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Advances in Brief

Disruption of the Murine *MRP* (Multidrug Resistance Protein) Gene Leads to Increased Sensitivity to Etoposide (VP-16) and Increased Levels of Glutathione[1]

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Abstract

The *mrp* (multidrug resistance protein) gene has been associated with the multidrug resistance of cancer cells *in vitro* and *in vivo*. To gain information on its physiological role, embryonic stem cells were used to generate mice homozygous for a disruption of the *mrp* gene, resulting in complete abrogation of *mrp* expression. No physiological abnormalities were observed, at least up to 4 months of age. Viability, fertility, and a range of histological, hematological, and serum-chemical parameters were similar in *mrp*(+/+) and *mrp*(−/−) mice. *mrp*(−/−) mice displayed an increased sensitivity to etoposide phosphate (2-fold) accompanied by greater bone marrow toxicity, whereas the acute toxicity of sodium arsenite was equivalent in *mrp*(+/+) and *mrp*(−/−) mice. Tissue levels of glutathione (GSH) were elevated in breast, lung, heart, kidney, muscle, colon, testes, bone marrow cells, blood mononuclear leukocytes, and blood erythrocytes of *mrp*(−/−) mice and were unchanged in organs known to express little if any *mrp*, such as the liver and small intestine. The increase in GSH was not due to an increase in the activity of γ-glutamylcysteine synthetase, the rate-limiting enzyme for GSH synthesis. The findings demonstrate that *mrp* is dispensable for development and growth but exerts a role in drug detoxification and GSH metabolism.

Introduction

MRP[3] is a recently identified member of the superfamily of ATP-binding cassette membrane transporters (1). To date, only MRP and another member of this superfamily, the 170-kDa P-glycoprotein, encoded by the *MDR1* gene (standard gene symbol, *PGY1*) have been shown in humans to be capable of conferring resistance to multiple classes of chemotherapeutic agents (MDR), a main cause of failure in the treatment of cancer (2). The *MRP* gene is ubiquitously expressed, with high levels present in both human and murine lung, kidney, heart, testes, and skeletal muscle (1, 3). In membrane vesicles isolated from MRP-transfected or -overexpressing cell lines, MRP reportedly exhibited the properties of a high-affinity transporter of cysteinyl leukotrienes as well as other GSH and glucuronide conjugates, but not of GSH alone in its reduced form (4, 5). These findings have led to the suggestion that MRP is the GS-X pump, a previously identified ATP-dependent export pump for multivalent organic anions, such as cysteinyl leukotrienes, GSH disulfide, and various other GSH *S*-conjugates (6). Human and murine MRPs have 88% amino acid identity, and both proteins can induce MDR when transfected into drug-sensitive cells (3, 7–9). Therefore, it is likely that most results obtained in the mouse model can be extrapolated to humans.

We have previously generated *in vitro* two sublines of murine ES cells in which both copies of the *mrp* gene have been disrupted, resulting in total abrogation of *mrp* expression (10). The abrogation of *mrp* expression resulted in hypersensitivity to many natural toxins, including the epipodophyllotoxin derivatives etoposide and teniposide, sodium arsenite, vincristine, and the anthracyclines doxorubicin and daunorubicin (10), but not to alkylating agents, which are known to undergo intracellular GSH conjugation (11). In this *in vitro* model, no evidence was obtained to support the hypothesis that MRP is the GS-X pump; instead, we found that baseline *mrp* expression protected ES cells from the toxic effects of xenobiotics by the co-transport of GSH and the xenobiotic from the intracellular compartment to the extracellular medium (11). To study the physiological role of *mrp* as well as the impact of *mrp* on the host response to toxins, we have generated and characterized mice in which *mrp* expression is totally abrogated.

