BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **243,** 277–283 (1998)
ARTICLE NO. RC978036

Reduced Expression of the Macrophage Scavenger Receptors in Macrophage-like Cell Mutants Resistant to Brefeldin A

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Received December 15, 1997

Brefeldin A (BFA)-resistant mutants, JB15, JB23 and JB33, were isolated from mutagenized murine macrophage-like (J774) cells and their modified low density lipoprotein (LDL) metabolism was studied. When JB23 cells, the most resistant clone, were incubated with acetylated LDL, intracellular accumulation of cholesteryl esters (CE) was reduced by 31% as compared with J774 cells. The cell-association of ^{125}I-acetyl-LDL with, and subsequent endocytic degradation by JB23 cells were reduced by 40–60% compared with J774 cells. Western and Northern blot analyses showed that the protein and mRNA levels of the macrophage scavenger receptors (MSR) were reduced by 68% and 55% respectively in JB23 cells as compared with those in J774 cells. These results indicate that a putative BFA-target molecule(s) might regulate MSR gene expression as well as macrophage-derived foam cell formation.

Several genes essential for cellular cholesterol metabolism are identified by molecular and somatic cell genetics approach [1]. For one thing, cDNA for 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a rate limiting enzyme of the mevalonate pathway, was cloned by using a somatic cell mutant overexpressing this enzyme [2]. The second example was the recent study of a Chinese hamster ovary (CHO) cell mutant deficient in acyl-coenzyme A:cholesterol acyltransferase (ACAT), an intracellular cholesterol esterification enzyme, by Chang and his associates, which led to a successful molecular cloning of human ACAT cDNA [3]. Furthermore, Chang and Limaneck [4] isolated a mutant line resistant to the cytotoxic effect of oxysterol (25-RA) which lacked negative feedback regulation of cholesterol homeostasis by the low density lipoprotein (LDL) receptor or HMG-CoA reductase. Subsequent analysis of this mutant has revealed a molecular structure of sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP), a protein crucial for cellular cholesterol homeostasis [5]. These examples demonstrated that somatic cell mutants provide an invaluable tool to explore the cellular regulation of cholesterol metabolism.

The regulation of cholesterol accumulation in macrophages is believed to be a key step in atherogenesis, because the early atherosclerotic lesions are characterized by the presence of cholesteryl ester (CE)-loaded foam cells derived from macrophages [6]. Macrophages are known to take up chemically modified LDLs, such as acetylated LDL (acetyl-LDL) and oxidized LDL (Ox-LDL) by the macrophage scavenger receptor pathways [7, 8], leading eventually to CE accumulation in the cytoplasm as lipid droplets which are recognized as foam cells [9]. However, the regulatory mechanism for macrophage-derived foam cell formation, especially regulatory molecules involving in this process, is not fully understood. Therefore, somatic cell genetical approach using macrophages is a thinkable method to study the regulation of foam cell formation.

Brefeldin A (BFA), a lipophilic fungal metabolite, is known to affect intracellular cholesterol metabolism presumably by disassembling the Golgi stacks [10-12]. BFA stimulates CE synthesis in CaCo2, HepG2, mouse peritoneal macrophages and J774 cells [13, 14] and inhibits high density lipoprotein (HDL)-mediated cholesterol efflux from human skin fibroblasts [15]. On the other hand, it was also reported that BFA does not

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Abbreviations used are: BFA, brefeldin A; MSR, macrophage scavenger receptors; acetyl-LDL, acetylated low density lipoprotein; ACAT, acyl-coenzyme A:cholesterol acyltransferase; CE, cholesteryl esters, MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; EMS, ethyl methanesulfonate; β-Me, 2-mercaptoethanol.

0006-291X/98 $25.00

influence the transport of newly synthesized (endogenous) cholesterol [16] and phosphatidylethanolamine [17] from sites of synthesis to the plasma membrane. However, the exact molecular mechanisms of BFA effects on cellular cholesterol metabolism are not clear at present. Therefore, somatic cell genetics using macrophage cell mutants resistant to BFA may be an appropriate approach to study the Golgi-related mechanism for foam cell formation.

