Lipopolysaccharide Increases Cell Surface P-glycoprotein that Exhibits Diminished

Activity in Intestinal Epithelial Cells.

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Abbreviations: BCRP, breast cancer resistance protein; DMEM, Dulbecco's modified Eagle's

media; HRP, horseradish peroxidase; LPS, lipopolysaccharide; MRP-2, multidrug resistance-

associated protein 2; P-gp, P-glycoprotein; PMSF, phenylmethylsulphonylfluoride; Rh123,

Rhodamine 123; TLR4, Toll-like receptor 4.

Abstract

Increasingly, it is recognized that commensal microflora regulate epithelial cell processes through the dynamic interaction of pathogen-associated molecular patterns and host pattern recognition receptors such as Toll-like receptor 4 (TLR4). We therefore investigated the effects of bacterial lipopolysaccharide (LPS) on intestinal P-glycoprotein (P-gp) expression and function. Human SW480 (P-gp+/TLR4+) and Caco-2 (P-gp+/TLR4-) cells were treated with media control or LPS (100 ng/ml) for 24 hours prior to study. P-gp function was assessed by measuring the intracellular concentration of Rhodamine 123 (Rh123). To confirm P-gp specific effects, breast cancer resistance protein (BCRP/ABCG2) and multidrug resistance-associated protein 2 (MRP-2/ABCC2) were also analyzed. Treatment of SW480 cells with LPS led to diminished P-gp activity which could be prevented with polymyxin B (control: 207 ± 16 vs. LPS: 402 ± 22 vs LPS + polymyxin B: 238 ± 26 pmoles Rh123/mg protein, $p<0.05$ control vs. LPS). These effects could be blocked by using polymyxin B and were not seen in the P-gp+/TLR4- Caco-2 cell line (control: 771 ± 28 vs. LPS: 775 ± 59 pmoles Rh123/mg protein). Total cellular levels of P-gp did not change in LPS treated SW480 cells however a significant increase in cell surface P-gp was detected. No change in activity, total protein, or apically located MRP-2 was detected following LPS treatment. Sequence analysis confirmed wildtype status of SW480 cells. These data suggest that activation of TLR4 in intestinal epithelial cells leads to an increase in plasma membrane P-gp that demonstrates a diminished capacity to transport substrate.

P-glycoprotein (P-gp) is arguably one of the best studied members of the ATP-binding cassette (ABC) transporter superfamily. In the last two decades, great advances have been made towards the elucidation of regulatory mechanisms controlling P-gp expression and function in tissues such as the intestinal epithelium.(Geick et al., 2001; Scotto, 2003; Albermann et al., 2005; Burk et al., 2005) Genetic factors such as polymorphisms in the ABCB1 gene and concomitant administration of P-gp inducers and inhibitors influence the level of expression and functionality of the transporter.(Hoffmeyer et al., 2000; Geick et al., 2001) One area which requires greater elucidation relates to the local intestinal environment. Diet has been purported to influence P-gp expression yet more research is required before distinct associations can be made.(Lo and Huang, 1999) In addition to diet, there are undoubtedly other environmental factors which may play a role.

One important environmental interaction that is not well characterized and may influence P-gp relates to the interface of intestinal epithelial cells with commensal bacteria. The human body is constantly exposed to microorganisms that reside in the gastrointestinal tract as part of the commensal flora.(Hooper et al., 1999; Eckburg et al., 2005; Gill et al., 2006) These commensals are vital for both health and disease and serve many important functions including important metabolic activities. Examples include serving as a functional barrier against pathogens and priming of the intestinal immune system.(Hooper and Gordon, 2001; Hooper et al., 2001; MacDonald and Gordon, 2005; Ley et al., 2006; Turnbaugh et al., 2006)

