effects, causing a higher inhibition of the later processes of glycan maturation, which take place on the trans-side of the Golgi. Thus, MGM caused an inhibition in the formation of complex-type oligosaccharides paralleled to an increase of the high mannose type. In addition, after shorter incubations, MGM had no detectable effect on the maturation of VSV G protein to an endo-H-resistant form, a process that is dependent on the activity of the medial-Golgi located enzyme mannosidase II, indicating that MGM does not inhibit, to a substantial degree, the access to the medial-Golgi compartment. Furthermore, in the presence of MGM, VSV G protein was undersialylated. A possible explanation for these results could be that MGM inhibits the activities of either sialyltransferase or the previous acting galactosyltransferase that would generate the substrate for the sialyltransferase. However, MGM did not inhibit the activities of galactosyl- and sialyltransferases in vitro (Table I). An alternative non-excluding explanation would be that the swelling induced by the drug could result in a decreased active lumenal concentration of the sugar nucleosides and hence reduced modification rates. MGM could directly inhibit sugar nucleotide transporters which would account for some of the MGM effects described. Such idea would imply that MGM preferentially inhibits the UDP-Gal and/or CMP-NeuAc transporters but not the GDP-Man or the UDP-GlcNAc transporters. However, as far as the literature surveyed (for review, see Hirschberg and Snider (1987) and Milla et al. (1989)), all the sugar nucleoside transporters described so far share the same biochemical properties, and there is no structural homology between MGM to any sugar nucleotide to act as a selective inhibitor. It is clear that direct testing of MGM in a sugar nucleotide transport assay will answer that point. However, we have tested CHO mutant cell lines lacking UDP-Gal (CHO Lec 2, Deutscher et al., 1984) or CMP-NeuAc (CHO Lec 8, Deutscher and Hirschberg, 1986) transporters for their MGM sensitivity. Both cell lines behave like the parental cell line in the sense that MGM induces the cisternal swelling to the same extent.2 Finally, MGM could be inhibiting the access of the proteins to the compartments where the glycosyltransferases are located. This possibility is strongly supported by the effect of MGM in an established in vitro intra-Golgi transport assay. Although in the in vitro assay, MGM inhibited cis- to medial- and medial- to trans-Golgi transport of VSV G protein.
protein, only this last effect was correlated with the effect of MGM in vivo. This is easily explained, considering that the maturation of glycans is a sequential process relying on the sequential action of compartmentalized enzymes, and therefore, the effects of transport inhibition accumulate through successive compartments.

The inhibition of glycoprotein sialylation was in agreement with previous data showing that MGM inhibited the addition of galactose to HSV glycoproteins (Alarcón et al., 1988). The intra-Golgi transport inhibition shown in this paper could explain the activity of MGM as an antiviral agent. MGM inhibited normal glycosylation of HSV, but not the synthesis of viral proteins nor the formation of viral particles, which were, nevertheless, non-infectious (Alarcón et al., 1984). The antiviral effect of MGM is nevertheless difficult to explain, because, as shown in this paper, MGM was not selective for viral glycoproteins, causing the secretion of incompletely processed glycoproteins and an inhibition in the sialylation of cell membrane glycoproteins. Although dramatic, the effects of MGM on the Golgi of noninfected cells were not correlated with an apparent toxicity to cells grown with MGM added once a week (data not shown). The observed effect could be due to an adaptation of the

![Fig. 5. Analysis of glycans synthesized in the presence of MGM. A and B, oligosaccharides were isolated from COS cells that had been labeled overnight with [3H]mannose in the presence of 50 μM MGM or mock-treated. The oligosaccharides from the cell lysates (A) or from the culture supernatants (B) were fractionated by concanavalin A-Sepharose chromatography. Complex type corresponded to the unbound material. Hybrid type made the major fraction eluted with 10 mM α-methylglucoside, and the high mannoside fraction was eluted with 500 mM α-methylmannoside. C, VSV-infected NRK cells were labeled for 4 h with [3H]mannose in the presence (closed bars) or absence (open bars) of 50 μM MGM. Cell lysates were immunoprecipitated with VSV G protein antibody P5D4, and oligosaccharides on the VSV G protein were fractionated on a Q-Sepharose column. Fraction 1 was the unbound material and corresponded to neutral oligosaccharides. Fractions 2, 3, and 4 were obtained by elution with increasing concentrations of NaCl (20, 75, and 120 mM) and corresponded to monosialylated, disialylated, and trisialylated oligosaccharides, respectively.](http://www.jbc.org/)

