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Use of brefeldin A to define sites of glycosphingolipid synthesis: GA2/GM2/GD2 synthase is trans to the brefeldin A block

(gangliosides/biosynthesis/Golgi/sialyltransferase/*N*-acetylgalactosaminyltransferase)

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ABSTRACT **Brefeldin A (BFA) induces the rapid redistribution of the Golgi complex into the endoplasmic reticulum (ER), causing the glycoproteins that are retained in the ER to be processed by Golgi enzymes. We have examined the effects of BFA on the synthesis of glycosphingolipids (GSL) to map the intracellular sites of GSL synthesis. In several cultured cell types, BFA inhibited the synthesis of the neutral GSL gangliotriaosylceramide (GA2) and monosialoganglioside GM2 and disialoganglioside GD2, where GD2 is GalNAc(β1→4)-[NeuAc(α2→8)NeuAc(α2→3)]Gal(β1→4)GlcCer, GM2 lacks the NeuAc(α2→8) unit, and GA2 lacks both NeuAc(α2→8) and NeuAc(α2→3) units. The observed decrease in labeling of GA2, GM2, and GD2 in the presence of BFA was not due either to enhanced degradation of these glycolipids or to shedding of these glycolipids from the cells. In rat liver all three of these glycolipids have been shown by others to be synthesized by the same enzyme, GA2/GM2/GD2 synthase, which catalyzes the addition of *N*-acetylgalactosamine to lactosylceramide (LacCer), GM3 [NeuAc(α2→3)Gal(β1→4)GlcCer], and GD3 [NeuAc(α2→8)NeuAc-(α2→3)Gal(β1→4)GlcCer], respectively. Studies with a fluorescent glycolipid analog indicated that BFA redistributed the trans-Golgi stacks into a reticular pattern characteristic of the ER. These studies localize GA2/GM2/GD2 synthase, a key enzyme involved in the synthesis of complex gangliosides, to a compartment late in the intracellular trafficking pathway, which remains functionally distinct from the ER in the presence of BFA.**

The subcellular locations of many of the enzymes involved in the synthesis of *N*-asparagine-linked oligosaccharide chains have been identified for many cultured cells and tissues (1). In contrast, comparatively little information exists concerning the glycosyltransferases responsible for synthesis of the glycosphingolipids (GSL). Biosynthesis of GSL begins with ceramide formation in the endoplasmic reticulum (ER), followed by addition of either glucose or galactose, which may take place in either the ER or the early cisternae of the Golgi apparatus (reviewed in ref. 2). Although subsequent steps of GSL synthesis have been attributed to the Golgi apparatus, the recent report of Trinchera and Ghidoni (3) provided direct evidence in support of a model for GSL biosynthesis that involves maturation of the forming oligosaccharide along the different Golgi cisternae.

In the present report we have used brefeldin A (BFA) to localize certain key enzymes of ganglioside biosynthesis. BFA rapidly redistributes the Golgi into the ER, leaving no definable Golgi structure but causing the glycoproteins retained in the ER to be processed by Golgi enzymes (4–7). Our results suggest that the *N*-acetylgalactosamine (GalNAc)-transferase responsible for synthesis of the more complex GSL of the ganglio series is located in a compartment that remains functionally discrete from the ER in the presence of BFA.

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MATERIALS AND METHODS

Cells. Chinese hamster ovary (CHO) cells were a gift from R. Simoni (Stanford University) and were grown in α minimal essential medium containing 5% (vol/vol) fetal calf serum. Human melanoma M1733 cells (8) and murine EL4 lymphoma cells were gifts of S. Hakomori (University of Washington) and J. Sando (University of Virginia), respectively. Other cells were grown as described previously: subclone CE4 of L5178Y murine lymphoma cells (ref. 9; parental cells a gift from C. S. Henney, Immunex, Seattle, WA) and Kirsten murine sarcoma virus (Ki-MSV)-transformed BALB/3T3 (clone K-234) cells [ref. 10; 3T3KiMSV cells also known as K-BALB (K-234)]. Human melanoma SK-MEL-28 cells were obtained from the American Type Culture Collection.