Materials and Methods

Targeted Disruption of the *mrp* Locus and Generation of Knockout Mice. A 6-kb clone of the 3′ end of the murine *mrp* gene was isolated from a mouse strain 129-derived ES cell genomic library as described previously (10). To gain information on the physiological function of *mrp*, the murine *mrp* gene in W9.5 ES cells was disrupted by replacing a 0.7-kb *Sma*I-*Bam*HI fragment containing part of two exons with a 1.6-kb neomycin resistance gene cassette. This deletion removed part of the second putative ATP-binding domain of the gene, in particular, the B motif, which is one of the characteristic signatures of the ATP-binding domain of the MDR family of proteins (1). Electroporation into W9.5 ES cells, positive/negative selection with neomycin and gancyclovir, and analysis of the genotypes by PCR and Southern blotting were performed as described previously (10). Chimeric mice were derived from ES clones injected into C57/BL6 blastocysts that were reimplanted as described previously (12). Genetic transmission of the disrupted allele was checked by Southern blotting of isolated tail DNA. Heterozygous mice (129 × C57/BL6 genotype) were interbred to generate homozygous mice. All studies were performed on sex- and age-matched 10–16-week-old mice of the F3 generation.

Southern Blotting. Genomic DNA was extracted from the tails of mice by the method of Laird *et al.* (13). After digestion of genomic DNA with *Sac*I, size separation using a 0.8% agarose gel, and transfer to a nylon filter, the 5′ junction was checked by hybridization with a 0.4-kb *Xba*I-*Bam*HI probe positioned immediately 5′ of the targeting construct. The presence of an ≈5-kb band instead of an ≈2-kb band is diagnostic of the mutated allele.

Immunoblotting. Immunochemical detection of mouse mrp by the MRPr1 monoclonal antibody (Signet Laboratory, Dedham, MA) was performed according to Flens *et al.* (14), with the following modifications. The concentration of Tween 20 in the blocking solution, incubation buffers, and washing buffers was 0.01% instead of 0.05%, and the enhanced chemiluminescence method was used for detection (Amersham Corp., Arlington Heights, IL).

Clinical Chemistry, Histology, and Hematology. Serum levels of glucose, urea nitrogen, creatinine, total protein, albumin, globulin, transaminases,

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[3] The abbreviations used are: MRP, human multidrug resistance protein; MDR, multidrug resistance; mrp, murine multidrug resistance protein; GSH, glutathione; γ-GCS, γ-glutamylcysteine synthetase; ES cells, embryonic stem cells.

calcium, phosphorous, and uric acid were determined in both male and female mice at 6 weeks of age. Mice were examined macroscopically before and during dissection. Autopsies were performed on both male and female mice at 6 weeks and 3 months of age. Organs were fixed in 10% buffered formalin, and sections were cut at 5 μm and stained with H&E in a standard fashion. Sections were viewed by light microscopy. Hemoglobin levels, hematocrits, and erythrocyte, leukocyte, and thrombocyte counts were determined in heparinized blood.

Flow Cytometric Analysis of the GSH-bimane Conjugate. To measure GSH levels in suspension cells, mononuclear blood leukocytes and bone marrow cells were isolated by density gradient centrifugation. Cells were incubated with 40 μM monochlorobimane (Molecular Probes, Eugene, OR) for 15 min at 37°C. In murine cells, monochlorobimane binds to GSH via a GSH *S*-transferase-mediated reaction, producing a fluorescent conjugate. At 40 μM monochlorobimane, a plateau of intracellular fluorescence was reached in most murine cells, with the level of fluorescence being proportional to the intracellular level of GSH (15). The cellular fluorescence of GSH-bimane conjugates was analyzed using a FACS Vantage flow cytometer (Becton Dickinson, San Jose, CA). Cells were excited at 356 ± 6 nm, and emission was collected through a 460 ± 35 nm bandpass filter. To standardize the assay, the mean fluorescence of Hoechst UV calibration beads (Flow Cytometry Standards Corp., Research Triangle Park, NC) was recorded, and the mean cellular fluorescence was compared to that of the beads. A minimum of 20,000 cells was analyzed for each sample.