![Fig. 6. Inhibition of glycoprotein sialylation in MGM-treated cells. Flow cytometry analysis of NRK cells stained with fluorescein-labeled lectins M. amurensis agglutinin (MAA), S. nigra agglutinin (SNA) and L. polyphemus agglutinin (LPA). The bar represents the point of maximum fluorescence for 98% of the cell population in unstained samples used as controls. The numbers in each quadrant represent the percentage of fluorescence positive cells (numbers on top) and the mean fluorescence intensity (numbers at bottom).](http://www.jbc.org/)

![Table I](http://www.jbc.org/)

| Activity | GalT | ST |
|----------|------|----|
| Homogenate | 6.21 ± 0.45 | 0.98 ± 0.11 |
| 50 μM MGM | 5.99 ± 0.64 | 1.02 ± 0.15 |
| Golgi-enriched fraction | 111.4 ± 9.78 | 14.7 ± 1.34 |
| Control | 118.9 ± 12.74 | 13.2 ± 2.09 |

![FIG. 5. Analysis of glycans synthesized in the presence of MGM. A and B, oligosaccharides were isolated from COS cells that had been labeled overnight with [3H]mannose in the presence of 50 μM MGM or mock-treated. The oligosaccharides from the cell lysates (A) or from the culture supernatants (B) were fractionated by concanavalin A-Sepharose chromatography. Complex type corresponded to the unbound material. Hybrid type made the major fraction eluted with 10 mM α-methylglucoside, and the high mannoside fraction was eluted with 500 mM α-methylmannoside. C, VSV-infected NRK cells were labeled for 4 h with [3H]mannose in the presence (closed bars) or absence (open bars) of 50 μM MGM. Cell lysates were immunoprecipitated with VSV G protein antibody P5D4, and oligosaccharides on the VSV G protein were fractionated on a Q-Sepharose column. Fraction 1 was the unbound material and corresponded to neutral oligosaccharides. Fractions 2, 3, and 4 were obtained by elution with increasing concentrations of NaCl (20, 75, and 120 mM) and corresponded to monosialylated, disialylated, and trisialylated oligosaccharides, respectively.](http://www.jbc.org/)
the mechanism of adaptation has been suggested for concana-
mycin B (Yilla et al., 1993), where an acidification inhibition of the
TGN could cause a default pathway to be utilized. The
authors propose that proteins would be shunted into a different
pathway causing proteins to reach the cell surface and to be
secreted although at a lower rate.

The effects of MGM on the Golgi complex organization were
different to those seen with BFA and IQ, which inhibit vesicu-
lar transport by acting on early steps of vesicle formation. BFA
causes the cis-, medial-, and trans-Golgi to redistribute into the
ER by blocking the anterograde vesicular transport, whereas
the trans-Golgi network is fused with endosomes (for a review,
see Klausner et al. (1992)). IQ, on the other hand, causes the
fragmentation and vesiculation of the Golgi and inhibits the
transport beyond the cis-Golgi (Takizawa et al., 1993). The
molecular target of MGM is at present unknown. It seems clear
that it is different to the target recognized by BFA, because
β-COP is not dissociated from the Golgi upon MGM treatment
(data not shown). In addition, transport took place at a normal
extension when cytosol from MGM-treated cells was used in
the in vitro transport assay (data not shown). This suggested
that MGM does not inhibit intra-Golgi transport by binding to
a soluble component.

On the other hand, it is difficult to understand how the
swelling of Golgi cisternae may impair the access of docking
vesicles from earlier Golgi compartments. In this regard, the
effect of MGM would be similar to the effects of mastoparan
and ARFp13, which have been recently described to inhibit
intra-Golgi transport by damaging Golgi membranes (Wed-
man and Winter, 1994). However, unlike these peptides, the
effect of MGM was reversible and affected specifically the last
steps of glycoprotein maturation.

In accordance with the effect on intra-Golgi transport, MGM
at 50 μM produced a partial inhibition (25–35%) of total protein
secretion (data not shown), pointing to an impairment in the
transport of secretory proteins beyond the trans-Golgi. The
presence of undersialylated glycoproteins in the secreted pro-
teins may be due to sialylation by sialyltransferases in earlier
Golgi compartments, perhaps due to mislocalization of the en-
zymes caused by MGM. This would not be surprising since
other drugs, like BFA, produce anomalous processing of glyco-
proteins, due to the accumulation of Golgi enzymes in the ER
(Chawla and Hughes, 1991).