Materials. BFA was a gift from J. Lippincott-Schwartz and R. Klausner (National Institutes of Health) and also was purchased from Epicentre Technologies (Madison, WI); [9,10-^{3}H]Palmitic acid (30 Ci/mmol; 1 Ci = 37 GBq) was purchased from New England Nuclear; 7-nitrobenz-2-oxa-1,3-diazol-4-yl-tagged ceramide (NBD-Cer) was purchased from Molecular Probes.

NBD-Cer Labeling. CHO cells were grown on 12-mm round glass coverslips. After appropriate treatment with or without BFA (see the legend to Fig. 1), the cells were fixed and stained with NBD-Cer as described (11). Briefly, cell monolayers grown on glass coverslips were fixed in 0.5% glutaraldehyde in 10% sucrose/100 mM Pipes, pH 7, for 10 min at room temperature. The fixed cells were then incubated three times for 5 min with freshly prepared $NaBH_4$ (0.5 mg/ml) in ice-cold PBS (137 mM NaCl/3 mM KCl/9.5 mM sodium phosphate, pH 7.4). After several rinses in cold PBS, the cells were incubated for 30 min at 2°C with a preformed complex of NBD-Cer and defatted bovine serum albumin (*ca.* 5 μM with respect to both the fluorescent lipid and albumin). The cells then were washed in PBS and incubated for four 30-min "back-exchanges" with 3.4 mg of defatted albumin per ml at room temperature. The coverslips were then mounted and observed by fluorescence microscopy with a Zeiss Axiophot.

Metabolic Labeling. L5178Y and EL4 lymphoma cells were labeled in suspension; all other cells were labeled in monolayer culture. Cells were precultured in normal growth me-

Abbreviations: GalNAc, *N*-acetylgalactosamine; GlcCer, glucosylceramide; LacCer, lactosylceramide; GA2, GalNAc(β1→4)Gal(β1→4)-GlcCer; GM3, NeuAc(α2→3)Gal(β1→4)GlcCer; GM2, GalNAc-(β1→4)[NeuAc(α2→3)]Gal(β1→4)GlcCer; GD3, NeuAc(α2→8)-NeuAc(α2→3)Gal(β1→4)GlcCer; GD2, GalNAc(β1→4)[NeuAc-(α2→8)NeuAc(α2→3)]Gal(β1→4)GlcCer; GM1b, NeuAc(α2→3)-Gal(β1→3)GalNAc(β1→4)Gal(β1→4)GlcCer; BFA, brefeldin A; ER, endoplasmic reticulum; GSL, glycosphingolipid(s); NBD-Cer, 7-nitrobenz-2-oxa-1,3-diazol-4-yl-tagged ceramide; Ki-MSV, Kirsten murine sarcoma virus.

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dium in the presence or absence of BFA (1 μg/ml) for 30 min at 37°C, labeled in medium containing 25 μC of [^{3}H]palmitic acid per ml with or without BFA for 1 hr at 37°C, and chased in medium containing 8.5 μM palmitic acid with or without BFA for 4 hr at 37°C. Cell monolayers were rinsed with ice-cold saline, harvested with a rubber policeman, and frozen at −80°C. The protein content of cell aliquots was determined by the method of Markwell *et al.* (12).

BFA was added to the medium from a stock solution in methanol (5 mg/ml) to a final concentration of 1 μg/ml. Control cells were incubated with the corresponding concentration of methanol (0.02%).

GSL Analysis. Cell pellets were extracted twice on ice with at least 20 vol of chloroform/methanol, 2:1 (vol/vol), followed by chloroform/methanol, 1:2 (vol/vol), twice using an Omni Mixer (Omni International, Norwalk, CT). GSL were purified by the acetylation procedure (13) followed by separation of deacetylated neutral and acidic GSL on DEAE-Sephadex (14). Acidic GSL were desalted on Sephadex G25 as described (15). GSL samples were analyzed on high-performance TLC plates (Merck) in solvents A ($CHCl_3$/CH_3OH/H_2O, 62:30:6, vol/vol) and B ($CHCl_3$/CH_3OH/0.25% KCl in H_2O, 50:40:10, vol/vol). Radiolabeled TLC bands were visualized by autoradiography with fluorographic enhancement (EN^{3}HANCE spray; New England Nuclear) on Kodak XAR-5 film. Labeled GSL bands that had been visualized by fluorography were quantitated by scraping into scintillation vials and assaying in the presence of 10 ml of ACS scintillation fluid (Amersham).