Measurement of GSH Levels. For the measurement of GSH in plasma, blood was collected after decapitation, anticoagulated by the addition of 10 mM EDTA (pH 7.0), and immediately centrifuged at 10,000 × *g* for 90 s. The plasma was deproteinized by the addition of 5-sulfosalicylic acid (3%, w/v), centrifuged at 10,000 × *g* for 5 min, and immediately assayed for GSH according to the recycling method of Tietze (16). To measure oxidized GSH, 100 μl of each sample were incubated with 2-vinylpyridine for 1 h at 20°C, followed by the addition of 18 μl of triethanolamine (1:3, v/v, in H_2O) to bring the pH to 7.0–7.5, and GSH was assayed by the recycling reaction (16). Measurement of GSH in tissues and organs was accomplished by rapid removal of tissues, which were rinsed in water, blotted dry, and homogenized on ice in 5 volumes of 3% 5-sulfosalicylic acid per gram of wet tissue weight using a Polytron homogenizer (17). After centrifugation, the supernatants were assayed for GSH as described above.

Measurement of γ-GCS Activity. Enzymatic activity was determined by a coupled enzyme procedure in which the rate of transformation of ATP to ADP was obtained from a decrease in the absorbance of NADH at 340 nm (18). Reaction mixtures containing 0.1 M Tris-HCl (pH 8.0), 150 mM KCl, 5 mM Na_2ATP, 2 mM phosphoenolpyruvate, 10 mM L-α-aminobutyrate, 20 mM $MgCl_2$, 2 mM Na_2EDTA, 0.2 mM NADH, 17 μg/ml pyruvate kinase, and 17 μg/ml lactate dehydrogenase were incubated at 37°C in a final volume of 1.0 ml. Tissues and organs were homogenized on ice in PBS. After centrifugation, reactions were initiated by the addition of γ-GCS-containing supernatants to the prewarmed reaction mixtures, and the absorbance at 340 nm was monitored.

Results and Discussion

Generation and Characterization of *mrp*-deficient Mice. The *mrp* gene was inactivated by homologous recombination in ES cells as described previously (10). Male chimeras with a high contribution of ES cell-derived agouti pigmentation to the coat color were obtained. These chimeric males were mated with C57BL/6 mice, and genotypic analysis of agouti offspring with the 5′ DNA probe revealed the presence of a 5-kb band diagnostic of the mutant allele. Heterozygous F1 breeding pairs were caged, and the F2 offspring were genotyped by Southern blotting. A representative Southern analysis of tail DNA from offspring of a cross between two *mrp*(+/−) mice is shown in Fig. 1*A*. At birth, mice were indistinguishable from one another; the distribution of genotypes at weaning was 23.1% wild-type, 49.5% heterozygous, and 27.4% *mrp* homozygous (n = 372). These numbers approximate the expected Mendelian percentages of 25:50:25 and indicate that the mrp deficiency does not produce an embryonic lethal phenotype.