The effect of concanamycin B, a macrolide antibiotic, on the
Golgi apparatus has been described (Yilla et al., 1993). Con-
canamycin B, as well as bafilomycins, is a V-H⁺-ATPase inhib-
itor (Bowman et al., 1988; Woo et al., 1992). The almost com-
plete blockade of glycoprotein secretion by concanamycin B
(Yilla et al., 1993) and the impairment in the transport of viral
particles to the membrane by bafilomycin (Pakolangas et al.,
1994) suggested that V-H⁺-ATPases maintain a low pH in the
trans-Golgi, which is fundamental for protein trafficking
through the trans-Golgi and for the activity of sialyltrans-
ferases (Yilla et al., 1993). The undersialylation of proteins
induced by MGM could also be explained by an indirect inhi-
bition of the sialyltransferases, due to an alteration of the
intra-Golgi milieu essential to the enzymatic activity caused by
the drug. In this regard, as concanamycin B inhibits sialylation
(Yilla et al., 1993), it could be argued that MGM could also act
by raising the intraluminal pH of the Golgi through an inhibi-
tion of the V-H⁺-ATPases. An efficient recognition of the trans-
ported proteins (soluble and membrane) by the components of
the sorting machinery may rely on the compartment pH. How-
ever, in contrast to concanamycin and bafilomycin, MGM in-
hibited poorly the V-type H⁺-ATPase of the Golgi in an in vitro
test at the concentrations that were inhibitory in the intra-
Golgi transport assays. In addition, bafilomycin A₁, which did

![Fig. 7. Intra-Golgi transport inhibition by MGM. Standard transport reactions were carried out as described under "Materials and Methods." Where indicated the reactions were performed in the absence of one of the components of the reaction. In the other samples the assays were performed with all the components in the presence of the indicated concentrations of MGM or of 1 mM NEM. A shows the effect of MGM on cis- to medial-Golgi transport where the donor membranes were from CHO 158 cells, and the acceptor membranes were from CHO wild type cells. B shows the effect on medial- to trans-Golgi transport, where the donor membranes were obtained from BHK ricin-resistant mutant 17 and the acceptor membranes from BHK wild type cells. 1 μg/ml of MGM roughly equals 1 μM.](http://www.jbc.org/)

![Fig. 8. Effect of MGM on Golgi acidification. H⁺ pumping activity was determined by fluorescence quenching of quinacrine with Golgi-enriched membrane vesicles as described under "Materials and Methods." MGM (A) or bafilomycin A₁ (B) were added at the concentrations indicated, and the reaction was started by addition of ATP. Finally, 5 mM nigericin was added to reverse the acidification of the Golgi vesicles.](http://www.jbc.org/)
inhibit Golgi acidification, had no detectable effects on Golgi morphology as concanamycin and MGO do. The swelling of the Golgi induced by MGM could be due to the alteration of other ion gradients, different from proton, across the Golgi membrane. In this regard, monensin has been shown to cause a swelling of the trans-Golgi stacks that result from the dissipation of Na\(^+\) gradients (for review, see Mollenhauer et al. (1990)). The swelling of the Golgi cisternae is produced because there is a net flow of water into the Golgi stacks to compensate for the H\(^+\) gradient that is still maintained in the presence of monensin. Thus, the addition of a proton ionophore prior to monensin results in the abrogation of monensin-caused swelling (Mollenhauer et al., 1993). By contrast, the addition of the proton ionophore carbonyl cyanide p-trifluoromethoxyphenyl-hydrazone did not prevent the swelling effects of MGM on the trans-Golgi (data not shown), suggesting that the mechanism of MGM-induced swelling is different from that of monensin. Monensin, on the other hand, totally inhibits the acquisition of endo-H resistance, while MGM did not.