RESULTS

Effect of BFA on Cell Morphology. To demonstrate that the Golgi complex was morphologically disrupted under the conditions to be used for metabolic labeling, CHO cells were incubated with or without BFA, fixed, and labeled with NBD-Cer. This ceramide analog has recently been shown to stain specifically the trans-Golgi stacks of fixed cells (11). Fluorescence staining with NBD-Cer of fixed CHO cells revealed an intensely labeled cluster of punctate, perinuclear structures characteristic of the Golgi apparatus in these cells (Fig. 1*a*; compare with figure 2*d* in ref. 11). After a 30-min treatment with BFA, much of the NBD-Cer had redistributed from the Golgi staining pattern into a reticular pattern (Fig. 1*b*) characteristic of the ER (5). After a 2-hr treatment with BFA, this redistribution was complete (Fig. 1*c*). Reassembly of the Golgi apparatus into the tightly organized perinuclear clusters found in control cells was largely complete 30 min after removal of BFA from the cells (Fig. 1*d*). BFA caused a similar redistribution in 3T3KiMSV cells and melanoma M1733 cells (data not shown). These results with NBD-Cer staining indicate that BFA causes a redistribution not only of the cis- and medial-Golgi cisternae as described (5, 6) but also of the trans-Golgi stacks as others have reported (16, 17).

Effect of BFA on Ganglioside Synthesis in Cultured Cells. The GSL pattern of CHO cell monolayers labeled with tritiated palmitate consisted of the neutral GSL glucosylceramide (GlcCer) and lactosylceramide (LacCer) (data not shown) and the monosialoganglioside GM3 (Fig. 2), in agreement with previous reports (18, 19). GM3 continued to be synthesized when labeling was performed in the presence of BFA (Fig. 2). These results indicate that all of the enzymes required for the sequential conversion of ceramide to GlcCer, LacCer, and GM3 were able to act on their respective substrates after the BFA-induced redistribution of the Golgi. These findings are thus consistent with the localization of the GM3 synthase (sialyltransferase I; see Fig. 4) to the cis Golgi (3).

Human melanoma cells M1733 and SK-MEL-28 have been shown to contain two major gangliosides, GM3 and the disialoganglioside GD3 (8, 20, 21). Both of these structures were synthesized in the presence and absence of BFA (Fig. 2). Hence, both GM3 synthase (sialyltransferase I) and GD3 synthase (sialyltransferase II; see Fig. 4), responsible for conversion of GM3 to GD3 (see Fig. 4), must be located in