In the present study, we started to isolate mutant cells resistant to BFA from J774 cells, a cell line derived from mouse macrophages, and were successful in obtaining three mutant cells. Our subsequent characterization of one BFA-resistant mutant cell called JB23 showed that a modified LDL-induced CE accumulating capacity of this mutant cell is reduced due largely to the reduction of the macrophage scavenger receptors (type I MSR and type II MSR) activity, a first step of foam cell formation.

MATERIALS AND METHODS

Materials. BFA was purchased from Wako Pure Chemical Industries (Osaka, Japan) and was stored at −20°C as a stock solution of 5 mg/ml in 100% ethanol. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Dojindo Laboratories (Kumamoto, Japan). [α-^{32}P]dCTP (370 MBq/ml) and Na^{125}I (3.7 GBq/ml) were purchased from Amersham International (Amersham, Bucks, UK). Fetal calf serum (FCS) was purchased from HyClone Laboratories Inc. 2F8 (rat anti-mouse MSR monoclonal antibody) was purchased from Serotec (Oxford, England) [18]. Cell culture media and reagents were obtained from Life Technologies Inc. (NY). All other chemicals were the best grade from commercial sources.

Lipoproteins and their modifications. LDL (d=1.019−1.063 g/ml) was isolated by sequential ultracentrifugation of fresh human plasma and dialyzed against 0.15 M NaCl and 1 mM ethylenediamine tetra-acetic acid (EDTA) (pH 7.4). Acetyl-LDL was prepared by chemical modification of LDL with acetic anhydride as described previously [19, 20]. Iodination of acetyl-LDL with ^{125}I was performed as described previously [21] based on the McFarlane's method [22]. The protein concentration was determined by BCA protein assay reagent (Pierce Chemical Co.) using bovine serum albumin (BSA) as a standard [23].

Cell culture. Unless otherwise specified, cell cultures were performed at 37°C in 5% CO_2. J774 cells and their BFA-resistant cell lines were cultured in RPMI1640 medium containing 10% FCS, 10 mM N-2-hydroxyethyl-1-piperazine-N′-2-ethanesulfonic acid (HEPES) (pH 7.4), 0.1 mg/ml streptomycin and 100 U/ml penicillin (medium A). Dulbecco's modified Eagle's medium (DMEM) containing 3% bovine serum albumin (BSA), 10 mM HEPES (pH 7.4), 0.1 mg/ml streptomycin and 100 U/ml penicillin (medium B) was used for association and degradation assay.

MTT assay. Cell growth was determined by the MTT method [24]. Briefly, 50 μl of a suspension of JB23 cells or J774 cells (5×10^3 cells) was seeded to each well of 96-well tissue culture plates (7-mm diameter, Falcon). Cells were incubated for 2 h and BFA (0, 0.1, 0.2, 0.5, 0.7 and 1.0 μg/ml) was added to each well, followed by further incubation for 0, 1, 3, 5 and 7 days. At indicated time intervals, 10 μl of 5 mg/ml of MTT was added to each well, followed by 4-h incubation to reduce MTT to a blue formazan product. In the next step, 150 μl of 10% SDS in 0.01 N HCl was added to each well and the mixture was further incubated overnight to dissolve the blue formazan product. The absorbance of each well was then measured at 570 nm using a multiwell spectrophotometer.

Isolation of BFA-resistant mutants from J774 cells. To select BFA-resistant mutants, J774 cells (1×10^6) were plated into a 75-cm^2 tissue culture flask (Falcon) in 20 ml of medium A and incubated for 48 h. The medium was removed and the cells were treated with 400 μg/ml of ethylmethane sulfonate (EMS) in 15 ml of medium A for 16 h to a survival rate of 10% of the initial cell number [25]. The cells were washed three times with PBS and cultured for additional two days in 20 ml of medium A without the mutagenic agent. The harvested mutagenized cells (5×10^5/dish) were seeded into 10 dishes (10 cm in diameter) and incubated for two weeks with 1 μg/ml of BFA in 10 ml of medium A. Each colony thus formed was transferred with a cloning cup to a culture dish containing 1 μg/ml of BFA in 10 ml of medium and incubated for two weeks. Three colonies, named JB15, JB23 and JB33, were subjected to further incubation for 3 months in the absence of BFA, and their BFA-resistant property was determined by colony formation described below. Three BFA-resistant clones were independently isolated from different dishes. One of these clones, JB23, was investigated in the present study.