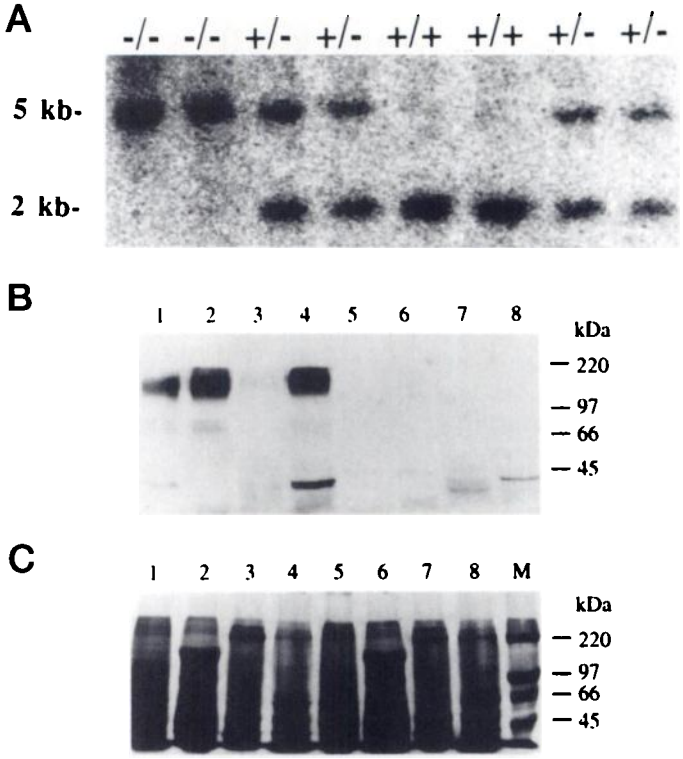

Fig. 1. Targeted disruption of *mrp*. *A*, Southern blot analyses of *Sac*I-digested genomic DNA of eight mice from one litter resulting from a cross between two *mrp*(+/−) mice. The blot was hybridized with the 0.4-kb *Xba*I-*Bam*HI probe positioned immediately outside of the 5′ end of the targeting construct (10). Fragment sizes and *mrp* genotypes as derived from the hybridization pattern are indicated. *B*, protein immunoblot analyses were performed in lung, muscle, liver, and colon of *mrp*(+/+) (*Lanes 1–4*) and *mrp*(−/−) (*Lanes 5–8*) mice using monoclonal antibody MRPr1. The *mrp* genotype and protein marker sizes are indicated. Total tissue proteins (30 μg/lane) were size-fractionated in a SDS/7.5% polyacrylamide gel and transferred to nitrocellulose by electroblotting. mrp was detected with monoclonal antibody MRPr1. Binding of antibody was visualized by enhanced chemiluminescence (Amersham). *Lanes 1* and *5*, lung; *Lanes 2* and *6*, muscle; *Lanes 3* and *7*, liver; *Lanes 4* and *8*, colon. *C*, identical SDS gel as in *A*, but stained with Coomassie Blue. *M*, marker proteins with the indicated molecular sizes.

To confirm that the mrp deficiency was a null allele, we assayed mice for expression of mrp by Western blotting using the monoclonal antibody MRPr1, raised in rats against a bacterial fusion protein containing a segment of the amino-proximal half of human MRP. This antibody has been shown by Flens *et al.* (14) and by our laboratory (11) to cross-react with murine mrp. In wild-type mice, a band of apparent M_r around 190,000 was clearly apparent in lung, colon, and muscle, three of the most abundant sources of mrp (1, 3), whereas the protein could not be detected in tissues from *mrp*(−/−) mice (Fig. 1*B*). In small intestine (data not shown) and liver, two organs in which mrp levels are reportedly extremely low (1, 3), mrp was undetectable in *mrp*(−/−) mice and was barely detectable in *mrp*(+/+) mice. A companion gel stained with Coomassie Blue is also shown in Fig. 1*C* to confirm the equal loading of protein and to exclude the presence of protein degradation. These findings confirm that the *mrp*−/− mutation is a null allele and that *mrp*(−/−) mice completely lack mrp. Also, because the MRPr1 antibody targets an epitope in the amino-proximal part of mrp, the results obtained exclude the possibility that a truncated mrp lacking the carboxyl-proximal portion of the protein was expressed in the tissues of *mrp*(−/−) mice.

The complete abrogation of *mrp* expression did not affect the viability of mice up to 4 months of age or their fertility. The litters of *mrp*(−/−) couples were of the same size as litters from wild-type mice, and the growth and behavior of *mrp*(−/−) mice were normal. Furthermore, hematological parameters and levels of a range of serum enzymes, proteins, and electrolytes did not differ between wild-type

and knockout mice. Gross anatomical and microscopic examination of most organs and tissues did not reveal any abnormalities. Several potential physiological substrates of MRP, including leukotriene C4 and 17β-estradiol-17(β-D-glucuronide), have been identified by studies using membrane vesicles from MRP-overexpressing cells (4, 5, 19). In spite of these possible functions of MRP, we did not observe any major biological anomalies in *mrp*(−/−) mice. In an analogous fashion, in spite of the relatively large number of functions speculatively attributed to mdr1-type P-glycoproteins, in mdr1a/1b(−/−) mice, no physiological abnormalities were found, with the only clear difference in phenotype being alterations in tissue distribution, cellular accumulation, and excretion of several drugs (20). It is conceivable that for the proposed biological functions of mrp, other protein systems exist that can substitute when mrp is not available. Alternatively, it is possible that mrp does not play a significant role in physiological functions and that its only role is to protect the organism against naturally occurring toxins. However, our previous finding that *in vitro*, in the absence of exposure to any xenobiotic, mrp mediates the export of GSH into the extracellular medium (11) argues against the latter possibility and suggests that, together with GSH, mrp co-transports one or more physiological substrates, the nature of which is at present unknown.