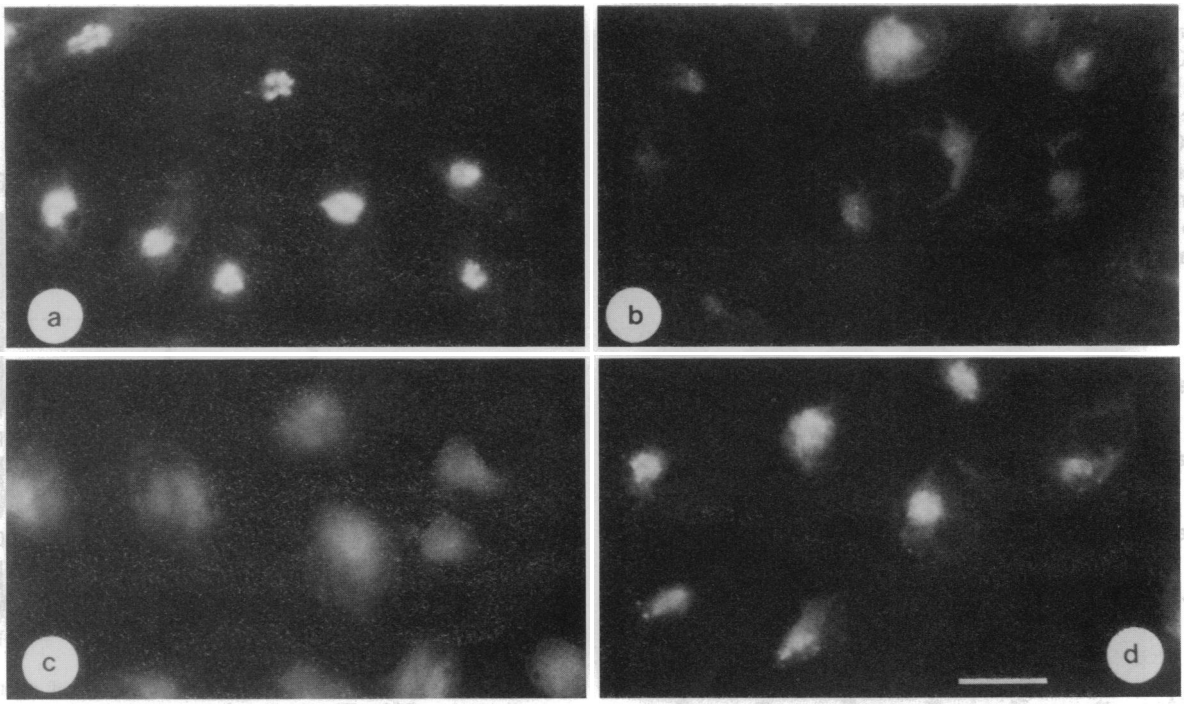

FIG. 1. Time course of BFA-induced disruption and recovery of the Golgi apparatus. CHO cells were incubated either in the absence of BFA (control, *a*) or with BFA at 1 μg/ml for 30 min (*b*) or for 2 hr (*c*). Alternatively, cells were incubated for 2 hr with BFA and then chased in fresh, untreated medium for 30 min to allow recovery from BFA treatment (*d*). The cells then were fixed, labeled with NBD-Cer, and back-exchanged as described. (Bar = 20 μm.)