The capacity to discriminate between commensal and pathogen relies in large part on a family of evolutionarily conserved receptors designated as pattern recognition receptors (PRR).(Cario and Podolsky, 2005) One important family of PRR is the Toll-like receptor (TLR) family. To date, eleven TLR have been identified, each with specific ligands.(Akira and Takeda,

2004) For example, the classic ligand which TLR4 recognizes is lipopolysaccharide (LPS) from Gram-negative bacteria.(Poltorak et al., 1998; Cario et al., 2000) Upon LPS binding, a complex signaling cascade is initiated leading to the activation or repression of a number of genes important for host response. Recent data indicate that microbial ligands signaling through TLRs are not limited to those from pathogenic organisms but also from non-pathogen bacteria that make up the commensal flora.(Rakoff-Nahoum et al., 2004) Therefore, it is essential to understand how commensal flora regulate homeostasis in the intestine through TLR signaling.

In the current study, we sought to elucidate the consequences of intestinal epithelial cell exposure to LPS on P-gp. The hypothesis was that LPS activation of TLR4 would lead to alterations in P-gp function. Our results indicate that LPS treatment leads to increased plasma membrane P-gp which exhibits attenuated transport ability. These results shed light on a potentially novel regulator of P-gp in the intestine.

Materials and Methods

Cell Lines and Reagents

SW480 and Caco-2 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Ultra-pure LPS from *E. coli* 0111 B4 strain was from InvivoGen (San Diego, CA). Rhodamine 123 (Rh123) and 5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate (CDFDA) was purchased from Invitrogen (Carlsbad, CA). The primary antibodies against human P-gp were MRK-16 (Kamiya Biomedical; Seattle, WA) and JSB-1 (Signet Laboratories; Dedham, MA). The primary antibody for multidrug resistance-associated protein 2 (MRP-2/ABCC2) was obtained from Kamiya. Species appropriate secondary antibodies were purchased from Invitrogen (Alexa Fluor 488) and Sigma-Aldrich (HRP-conjugated). SuperSignal West Dura Extended Duration Substrate was used for chemiluminescent detection (Pierce Biotechnology; Rockford, IL). The MRP-2 inhibitor, MK-571 was obtained from EMD Biosciences (La Jolla, CA). All other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Hampton, NH).

Cell Culture

For fluorescent probe uptake and confocal microscopy studies cells were seeded in 24 well tissue culture plates (Becton Dickenson, Franklin Lakes, NJ) with or without glass coverslips. SW480 cells were studied 7-9 days post-seeding and Caco-2 cells studied 14 days after seeding. Cells used for immunoblotting were grown in six well plates and studied at similar time periods. All cell lines were maintained in a humidified atmosphere at 37 °C. SW480 cells were maintained in Leibovitz's L-15 medium containing 10% (v/v) fetal bovine serum (FBS) and 2 mM L-glutamine. Caco-2 and growth media consisted of Dulbecco's modified Eagle's media

(DMEM) without antibiotics supplemented with 25 mM glucose, 10% (v/v) FBS, 1% (v/v) non-essential amino acids, 2 mM L-glutamine, and 1 mM sodium-pyruvate. For the measurement of transepithelial electrical resistance (TER), SW480 cells were seeded on porous (0.4 μm) Transwell® inserts (Costar, Cambridge, MA) in six well plates. On the day of study, TER was measured using a Millicell-ERS Electrical Resistance System (Millipore, Bedford, MA) as we have described previously.(Chiu et al., 2003)