Cell survival by colony formation. Cell survival of BFA-resistant mutant cells and J774 cells in the presence of BFA were measured by colony formation [26]. Three hundred cells were plated in duplicate at 10 cm dishes with medium A, incubated for 18 h and then exposed for 10 days to various doses of BFA. Colonies appearing after incubation for 10 days were counted.

Determination of cellular cholesterol mass. JB23 and J774 cells (2×10^5) in 1 ml of medium A were seeded into each well of 24-well plate (16 mm in diameter, Falcon) and incubated overnight. Cell monolayers were incubated for 12 h with 50 μg/ml of acetyl-LDL and cellular lipids were extracted, followed by determination of both free cholesterol (FC) mass and total cholesterol (TC) mass by a modification of the enzymatic fluorometric methods [27, 28]. Briefly, cellular lipid extracts were dried under nitrogen and dissolved in 180 μl of isopropanol. Aliquots (30 μl) of the lipid extract were added to 0.4 ml of each mixture of enzymes and incubated at 37°C for 1 h (for FC) or for 2 h (for TC), followed by the addition of 0.81 ml of 0.5 M NaOH to terminate the reaction. The enzyme mixture for determination of TC mass was identical to theirs [27] except that Carbowax-6000 was replaced by 0.01% Triton X-100 and enzyme concentrations were 2-times higher (cholesterol oxidase; 0.16 U/ml and cholesteryl ester hydrolase; 60 U/ml). The enzyme mixture for determination of FC mass was identical to the enzyme mixture for determination of TC mass except that cholesteryl ester hydrolase was not added. Fluorescence intensity was measured with an excitation at 320 nm and an emission at 407 nm. CE was calculated by subtracting the free cholesterol from total cholesterol. Cells were dissolved in 0.1 M of NaOH to determine cell proteins using a BCA protein assay reagent [23].

Association and degradation of ^{125}I-acetyl-LDL. To determine the cell-association of ^{125}I-acetyl-LDL, and their endocytic degradation by macrophages, JB23 and J774 cells (4×10^5) in 1 ml of medium A were seeded into each well of 24-well plate and incubated overnight. The monolayers thus formed were incubated for 12 h with various amounts of ^{125}I-labeled acetyl-LDL (0, 1, 2, 5 and 10 μg/ml) for total cell-association and degradation. To determine the nonspecific cell-association and degradation, parallel incubations were performed in the same way in the presence of 20-fold excess of unlabeled ligands. After incubation, 0.75 ml of the culture medium was taken from each well and used for determination of endocytic degradation, while the cells in each well were used for determination of cell-association. To determine endocytic degradation, 0.25 ml of ice-cold 40% TCA and 0.2 ml of 0.7 M $AgNO_3$ were added to 0.75 ml of culture medium and the mixture was incubated at room temperature for 30 min to precipitate free iodine [29]. After centrifugation at 700 g for 10 min, 0.6 ml of the resulting supernatant was used for the determination of radioactivity using a gamma-counter [30]. The cells were

washed once with 1 ml of PBS containing 0.3% BSA and twice with 1 ml of ice-cold PBS, and then dissolved in 0.1 M of NaOH. The cell-associated radioactivity was determined as described previously [31]. The specific cell-association and degradation was calculated by subtracting nonspecific cell-association and degradation from the total cell-association and degradation.

Northern blot. The probes for Northern blot were prepared as follows. Partial cDNA for murine MSR (Probe A) was synthesized from total RNA of J774 cells by RT-PCR [32, 33]. Sense and antisense primer pairs were 5′-CGCCGAGCGGCCGCGCTGTCTTCTTTA-CCAGC-3′ and 5′-CGCCGGTCTAGATTATACTGATCTTGATCCGC-3′. The cDNA was labeled with [α-^{32}P]dCTP by the random primer method. The random nonamer primer was added to 25 ng template cDNAs (nucleotides 1 to 1070 bp of mouse MSR coding sequence; nucleotides 71 to 1053 bp of human GAPDH coding sequence as a control [34]). After incubation for 5 min at 95°C, they were incubated with Klenow enzyme and [α-^{32}P]dCTP for 1 h at 37°C. Unincorporated radioactive nucleotides were removed by Sephadex G-50 Quick Spin Column.