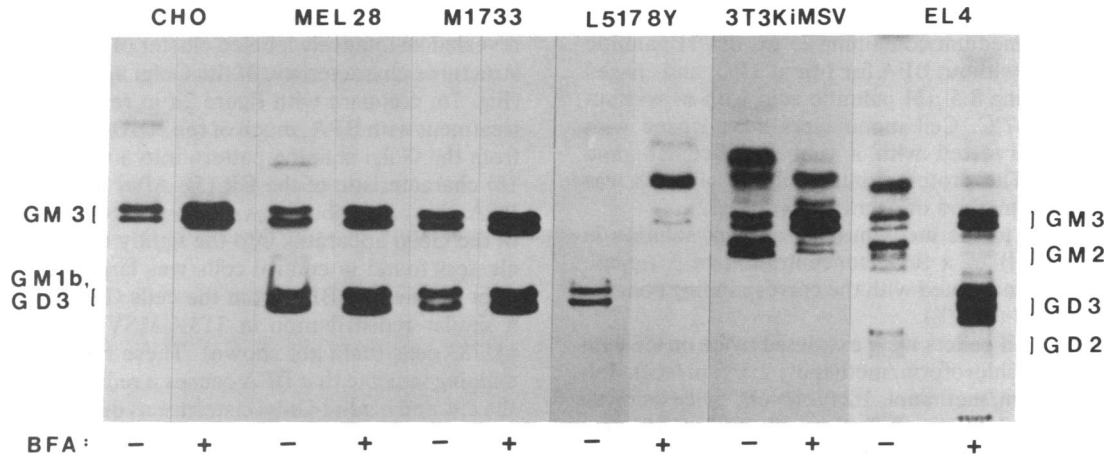

FIG. 2. Effect of BFA on the synthesis of gangliosides. Cells were labeled with [^{3}H]palmitate for 1 hr and chased for 4 hr in the presence or absence of BFA at 1 μg/ml as described. Cells were harvested, GSL were purified, aliquots of acidic GSL were chromatographed on a high-performance TLC plate in solvent B, and labeled bands were visualized by fluorography. The bands that migrated faster than GM3 were nonglycolipid substances that remained in the GSL fraction throughout purification. The monosialogangliosides (GM) and disialogangliosides (GD) are as follows: GM1b: NeuAc(α2→3)Gal(β1→3)GalNAc(β1→4)Gal(β1→4)GlcCer, GM2:GalNAc(β1→4)[NeuAc(α2→3)]-Gal(β1→4)GlcCer, GM3: NeuAc(α2→3)Gal(β1→4)GlcCer, GD2: GalNAc(β1→4)[NeuAc(α2→8)NeuAc(α2→3)]Gal(β1→4)GlcCer, GD3: NeuAc(α2→8)NeuAc(α2→3)Gal(β1→4)GlcCer. MEL28, SK-MEL-28 cells.

compartments that continued to have access to their substrates following BFA-induced redistribution.

As previously described (22), murine lymphoma cells L5178Y synthesized the neutral GSL GlcCer, LacCer, and GA2 [GalNAc(β1→4)Gal(β1→4)GlcCer] (Fig. 3) and ganglioside GM1b (Fig. 2) when labeling was performed in control medium. In striking contrast, the labeling of GA2 (Fig. 3) and GM1b (Fig. 2) was markedly decreased by incubation in the presence of BFA, while the levels of the precursors LacCer and GM3 were increased as compared with the control.

One possible explanation for these findings was that the enzymes responsible for GA2 and GM1b synthesis might be located in a compartment, possibly late in the intracellular trafficking pathway, which in the presence of BFA remained functionally distinct from the labeled substrates in the ER. Since GM1b is downstream from GA2 in the proposed biosynthetic pathway (Fig. 4), we explored the possibility that the GalNAc-transferase responsible for converting LacCer to GA2 might be located in such a compartment. In rat liver this enzyme also catalyzes the conversion of GM3 to GM2 and GD3 to GD2 (23). Therefore, we tested other cell lines to determine if the synthesis of GA2, GM2, and GD2 was inhibited by BFA.

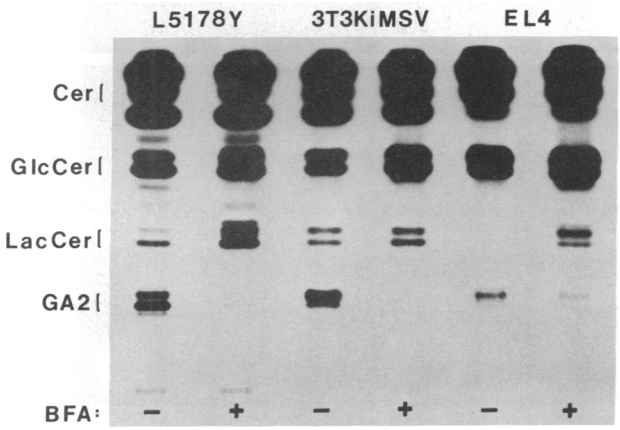

FIG. 3. Effect of BFA on the synthesis of neutral GSL. Neutral GSL were purified from labeled cells as described and chromatographed in solvent A.

Ki-MSV-transformed BALB/3T3 cells contain GM2 as the major ganglioside plus a lesser amount of GM3 (24, 25), and the K-234 clone of those cells, which we call 3T3KiMSV in this paper, also synthesizes high levels of GA2 (10). Whereas this pattern was obtained by palmitate labeling in control medium (Figs. 2 and 3), in the presence of BFA the labeling of GA2 and GM2 was markedly inhibited while the level of GM3 increased. Similarly, murine lymphoma cells EL4 have been shown to contain gangliosides GM2 and GD2 (26). In control medium, clone AF4 of EL4 synthesized the neutral GSL GlcCer, LacCer, and GA2 and the gangliosides GM3, GM2, and GD2 (Figs. 2 and 3). When labeling was performed in the presence of BFA, the levels of GA2, GM2, and GD2 were decreased while the labeling of their respective precursors LacCer, GM3, and GD3 increased (Figs. 2 and 3).