Uptake of Fluorescent Probe Substrates

To evaluate the effect of LPS on intestinal epithelial P-gp, SW480 and Caco-2 cells were employed. Both SW480 and Caco-2 cells express P-gp yet only SW480 cells can signal through TLR4.(Vora et al., 2004) Due to the poor expression of the TLR4 complex in Caco-2 cells, they serve as an excellent control. SW480 and Caco-2 cells were exposed to 100 ng/ml LPS, LPS plus the LPS antagonist, polymyxin B (10 μg/ml) or media control for 24 hours. Selected wells were treated with the P-gp antibody MRK16 (20 μg/ml) which binds to an external epitope of P-gp and is a functional inhibitor. Cells were then treated with 10 μM Rh123 in growth media for 1 hour, washed three times with ice-cold Hank's Balanced Salt Solution (HBSS) and harvested with 1% (v/v) Triton-X-100 in PBS. A 200 μl aliquot was saved for protein determination and the remaining mixture centrifuged at 14,000 rpm for 5 minutes at 4 °C. Equal amounts of the supernatant were collected and the intracellular fluorescence measured using a Wallac 1420 VICTOR[2] plate reader (PerkinElmer, Wellesley, MA). All intracellular Rh123 fluorescence values were corrected for protein. To confirm that altered uptake of Rh123 was not due to changes in MRP-2 activity, similar uptake experiments were conducted using 10 μM of the

fluorescent probe CDFDA and 25 μM of the MRP-2 inhibitor MK-571. All experiments

employed 4-6 wells per condition and were repeated on 2-3 separate occasions.

Immunoprecipitation and Western Blot Analysis

Cells were treated with 100 ng/ml LPS or media control for 24 hours and subsequently

washed with ice-cold PBS. One milliliter of lysis buffer (20 mM Tris (pH 8.0), 2 mM EDTA,

150 mM NaCl, 1 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10% (v/v) glycerol, 1%

NP-40 (v/v), 10 U/μl benzonase) was added and incubated for 15 minutes on ice. Cells were

collected and centrifuged at 14,000 rpm for 1 minute. Equal amounts of lysate were collected

and immunoprecipitated with 5 μg of the monoclonal anti-human P-gp antibody, JSB-1 and

incubated at 4 °C overnight with rotation. Immune complexes were then precipitated with

ProteinA-Sepharose (50 % slurry) and immunoprecipitated proteins re-suspended with 50 μl of

Lammelli buffer. Proteins were resolved on a 7.5% polyacrylamide gel and transferred to a

polyvinylidene difluoride membrane prior to immunoblotting with JSB-1 (1:200). Secondary

chemiluminescent detection was accomplished using a HRP-conjugated, anti-mouse IgG (whole

molecule) secondary antibody (1:2000) and the SuperSignal West Dura Extended Duration

Substrate kit. Individual bands were visualized and analyzed using a Bio-Rad Chemidoc XRS

System (Bio-Rad Laboratories, Hercules, CA) with accompanying densitometry software.

Results were confirmed using three independent experiments. In addition to P-gp, total cellular

amounts of MRP-2 were quantified using 8% SDS-PAGE and 1:50 dilution of primary, mouse

anti-human MRP-2 antibody. Experiments were conducted in triplicate and were repeated on 2-3

separate occasions.

Confocal Microscopy

SW480 cells grown on coverslips were exposed to 100 ng/ml LPS or media control for 24 hours and fixed in 3.7 % (v/v) formaldehyde in PBS. In selected wells, polymyxin B (10 µg/ml) was added to antagonize the effects of LPS. Slides were washed twice with PBS and permeabilized by incubating with 0.2 % Triton X-100 in PBS for 5 minutes at 4 °C. These cells were then washed with PBS three times and blocked with 0.1% (v/v) bovine serum albumin in PBS for 30 minutes. Five micrograms of MRK-16 was added in a total volume of 100 µl to the coverslip and kept for two hours at room temperature. Cells were washed three times with PBS followed by incubation with anti-mouse Alexa Fluor 488 secondary antibody (1:100) for one hour before a final washing. For controls, selected wells were incubated with secondary antibody in the absence of MRK-16. Fluorescence was examined by confocal laser microscopy using an MRC-1024 microscope (Bio-Rad). Immunofluoresence of MRP-2 was conducted in an identical manner using 5 µg of MRP-2 primary antibody and Alexa Fluor 488 conjugated secondary antibody. Experiments were conducted in triplicate and were repeated on 2-3 separate occasions.