Poly (A)$^+$ RNA was isolated from J774 cells or JB23 cells using Fast Track 2.0 Kit (Invitrogen). Two micro gram of Poly (A)$^+$ RNA per lane was separated by electrophoresis in a formaldehyde-containing 1% agarose gel [35]. The RNAs were transferred to Hybond-N+ nylon membrane (Amersham) by capillary transfer with 10 × SSC for 20 h and cross-linked by UV (FS 1500, Funakoshi). The membrane was prehybridized with a hybrid solution containing Hybridization Buffer Tablets (Amersham) in 50% formamide, 0.1 mg/ml salmon sperm DNA at 42°C for 2 h, and then heat-denatured [^{32}P]labeled probe of MSR (final 6 ×10^6 cpm/ml) was added into a fresh hybrid solution and hybridized for 24 h at 42°C. The membrane was washed in 1 × SSC/0.1% SDS and 0.2 × SSC/0.1% SDS at 46°C [35]. The count during successive washes was monitored by using a Geiger counter. The membrane was then exposed to a Fuji Imaging Plate BAS-III (Fuji Photo Film Co.) for 2 h at room temperature and analyzed using a BAS-2000 II (Fuji X). The intensity of MSR mRNA relative to that of GAPDH was calculated by densitometry of the radiograph.

Western blot. J774 cells and JB23 cells were plated in 10 ml of medium A in 10 cm dishes. After confluence, the cells (8 × 10^6 cells/dish) were harvested with PBS containing 10 mM EDTA. These cells were re-suspended in 0.5 ml of 50 mM Tris-HCl/1 mM EDTA (pH 7.7) and homogenized for 20 strokes with Teflon Homogenizer (EYELA). The cell lysates were boiled for 30 seconds with or without thiol reducing agents (2-mercaptoethanol, final concentration 5%) and 20 μg protein per lane were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 4-20% gel), transferring to nitrocellulose membrane. The nitrocellulose membrane was blocked with 5% non-fat dry milk (Carnation, CA) and 0.02% NaN_3 in buffer A (20 mM Tris-HCl, pH 7.6, 150 mM NaCl and 0.3% Tween-20) for 1.5 h at room temperature and then incubated with 1.25 μg/ml rat anti-mouse MSR IgG, 2F8 (Serotec, Oxford, UK) in buffer A containing 1% non-fat dry milk (Carnation) and 0.02% NaN_3 for 4 h at room temperature [18]. The membrane was washed with buffer A then incubated with buffer A containing 1% non-fat dry milk (Carnation) and peroxidase-conjugated goat anti-rat IgG antibody (1:1000) (Amersham) for 1 h. The membrane was washed with buffer A and then buffer B (20 mM Tris-HCl, pH 7.6 and 150 mM NaCl) and detected by chemiluminescence (ECL, Amersham). The intensity of MSR protein of JB23 cells relative to that of J774 cells was calculated by densitometry of the fluorograph.

RESULTS

Selection of BFA-Resistant Cell Lines from J774 Cells

Preliminary experiments with MTT assay in the parent cell line J774 showed that BFA at a concentration as low as 0.25 μg/ml inhibited the growth of J774 cells while concentrations in excess of 1 μg/ml of BFA induced death of all J774 cells. This demonstrated the feasibility of selecting resistant cell lines by adding the drug to the growth medium. BFA-resistant colonies were selected by incubating ethyl methanesulfonate (EMS)-mutagenized J774 monolayers with 1 μg/ml BFA as described under "Materials and Methods." Resistant colonies appeared after 2 weeks of continuous growth. One such selection yielded three individual cell lines from ten dishes (1 × 10^7 total cells), named JB15, JB23 and JB33. All these mutants did not show any significant morphological changes (data not shown).