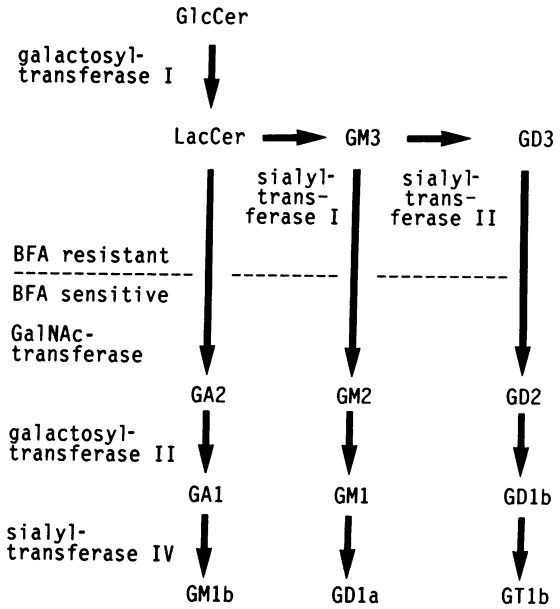

FIG. 4. Proposed site for inhibition by BFA of synthesis of ganglio-series GSL. The model for pathways of synthesis was adapted from ref. 23 in which the same GalNAc-transferase was shown to catalyze conversion of LacCer to GA2, GM3 to GM2, and GD3 to GD2. Synthesis of those GSL above the broken line continued in the presence of BFA, while those below the line were blocked.

Table 1. Effect of BFA on GSL biosynthesis

Exp.	Total cpm incorporated into GSL per mg of protein*	Relative labeling,† % of total cpm incorporated into GSL						
		GlcCer	LacCer	GA2	GM3	GM2	GD3	GD2
3T3KiMSV cells								
Control	50,000	62.3 ± 6.0	8.2 ± 1.2	10.9 ± 5.1	4.3 ± 1.6	14.4 ± 1.7	NA	NA
With BFA	250,000	90.2 ± 0.2	2.7 ± 0.1	0.2 ± 0.1	6.4 ± 0.3	0.6 ± 0.1	NA	NA
EL4 cells								
Control	300,000	94.4 ± 0.9	0.6 ± 0.1	2.1 ± 0.4	0.3 ± 0.1	1.4 ± 0.2	0.2 ± 0.04	1.1 ± 0.41
With BFA	910,000	94.9 ± 0.8	1.0 ± 0.3	0.2 ± 0.1	1.2 ± 0.2	0.2 ± 0.01	2.4 ± 0.5	0.3 ± 0.1

Cells were labeled with [^{3}H]palmitate for 1 hr and chased for 4 hr in the presence or absence of BFA at 1 μg/ml as described. Cells were harvested, GSL were purified, GSL aliquots were chromatographed on high-performance TLC plates, labeled bands were visualized by fluorography, and individual GSL regions were scraped and assayed for radioactivity.

*Because of differences in total incorporation of radiolabeled precursors between experiments, values are presented for individual experiments; however, incorporation into total GSL was consistently several fold higher in the presence of BFA as compared with control.

†Values are the mean ± SEM for two separate experiments for 3T3KiMSV cells and four experiments for EL4 cells. NA, not applicable (the GSL was not synthesized in these cells).

Quantitation of these labeling studies in 3T3KiMSV and EL4 cells is shown in Table 1. There was a marked increase in incorporation of labeled palmitate into the total GSL in the presence of BFA, which primarily was due to increased labeling of GlcCer. The most striking feature of these studies was the marked decrease in product to precursor ratio for each of the steps of GalNAc addition (Table 2).

Controls. One possible explanation for the apparent inhibition of synthesis of GalNAc-containing GSL by BFA was that these GSL were in fact being synthesized but were then being released from the cell. To address this possibility, the culture medium from an L5178Y cell labeling experiment was extracted, and the purified GSL fraction was analyzed. We previously showed that a low level of GSL was shed from these cells in culture; during a 46-hr labeling period 8–9% of labeled GA2 was released (9). During the 1-hr labeling and 4-hr chase period of the present experiments, no detectable GA2 was released into either the control or BFA medium (<2% of the control cell content of GA2).