So, at this point, the subtle differences between other macrolide antibiotics and MGM are more interesting than their similarities, and one relevant issue raised is that they are possibly revealing some hitherto unappreciated complexity in the family of vesicular ATPases not equally susceptible to inhibition (Alarco´n, B., Lacal, J. C., Fernández-Sousa, J. M., and Carrasco, L. (1984) Mol. Cell. Biol. 39, 12327–12341). Kelly, R. B. (1990) Cell 61, 5–7 Klaussner, R. D., Donaldson, J. C., and Lippincott-Schwartz, J. (1992) J. Cell Biol. 116, 1071–1080 Lewis, M. J. and Pelham, H. R. H. (1992) Cell 68, 353–364 Lippincott-Schwartz, J., Yuan, L. C., Bonifacino, J. S., and Klaussner, R. D. (1989) Cell 56, 801–813 Lippincott-Schwartz, J., Donaldson, J. C., Schweizer, A., Berger, E. G., Hauri, H. P., Yuan, L. C., and Klaussner, R. D. (1990) Cell 60, 821–836 Lippincott-Schwartz, J., Yuan, L. C., Tipper, C., Amherdt, M., Ordi, L., and Klaussner, R. D. (1991) Cell 67, 601–616 Mills, M., Capasso, J., and Hirschberg, C. B. (1989) Biochem. Soc. Trans. 17, 348–350 Misumi, Y., Misumi, M., Yiki, T., Takatsuki, A., Tamura, G., and Ikehara, Y. (1986) J. Biol. Chem. 261, 11398–11403 Mollenhauer, H. H., Morre, D. J., and Rowe, L. D. (1990) Biochim. Biophys. Acta 1031, 225–246 Monis, E., Bonay, P., and Hughes, R. C. (1987) Eur. J. Biochem. 168, 287–294 Moremen, K. W., Touster, O., and Robbins, P. W. (1991) J. Cell Biol. 169, 1610–1618 Moriyama, Y. and Nelson, N. (1989a) Biochim. Biophys. Acta 980, 241–247 Moriyama, Y. and Nelson, N. (1989b) J. Biol. Chem. 264, 18445–18450 Morelatois, Z., Adler, H., Hirano, A., Donnenfeld, H., Gonatas, J. O., and Gonatas, N. K. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4393–4395 Munro, S. (1991) EMBO J. 10, 3577–3588 Nelson, N., and Taiz, I. (1989) Trends Biochem. Sci. 14, 113–116 Oda, K., Nishimura, Y., Ikehara, Y., and Kato, K. (1991) Biochem. Biophys. Res. Commun. 178, 369–377 Palkanigans, H., Metsikko, K., and Vaananen, K. (1994) J. Biol. Chem. 269, 17577–17585 Pelham, H. R. B. (1991) Cell 67, 449–451 Rothman, J. E. (1987) J. Biol. Chem. 262, 12502–12510 Seeg, D. H., Yamazaki, M., and Takatsuki, M. (1994) Biochim. Biophys. Acta 12502–12510 Takizawa, P. A., Yucel, J. K., Veit, B., Faulkner, D. J., Deerinck, T., Soto, G., Ellisman, M., and Mahdavi, V. (1993) Cell 73, 1079–1090 van Weert, A. M. W., Dunn, K. W., Gauske, H. J., Maxfield, F. R., and Stoorvogel, W. (1995) J. Cell Biol. 130, 821–834 Varki, A., and Kornfeld, S. (1983) J. Biol. Chem. 258, 2808–2818 Vischer, P., and Hughes, R. C. (1981) Eur. J. Biochem. 117, 275–284 Weidman, P. J., and Winter, W. M. (1994) J. Cell Biol. 125, 1815–1827 Weinstein, M. J., Luederman, G. M., Ridge, G., Wagner, G. M., and Marquez, J. A. (1972) U. S. Patent 3,632,750 Woo, J., Shinhirasa, C., Sakai, K., Hasumi, K., and Endo, A. (1992) J. Biol. Chem. 267, 389–393 Xie, K. S., and Stone, D. K. (1986) J. Biol. Chem. 261, 2492–2495 Xu, H., and Shields, D. (1994) J. Biol. Chem. 269, 22875–22881 Yoda, M., Tan, A., Ito, K., Miwa, K., and Ploegh, H. L. (1993) J. Biol. Chem. 268, 19092–19100 Yoshimori, T., Yamamoto, A., Moriyama, Y., Futai, M., and Tashiro, Y. (1991) J. Biol. Chem. 266, 17707–17712 Zhang, J., Feng, Y., and Forgac, M. (1994) J. Biol. Chem. 269, 23518–23523

REFERENCES

Alarcón, B., Lacal, J. C., Fernández-Sousa, J. M., and Carrasco, L. (1984) Antibiol. Res. 4, 231–243 Alarcón, B., González, E., and Carrasco, L. (1988) FEBS Lett. 231, 207–211 Alarcon, B., Ley, S. C., Sánchez-Madrid, F., Blumberg, R., J., u. S. T., Fresno, M., and Terhorst, C. (1991) EMBO J. 10, 903–912 Aral, H., Berne, M., Terres, G., Terres, H., Puopolo, K., and Forgac, M. (1987) Biochemistry 26, 6632–6638 Balch, W. E., Dunphy, W. G., Braiell, W. A., and Rothman, J. E. (1984) Cell 39, 405–416

Effect of Megalomicin on the Golgi