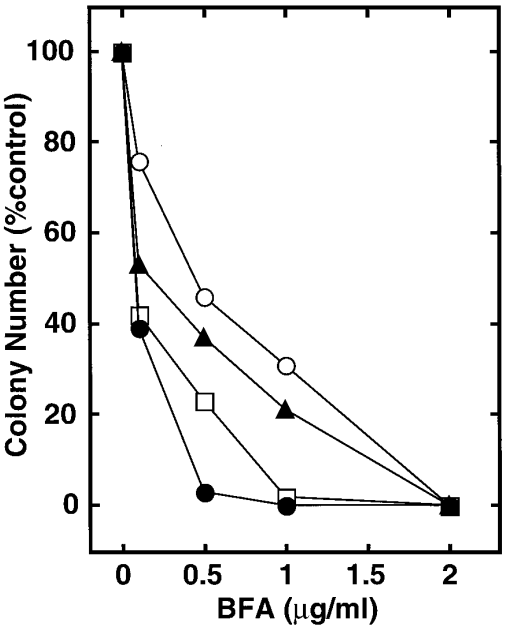

FIG. 1. Dose-response to BFA of BFA-resistant mutants and J774 cells. Cells of four clones were plated in 10-cm dishes at 300 cells/ dish with 10 ml of medium A. After 20 h, brefeldin (BFA) at different concentrations was added to each dish. After incubation at 37°C for ten days, the obtained colonies of J774 (●), JB15 (▲), JB23 (○) and JB33 (□) were counted. Data are representative of experiments with duplicate dishes.

Colony Formation Ability of BFA-Resistant Cell Lines

The mutant cell lines were first characterized by examining their resistance to the cytotoxic effect of BFA. Using the cell survival assay as described in "Materials and Methods", the colony-forming properties of wild type (J774) and 3 mutant lines (JB15, JB23 and JB33) were compared. As shown in Fig. 1, mutants and parent cells showed an apparent resistance to 0.1 μg/ml of BFA, but the surviving fraction of mutant cells was higher than J774 in the presence of 0.5 μg/ml BFA. At 1 μg/ml BFA, the colony formation of J774 cells was completely inhibited, while those of JB15, JB23 and JB33 cells decreased to 21%, 31% and 2%, respectively.

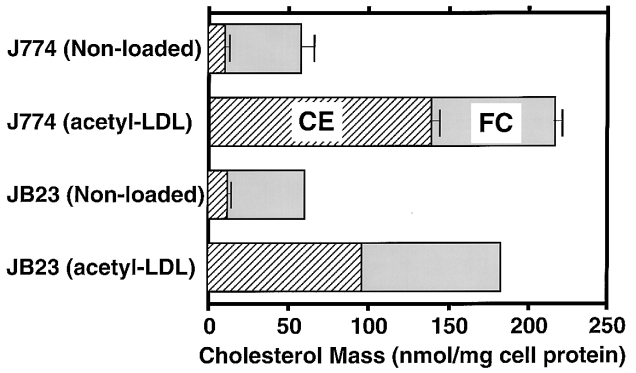

FIG. 2. The cellular cholesterol mass of JB23 cells. JB23 or J774 cells (2×10^5) in 16-mm plates were converted to foam cells by incubation for 12 h with 50 μg/ml of acetyl-LDL. After washing with PBS containing 0.3% BSA and PBS, the cellular lipids were extracted for determination of cholesterol mass by using the enzymatic fluorometric assay as described under "Materials and Methods." CE: cholesteryl esters, FC: free cholesterol. Data are representative of three separate experiments with quadruplicate wells. Bars represent SD.

These results indicate that the mutant cell lines are indeed resistant to BFA compared with the parent cell lines. Since JB23 cells were the most resistant to the cytotoxic effect of BFA among the three mutants, this line was used in the following study.

Effect of Acetyl-LDL on CE Accumulation in JB23 Cells

In the next step, we examined the capacity of JB23 cells to accumulate CE intracellularly when incubated with acetyl-LDL. JB23 and J774 cells as a control were incubated for 12 h with or without 50 μg/ml of acetyl-LDL and cellular lipids were extracted, followed by determination of FC and CE masses. As shown in Fig. 2, without acetyl-LDL (Non-loaded), the levels of cellular CE and FC in JB23 cells were indistinguishable from those in J774 cells. In contrast, upon incubation with acetyl-LDL, the CE mass in JB23 was 31% lower than that in J774 cells. The level of cellular total cholesterol was also decreased by 16% in JB23 cells. Based on these results, we concluded that the ability of JB23 cells to accumulate CE following exposure to acetyl-LDL was markedly lower than that of J774 cells.