An alternative explanation for the apparent inhibition of synthesis of GalNAc-containing GSL by BFA was that these GSL were being synthesized but were then being degraded, perhaps by a hexosaminidase that could interact with substrate in the redistributed Golgi/ER. To address this question, 3T3KiMSV cells were labeled for 1 hr and chased for 4 hr in control medium. Samples were then either harvested or chased for an additional 4 hr in the presence or absence of BFA. In control medium the labeling of GM2 increased during the additional 4 hr of chase (Table 3), resulting in an increase in the ratio of GM2 to GM3. When the additional chase was performed in the presence of BFA, there was a more modest increase in GM2, while the labeling of GM3 increased significantly, thus causing the GM2/GM3 ratio to decrease below the 4-hr value. However, this ratio was still much higher than that for cells in which BFA was present during the entire labeling and chase period. The fact that the GM2 level increased slightly during the second chase period in the presence of BFA rather than being lower than the value at the end of the first 4-hr chase argues against degradation of GalNAc GSL as being responsible for the low levels of these compounds found when labeling and chase were performed in the presence of BFA.

One feature of BFA is its reversibility (5, 6). In contrast to the rapid restoration of Golgi morphology (Fig. 1*d*) and secretion of glycoproteins after BFA washout (5, 6), preliminary studies in L5178Y cells indicated that the inhibition by BFA of GA2 and GM1b synthesis was slowly reversed over

Table 2. Ratio of GalNAc-transferase product to precursor synthesized in the presence or absence of BFA

Experimental conditions	Ratio of GalNAc-containing product to precursor				
	3T3KiMSV cells		EL4 cells		
	GA2/ LacCer	GM2/ GM3	GA2/ LacCer	GM2/ GM3	GD2/ GD3
4-hr chase					
Control	1.7	2.2	1.3	4.0	3.5
With BFA	0.1	0.1	0.1	0.2	0.1
17-hr chase					
Control	1.2	6.8	2.4	1.1	3.3
Washout	0.5	2.0	1.1	1.9	1.6

Cells were treated as follows. In the control experiment with 4-hr chase, cells were preincubated in control medium in the absence of BFA for 30 min, labeled with [^{3}H]palmitate for 1 hr in the absence of BFA, and chased for 4 hr in the absence of BFA. In the control experiment with 17-hr chase, cells were treated as described for the 4-hr-chase control sample, rinsed in BFA-free medium, and cultured without BFA for an additional 13 hr. In the experiment with BFA and a 4-hr chase, cells were preincubated for 30 min in medium containing 1 μg of BFA per ml, labeled with [^{3}H]palmitate for 1 hr in the presence of BFA, and then chased for 4 hr in the presence of BFA. In the 17-hr-chase experiment with washout, cells were treated as described for the sample chased for 4 hr in the presence of BFA, rinsed in BFA-free medium, and then cultured without BFA for an additional 13 hr. Incorporation into individual GSL was quantitated as described in Table 1.

Table 3. Degradation control experiment with 3T3KiMSV cells

Protocol		[^{3}H]Palmitate incorporation, cpm per flask		Ratio
No.	Description	GM3	GM2	GM2/GM3
I	4-hr chase	9,240	49,900	5.4
II	8-hr chase	8,460	75,520	8.9
III	Protocol I + 4-hr chase with BFA	31,380	60,140	1.9
IV	BFA in preincubation, labeling, and 4-hr chase	107,030	8,066	0.08

Cells were treated as follows. In protocol I, cells were labeled with [^{3}H]palmitate for 1 hr without BFA and then chased for 4 hr without BFA. In protocol II, cells were labeled with [^{3}H]palmitate for 1 hr without BFA and chased for 8 hr without BFA. In protocol III, cells were labeled with [^{3}H]palmitate for 1 hr without BFA, chased for 4 hr without BFA, and then chased for 4 hr in medium containing BFA at 1 μg/ml. In protocol IV, cells were preincubated for 30 min in medium containing BFA at 1 μg/ml, labeled with [^{3}H]palmitate for 1 hr in the presence of BFA, and chased for 4 hr in the presence of BFA. Cells were harvested, acidic GSL were purified, aliquots were chromatographed on high-performance TLC plates, labeled bands were visualized by fluorography, and individual GSL regions were scraped and counted.