Sequencing of BCRP/ABCG2 Around Position 482

In order to confirm that changes in Rh123 transport in LPS treated SW480 cells was not due to changes in BCRP activity, the ABCG2 gene was sequenced to determine the amino acid at position 482. The BCRP wild type allele accession number is XM_032424 and served as the basis for primer design (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/). Total RNA from of SW480 was isolated using the Trizol reagent (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Two micrograms of total RNA was reverse transcribed and full length BCRP cDNA was amplified using the Qiagen One Step RT-PCR Kit (Valencia, CA).

ABCG2 primers were as follows: (F) 5'-GCACGCATCCTGAATCC-3'and (R) 5'-

AACAATTGCTGCTGTGCAAC3'. A 1:10 dilution of the first round of ABCG2 cDNA was

amplified a second time with nested primers: (F) 5'-CTGAGATCCTGAGCCTTTGG-3' (R)

5'GGCAAGGGAACAGAAACAGAAAACAA-3'. To amplify the region spanning position

482, the primers (F) CTGCGGATCCTACTTTGGGCTAAAAAATGATTC3' and (R)

5'GCGACTCGAGCAATCATACAAGCCAAGGCCACGTG3' were employed. The amplified

PCR product was sequenced at the University of Tennessee Health Science Center Molecular

Resource Center using the sequencing primer

5'GATTTATTACCCATGAGGATGTTACCAAGT 3'. The sequence was analyzed and aligned

with the human ABCG2 cDNA using Applied Biosystems (Foster City, CA) pairwise alignment

software.

Statistical Analysis

Data were analyzed using a one-way analysis of variance using SigmaStat® Statistical

Software 2.03 (SPSS, Chicago, IL). If significant differences were detected, pair-wise

comparisons were made using a Tukey post-hoc test. Significance was defined as $p < 0.05$ for all

analyses.

Results

LPS Treatment Leads to Attenuated P-gp Function in SW480 Cells.

To assess the functional capacity of P-gp, LPS or control treated cells were incubated

with Rh123 and the intracellular concentration measured after 24 hours. Intracellular Rh123 was

significantly increased in SW480 cells compared to control after LPS exposure, indicative of

decreased P-gp function (Fig. 1). Polymyxin B alone had no effect on intracellular

concentrations of Rh123. These changes could be prevented with the LPS antagonist polymyxin

B (Fig. 1) and were not seen in the Pgp+/TLR4- Caco-2 cell line (control: 771±28 vs. LPS: 775

±59 pmoles Rh123/mg protein). The P-gp inhibitor MRK16 increased intracellular Rh123 in

both control and LPS treated cells (Fig. 2). To confirm that increased intracellular Rh123

concentrations were not due to an increase in paracellular permeability, TER was measured. No

reductions in TER were detected with 24 hours of LPS treatment compared to control (LPS: 520

$\pm$ 5 vs. Control: 512 $\pm$ 3 $\Omega \cdot cm^2$).

Exposure to LPS Results in Increased Cell Surface P-gp.

To further clarify the effects of LPS on P-gp, the total cellular amounts and localization

of P-gp were determined following 24 hours of LPS exposure. Immunoprecipitation and Western

blot of total cellular P-gp revealed no differences between control and LPS treated SW480 cells

at 24 hours (Fig. 3A). However, confocal microscopy showed that P-gp on the apical membrane

was significantly increased in LPS treated cells compared to control (Fig. 3B). Antagonism of

TLR4 signaling with polymyxin B prevented the increase in membrane targeted P-gp.

Polymyxin B alone did not affect localization of P-gp (data not shown). Lastly, LPS did not alter

P-gp localization or protein levels in Caco-2 cells (data not shown).