Cell-Association of ^{125}I-Acetyl-LDL with, and Subsequent Endocytic Degradation by JB23 Cells

In the next series of experiments, we used cellular and molecular biological approaches to elucidate the molecular mechanism(s) of the reduced ability of acetyl-LDL-induced foam cell formation in JB23 cells. Since acetyl-LDL is known as a representative ligand for MSR, the interaction of acetyl-LDL with JB23 cells was determined by cell-association and endocytic degradation. As shown in Fig. 3A, the specific cell-association of ^{125}I-acetyl-LDL increased dose-dependently in a saturable manner in both JB23 and J774 cells. However, the specific cell-association of ^{125}I-acetyl-LDL with JB23 cells (0.857 μg/mg cell protein/12 h at 10 μg/ml of acetyl-LDL) was 46% lower than that of J774 cells (1.576 μg/mg cell protein/12 h at 10 μg/ml of acetyl-LDL, Fig. 3A). Similarly, the specific degradation of ^{125}I-acetyl-LDL increased dose-dependently in a saturable manner in both JB23 and J774 cells (Fig. 3B). However, the amount of ^{125}I-acetyl-LDL degraded by JB23 cells (0.668 μg/mg cell protein/12 h at 10 μg/ml of acetyl-LDL) was only 59% of that degraded by J774 cells (1.139 μg/mg cell protein/12 h at 10 μg/ml of acetyl-LDL) (Fig. 3B). These results strongly suggested that the MSR activity of JB23 cells was significantly weaker than that of J774 cells.

Northern and Western Blot Analyses of MSR in JB23 Cells

Northern blot analysis was performed to determine whether MSR expression in JB23 cells was reduced at the mRNA level. Previous studies have demonstrated that two isoforms (type I and type II) of MSR are formed by alternative splicing of transcripts derived from a single gene [36, 37]. Since type I MSR has a single transcript whereas type II MSR has three transcripts [32], we designed probe A to recognize all these four species of transcripts. As shown in Fig. 4A, the experiment using [^{32}P]-labeled probe showed three bands representing type II MSR transcripts

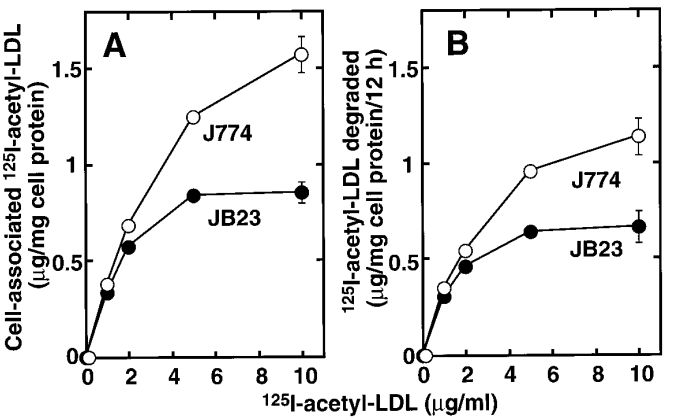

FIG. 3. Cell-association (A) of ^{125}I-acetyl-LDL with, and subsequent endocytic degradation (B) by JB23 cells. JB23 cells and control J774 cells (4×10^5) were incubated in medium A for 20 h at 37°C. After washing three times with PBS, cells were incubated in medium B for 12 h with indicated concentrations of ^{125}I-acetyl-LDL in absence or presence of 20-fold excess unlabeled acetyl-LDL. The amount of the cell-associated (A) ^{125}I-acetyl-LDL and that degraded (B) by J774 (○) and JB23 (●) cells was determined as described under "Materials and Methods." Data are the mean ± SD of representative three separate experiments with duplicate wells. The SD values of some data points are too small and the corresponding bar is within the symbol.