a period of several hours (data not shown). This slower recovery of GSL synthesis may reflect the slow kinetics of GSL synthesis seen in these cells in control medium (22). Consequently, recovery from BFA inhibition was assayed by washing out BFA after 4 hr of chase and harvesting cells after 17 hr of chase in all cell types tested in the present study. Table 2 indicates that labeled precursors that had accumulated during the BFA block were converted to GalNAc-containing products during the washout period. Thus, the inhibition of synthesis of GalNAc-containing ganglio-series GSL by BFA was reversible following BFA washout and was not due to irreversible toxicity of the drug.

DISCUSSION

BFA causes the accumulation of secretory proteins in the ER because of disassembly of the Golgi complex (4). Initial results indicated that BFA caused the redistribution of the cis- and medial-Golgi cisternae such that they became morphologically and functionally a part of the ER (5). However, recent results have demonstrated that the trans-Golgi stacks are redistributed by BFA as well (7, 16, 17). The BFA-induced redistribution of NBD-Cer (Fig. 1), a marker for the trans Golgi in fixed cells (11), is consistent with these latter findings.

In agreement with the recent report of van Echten *et al.* (27), the sialyltransferases responsible for synthesizing GM3 and GD3 continue to act on their substrates in the presence of BFA (Fig. 2), thus localizing them to Golgi stacks, which are redistributed by BFA. In contrast, GA2/GM2/GD2 synthase must be located in a compartment that remains functionally distinct from the ER in the presence of BFA because BFA blocks the synthesis of the products of this enzyme. Because the trans- as well as the cis- and medial-Golgi stacks are redistributed by BFA, the most likely location for this GalNAc-transferase is the trans-Golgi network. In a proposed model for ganglioside synthesis (ref. 23; Fig. 4), this enzyme catalyzes an early step in the synthesis of all higher gangliosides. Therefore, all enzymes acting subsequent to GalNAc addition must also be located in a late, post-Golgi compartment. These results are consistent with the recent localization of GM3 synthase (sialyltransferase I; Fig. 4) to Golgi stacks of rat liver that are cis to the location of sialyltransferase IV (Fig. 4), which synthesizes GD1a (3). Thus, the present findings using BFA provide additional support for a model of maturation of GSL oligosaccharide formation occurring during passage through the ER-to-Golgi intracellular pathway.

Although the BFA-induced block in synthesis of GalNAc-containing ganglio-series GSL was dramatic (Table 2), it was not absolute as shown in Figs. 2 and 3 and Table 1. This lack of complete inhibition could be due to several possibilities. The first, that the dose of BFA was insufficient, is unlikely because experiments using 5 μg/ml produced the same level of inhibition (data not shown) as those with 1 μg/ml. Second, the BFA block may have been incomplete because of metabolism of BFA by the cells. This point merits further experimentation because of conflicting reports in the literature. Whereas Fujiwara *et al.* (16) reported reassembly of the Golgi when cells were exposed to 2.5 μg of BFA per ml for 4 hr, Ulmer and Palade (7) could not detect morphologic evidence for Golgi reorganization even after 6 hr of exposure to 1 μg of BFA per ml. Third, the incomplete block by BFA may reflect the dynamic relationship between the Golgi and the trans-Golgi network (28). While the majority of GA2/GM2/GD2 synthase may be located trans to the BFA block, a percentage of enzyme molecules may reside in elements of the trans-Golgi stacks that are redistributed by BFA. The relative proportions of enzyme found in the two locations may vary between cell types, which could explain the greater inhibition seen in 3T3KiMSV cells than in L5178Y and EL4 cells (Figs. 2 and 3).

BFA should be useful in future kinetic studies to determine more precisely the locations of early glycosylation steps and the enzymes responsible for addition of the terminal sugars for other families of GSL such as the lacto-, globo-, and galactosylceramide-based series (29).

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