Altered Uptake of Rh123 Is Not Due to Changes in BCRP or MRP-2

To rule out contributions of BCRP or MRP-2, we assessed these 2 transporters. We

sequenced the BCRP gene (ABCG2) to confirm wildtype status at position 482 since this

isoform cannot transport Rh123. We also evaluated MRP-2 by immunoblot, confocal

microscopy, and uptake of a fluorescent substrate to confirm that changes in this protein did not

occur with LPS treatment. Sequence analysis confirmed that SW480 cells were wildtype at

position 482 encoding for arginine. No changes in MRP-2 total protein, apical distribution, or

function were detected in SW480 cells treated with LPS for 24 hours compared to vehicle

(media) controls (Fig. 4).

Discussion

One of the best characterized roles for P-gp in the gastrointestinal tract relates to decreased absorption of orally administered substrates.(Cascorbi, 2006) Despite this knowledge, the specific physiologic roles of P-gp are still unclear. Understanding the normal physiological mechanisms which regulate the expression and function of intestinal P-gp are therefore required. Given the close proximity of the microflora to P-gp on the apical membrane of intestinal epithelial cells, it is reasonable that these bacteria may affect the expression and function of this important transporter. Recently, important contributions of the intestinal microflora to epithelial homeostasis have been recognized.(Hooper et al., 1999; Rakoff-Nahoum et al., 2004; Shirkey et al., 2006) One mechanism by which commensal bacteria modulate epithelial physiology is through TLRs which interact with ligands including LPS.(Rakoff-Nahoum et al., 2004; Vora et al., 2004; Fukata et al., 2005) In the current investigation, we hypothesized that activation of intestinal TLR4 receptors by LPS may affect P-gp expression and function. Our findings indicate that exposure of intestinal epithelial cells to LPS results in increased cell surface localization of P-gp which exhibits attenuated transport ability. No alterations in total cellular P-gp were detected suggesting that the increase in surface expression of P-gp was the result of cellular redistribution rather than increased synthesis.

Previous investigations have evaluated the effects of LPS on P-gp expression in rodents and have found decreased expression or function of the transporter.(Goralski et al., 2003; Kalitsky-Szirtes et al., 2004; Hartmann et al., 2005; Wang et al., 2005) LPS administered via the intraperitoneal (I.P.) route resulted in dramatic reduction in *mdr1a* mRNA levels in the brain, heart, liver and small intestine. In addition to transcriptional alterations, significant decreases in the transport of a number of known P-gp substrates were demonstrated. Maezono and colleagues

employed an LPS-induced intestinal damage model using excised rat intestinal segments to assess P-gp activity. Similar to the studies described above, they found decreased P-gp activity.(Maezono et al., 2005) Once again, however, LPS was administered I.P. prior to segment excision. Thus, it is impossible to separate the local effects of LPS on intestinal epithelial cells from global ischemia which, in turn, may have affected the intestine. Although these studies were eloquent in design, they do not shed light on the direct, local or direct effects of LPS on P-gp expression and function. Rather, they may reflect the effects of systemic inflammation and/or LPS-induced hypotension on P-gp in peripheral organs. Certainly limitations are inherent with in vitro studies as well, however, use of a TLR4 +/P-gp + system such as the SW480 cell line circumvents the difficulties presented with systemic administration of LPS.

Exposure of SW480 cells to LPS led to decreased P-gp activity demonstrated by increased intracellular accumulation of Rh123 at 24 hours. Involvement in TLR4 signaling was supported by the findings that co-incubation of polymyxin B prevented these changes and no alterations in Rh123 accumulation occurred in P-gp+/TLR4- Caco-2 cells. LPS did not appear to totally abolish P-gp transport activity since MRK16 treatment could further increase the intracellular accumulation of Rh123. One could postulate that increases in intracellular Rh123 may be due to increased permeability from leaky tight junctions caused by LPS. However, TER values did not change in LPS treated cells and therefore this explanation is unlikely.