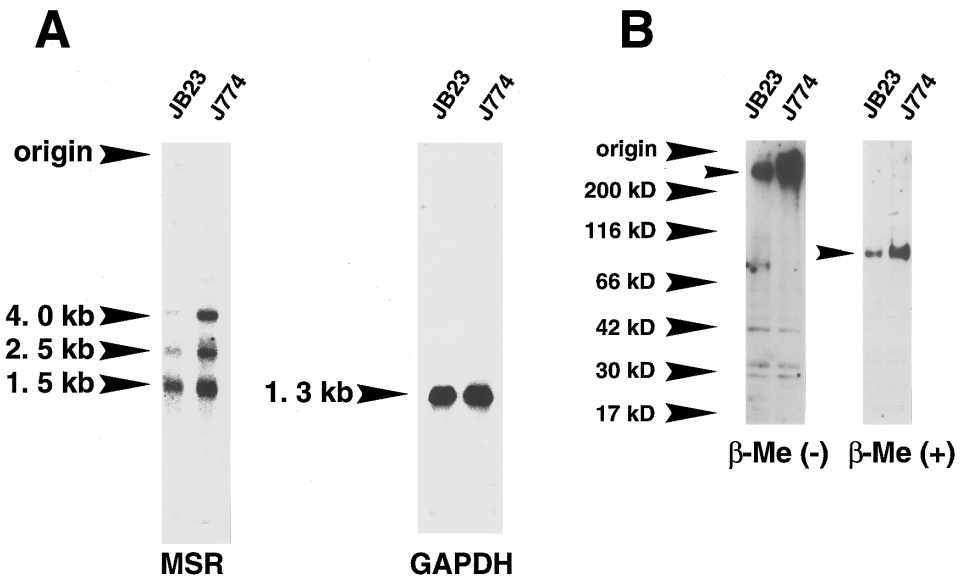

FIG. 4. Northern blot (A) and Western blot (B) analyses of MSR in JB23 cells. (A) Poly $(A)^+$ RNA was isolated from J774 or JB23 cells and 2 μg of each RNA was separated by 1% agarose electrophoresis in a formaldehyde-containing gel. The RNAs were transferred to Hybond-N+ nylon membrane and cross-linked to the membrane by UV irradiation. The membrane was prehybridized for 2 h at 42°C and hybridized with heat-denatured [^{32}P]-labeled probe of MSR and GAPDH for 24 h at 42°C. After washing, the membrane was exposed to a Fuji Imaging Plate BAS-III for 2 h and analyzed. Arrowheads indicate mRNA bands in kb. (B) Cell lysate of JB23 or J774 cells was mixed with sample buffer and boiled for 30 seconds in the absence [β-Me (−)] or presence [β-Me (+)] of 5% 2-mercaptoethanol. Total proteins were separated by 4-20% SDS-PAGE and the MSR protein was recognized by antibody 2F8 as described under "Materials and Methods." The picture presented here is representative of three independent experiments. Arrowheads indicate 70 kDa (monomer) and 210 kDa (trimer) proteins reacted with 2F8 monoclonal antibody.

of 4.0, 2.5 and 1.5 kb, in which type I MSR transcript was probably overlapped by 1.5 kb of type II MSR transcript. The amounts of these four transcripts were significantly lower in JB23 cells than in J774 cells. Parallel experiment showed that the mRNA levels of GAPDH in JB23 cells and J774 cells were indistinguishable from each other. The densitometry units for all MSR bands were divided by the corresponding units of GAPDH. The ratio of MSR mRNA level to the GAPDH level in JB23 cells (1: 0.268) was only 45% of that in J774 cells (1: 0.595). Therefore, the expression of MSR mRNA in JB23 was significantly reduced compared with that in J774.

In parallel studies, we determined the amount of MSR protein by using 2F8, a monoclonal antibody that recognizes both type I MSR and type II MSR [18]. As shown in Fig. 4B, MSR was visualized both in JB23 and J774 cells as a reduced monomeric form of 70 kDa and a nonreduced trimeric form of 210 kDa. The densitometry units for the reduced form in JB23 cells was 32% of that in J774 cells, consistent with the data for mRNA expression (Fig. 4A). Thus, it is likely that the reduced MSR activity in JB23 cells is due to a decreased expression of MSR at both mRNA and protein levels.