One difficulty in assessing the functional characteristics of P-gp is that the protein exhibits broad substrate affinity with considerable overlap with substrates for other membrane transporters. Many so-called P-gp substrates are also in fact transported by other transporters making the ability to draw conclusions about P-gp specificity difficult.(Sarkadi et al., 2004)

Besides P-gp, MRP-2 and BCRP are the two principal apically located efflux transporters in intestinal epithelial cells. In cell lines not subjected to drug selection, Rh123 transport correlates well with P-gp activity in a variety of cell lines, however there is concern that it may be transported by other proteins.(Lee et al., 1994) The wild-type form of BCRP contains an arginine at amino acid position 482 and is not able to transport Rh123.(Ozvegy et al., 2002) Drug-selection of cell lines, however, can lead to mutations producing either a glycine or threonine at this position. These changes result in gain of function and the ability to transport Rh123.(Honjo et al., 2001) Therefore, we sought to exclude the possibility that changes in BCRP activity resulted in altered Rh123 uptake in LPS treated SW480 cells. Sequence analysis of SW480 cells revealed that these cells were wild-type at position 482 and thus unable to transport Rh123. The other possibility for the Rh123 data would be that MRP-2 activity was altered. No change in total MRP-2 protein levels or distribution on the plasma membrane were detected. In addition, functional assays employing the fluorescent MRP-2 substrate CDFDA were conducted and revealed no change with LPS treatment. Taking into consideration the ABCG2 sequence data, MRP-2 studies, and TER results, we are confident that changes in Rh123 uptake with LPS were due to diminished P-gp activity.

The exact mechanism for decreased P-gp activity in cells subjected to an inflammatory stimulus such as LPS is unclear. The ability to transport substrate would be diminished if endocytic trafficking defects of P-gp occurred, ultimately affecting localization of the protein on the plasma membrane.(Kim et al., 1997; Fu et al., 2004) Elferink and colleagues found that treatment of human liver slices with LPS virtually abolished the presence of the ABC-transporters ABCB11 (BSEP) and ABCC2 (MRP2) in the canalicular membrane.(Elferink et al., 2004) We however, found the opposite in our system with increased localization of P-gp on the

plasma membrane of SW480 cells with total cellular levels unchanged. Reductions in P-gp

activity occurred in spite of increased P-gp localization on the cell surface. One possibility for

these findings is that LPS may have adversely affected protein folding or led to mislocalization

of P-gp on the plasma membrane leading to a reduced capacity to transport substrate. Even

though there was an increase of P-gp in the plasma membrane in LPS treated cells, defects in the

way it anchored to the cytoskeleton may have led to impairment in function.(Liang et al., 2003)

A second potential explanation relates to the ATP-dependent nature of transport for P-gp.

Substrate binding to key regions in the transmembrane domains and subsequent hydrolysis of

ATP ultimately lead to drug efflux.(Schinkel and Jonker, 2003) Interference with ATP-

hydrolysis results in impairment of transport capacity.(Batrakova et al., 2001) One could

postulate that the signal transduction initiated by TLR4 activation over a 24 hour period led to

impairment of this key process.

A final potential mechanism for our findings may relate to inflammatory mediators as

endothelin-1 (ET-1) released in response to TLR4 activation. Hartz and colleagues eloquently

demonstrated in isolated rat brain capillaries that LPS mediated activation of TLR4 led to the

release of tumor necrosis factor-α (TNFα) which bound to the TNF-R1 receptor leading to the

release of ET-1.(Hartz et al., 2006) Binding of ET-1 to the ET_B receptor was believed to initiate a

signal transduction cascade resulting in the activation of nitric oxide synthase (NOS) and protein

kinase C (PKC) ultimately leading to a loss of P-gp function. Similar to our study, no change in

total P-gp protein levels were documented. Given the multitude of mediators released in response

to LPS, this certainly may help to explain the decreased P-gp function in SW480 cells.

In summary, the present work indicates that the activation of TLR4 by LPS in intestinal

epithelial cells induces a mobilization of P-gp to the plasma membrane. Despite an increased

number of transporters, P-gp exhibited decreased transport ability. Under normal conditions, the

intestinal epithelium is constantly exposed to LPS from commensal bacteria. Signaling through

TLRs may represent one mechanism by which the intestinal microflora regulate the expression

and function of P-gp.

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Footnotes

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Figure legends

Figure 1: Binding of LPS to TLR4 leads to diminished P-gp function and increased intracellular

Rh123 in SW480 cells. SW480 cells were treated with media control or 100 ng/ml of LPS for 24

hours. Selected wells were incubated with 10 μg/ml polymyxin B (PMB) prior to Rh123 loading.

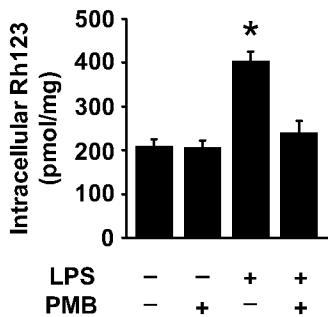Values are mean ± S.D. * denotes p < 0.05 compared to vehicle control.

Figure 2: Inhibition of P-gp function with MRK16 augments LPS mediated intracellular

accumulation of Rh123. SW480 cells were incubated with media control or 100 ng/ml LPS for

24 hours. Cells were then loaded with Rh123 in the presence or absence of 20 μg/ml MRK16.

MRK16 resulted in increased Rh123 accumulation in both control and LPS treated cells. Values

are mean ± S.D. * denotes p < 0.05 compared to vehicle control.

Figure 3: LPS leads to increased cell surface P-gp with no alterations in total cellular levels (A)

P-gp immunoblot of SW480 cells treated with media control or 100 ng/ml LPS for 24 hours. No

difference in total cellular P-gp between control and LPS treated cells. (B) Immunofluorescent

detection of cell surface P-gp. SW480 cells grown on coverslips were treated with media

control, LPS 100 ng/ml, or LPS 100 ng/ml plus 10 μg/ml polymyxin B (PMB) for 24 hours prior

confocal microscopy (60X). LPS treatment resulted in increased apical staining of P-gp which
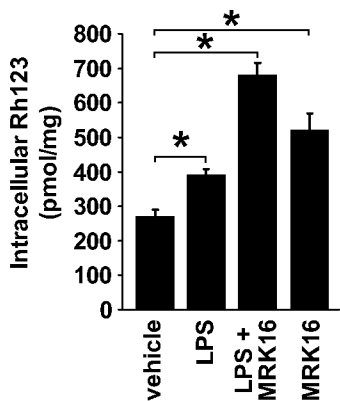
could be antagonized with polymyxin B.

Figure 4: Changes in MRP-2 do not explain LPS-mediated intracellular Rh123 accumulation.

SW480 cells were treated with vehicle control or 100 ng/ml of LPS for 24 hours. (A)

Intracellular accumulation of CDFDA in SW480 cells treated with media control or LPS 100

ng/ml for 24 hours. No differences (ND) were found between LPS and control cells. The MRP-2

inhibitor, MK-571, significantly increased intracellular accumulation of CDFDA in both control

and LPS treated cells. (B) Immunoblot and (C) confocal analysis of MRP-2. No differences in

total cellular amount of protein or apically located MRP-2 was detected.
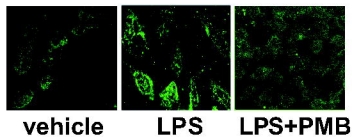
Figure 1

Figure 2

Figure 3

A

170 kDa - **Pgp**

vehicle LPS

B

vehicle   LPS   LPS+PMB

Figure 4

A

Intracellular CDFDA (pmol/mg)

NS

vehicle · MK571 · LPS · LPS + MK571

B

225 kDa-
150 kDa- MRP-2
42 kDa- β-actin

C

Control · LPS