DISCUSSION

In the present study, we were successful in preparing BFA-resistant mutant cells from J774 cells. To our knowledge, this is the first report of isolation and characterization of macrophage-like BFA-resistant mutants. BFA is a unique fungal product that selectively inhibits the function of the Golgi apparatus [38]. Incubation of cells with BFA causes the complete disassembly of the Golgi apparatus, resulting in a disruption of anterograde transport from endoplasmic reticulum (ER) to the Golgi apparatus [11]. Under these conditions, the cis- and medial-Golgi membranes and ER membranes fuse with each other to form an ER-Golgi mixed compartment, leading to a dysfunction of the Golgi apparatus and ultimately cell death. In order to understand the mechanism of action of BFA, several somatic cell mutants resistant to the cytotoxicity of BFA have been selected from mutagenized populations of African green monkey kidney Vero cells [39], human epidermoid carcinoma KB cells [40] and Chinese hamster ovary (CHO) cells [41]. Because it is possible that a molecule(s) responsible for resistance in these mutants plays a crucial role in the Golgi function, these BFA-resistant cell lines would be useful to identify a cellular target(s) of BFA or a protein(s) that interacts with such a target(s). Furthermore, if there is a macrophage-specific regulation of Golgi function, our mutant cells may also be useful to identify Golgi-dependent cellular phenomena such as modified LDL-induced CE-accumulation in macrophages.

Membrane proteins recognizing modified LDLs as a

ligand are collectively categorized as a scavenger receptor family which include MSR [36, 37], FcγRII-B2 [42], CD36 [43], SR-BI [44], MARCO [45] and macrosialin [46]. Among these receptors, it is highly likely that MSR plays a central role in the endocytic uptake of acetyl-LDL and Ox-LDL by macrophages or macrophage-derived cells, because the capacity of peritoneal macrophages obtained from MSR-knockout mice to degrade acetyl-LDL and Ox-LDL was 25%, and 35% of those by wild type-macrophages [47]. Two types of proteins, type I MSR and type II MSR, are generated from a single gene by alternative splicing [36, 37]. However, experiments using CHO cells transfected with either type of cDNA showed that the ligand specificity of type I MSR for acetyl-LDL and Ox-LDL was indistinguishable from that of type II MSR [48]. The apparent molecular mass of type I MSR and type II MSR was reported to be ~70 kDa and ~90 kDa, respectively [18]. The present results of Western blot (Fig. 4B) showed that both JB23 cells and J774 cells preferentially express type II MSR rather than type I MSR, because the band detected by 2F8 was a single band of 70 kDa. The dominant expression of type II MSR was also observed in mouse macrophage cell line, RAW264 cells [18]. It is not clear why type II MSR is preferentially expressed in RAW264 cells as well as JB23 cells or J774 cells.

One of the major findings in the present study was that the type II MSR expression in JB23 cells was reduced both at mRNA and protein levels (Fig. 4). We have been investigating the mechanisms of modified LDL-induced macrophage foam cell formation via MSR [21, 24, 31]. A morphological study showed that MSR protein expression is highly restricted in macrophages and macrophage-derived cells in the human body [49]. *In vitro* study showed that MSR mRNA expression is markedly increased when monocytes are differentiated into macrophages [50]. Moreover, previous studies using macrophages have demonstrated that MSR mRNA expression is up-regulated by macrophage colony-stimulating factor, while it is down-regulated by several cytokines such as interferon-γ, tumor necrosis factor-α and transforming growth factor-β [51]. All these results suggest that MSR expression is mainly regulated at a mRNA level in a macrophage specific manner. However, the molecular machinery for the regulation of MSR expression and subsequent macrophage foam cell formation is not fully understood at the present. The present study suggest that one of the regulators of foam cell formation may relate to a target(s) of BFA [13, 14]. However, it is difficult to explain how BFA-resistant phenotype is related with decreased expression of MSR in JB23 cells. Further studies will be needed to explore the molecular basis of this alteration, which would help understand a regulatory role of type II MSR in foam cell formation from macrophages.

ACKNOWLEDGMENTS

We are grateful to Drs. Masakazu Sakai, Takeshi Biwa, and Yu-Ichiro Sakamoto from our laboratory for their endeavor throughout this study. This work was supported by Grant-Aid for Scientific Research (No. 08044304, 09877200 and 09770789) from the Ministry of Education, Science and Culture of Japan.

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