The Effects of mrp on the Toxicity of Etoposide. To ascertain whether the baseline expression of *mrp* protects mice from the toxic effects of xenobiotics, limited toxicity tests were conducted with etoposide and sodium arsenite, two well-documented substrates for MRP (2, 10). Because in the past, it was not possible to precisely determine the acute toxicity of etoposide in animals, including mice, because of interference by the solubilizing mixture (21), we have used etoposide phosphate (etopophos), a water-soluble etoposide ester that is completely and rapidly dephosphorylated to etoposide in plasma. Etoposide phosphate, injected i.p. as a single dose, was twice as toxic to *mrp*(−/−) mice than to *mrp*(+/+) mice, with calculated LD_{50} values of 95 and 190 mg/kg, respectively (Table 1). One of the main toxicities of etoposide in humans as well as in mice is to the bone marrow. To determine whether treatment with etoposide phosphate resulted in differential bone marrow toxicity to *mrp*(+/+) and (−/−) mice, the total WBC count was measured in three mice of each strain at different times after the i.p. injection of 150 mg/kg etoposide phosphate. After a rapid initial drop in the WBC count, a nadir was reached between days 2 and 3 in both *mrp*(+/+) and (−/−) mice (Fig. 2). Subsequently, the leukocyte count recovered in wild-type animals, but not in *mrp* knockouts. This result implies that etoposide phosphate exerted a differential toxicity to the bone marrow of wild-type and *mrp* knockout mice. These findings were corroborated by a pathological examination of the bone marrow and spleen of wild-type and *mrp* knockout mice 5 days after treatment with 150 mg/kg etoposide phosphate. Whereas in *mrp* knockout animals, the bone marrow exhibited a severe depletion of nucleated cells, and the spleen exhibited a depletion of myeloid activity in the red pulp, in wild-type animals, the bone marrow and spleen were either normal or hypercellular (data not shown).

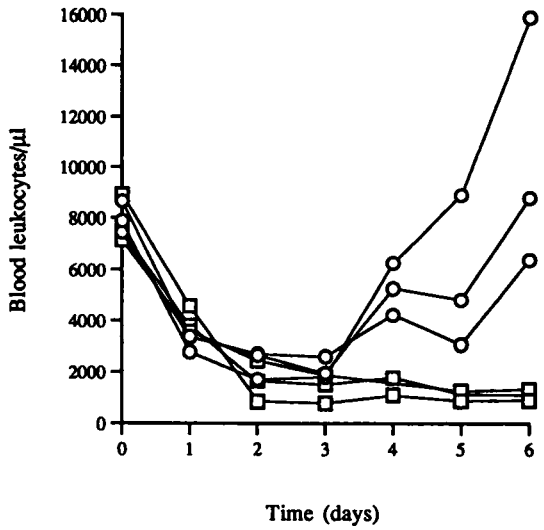

Fig. 2. Blood leukocyte counts in *mrp*(+/+) and *mrp*(−/−) mice after treatment with 150 mg/kg etoposide phosphate. ○, *mrp*(+/+) mice; □, *mrp*(−/−) mice.

Table 1 *Toxicity of etoposide phosphate and sodium arsenite in mrp(+/+) and (−/−) mice*

Drug	Dose (mg/kg)	Survival (%)[a]	
		mrp(+/+)	*mrp*(−/−)
Etoposide phosphate	50	100	100
	75	100	100
	87.5	ND[b]	83.3
	100	100	42.9
	125	100	33.3
	150	66.6	0
	175	57.1	0
	225	0	ND
Sodium arsenite	3	100	100
	5	100	100
	10	100	83.3
	15	83.3	50
	17.5	16.7	0
	22.5	0	0

[a] The survival percentage at each dose of etoposide phosphate, in mg of etoposide equivalents per kg of body weight, or of arsenite, in mg/kg of body weight, injected i.p. is listed. Each dose represents 6–12 age- and sex-matched animals.
[b] ND, not determined.

Sodium arsenite, injected i.p. as a single dose, was equivalent in toxicity to *mrp*(+/+) and (−/−) mice, as shown in Table 1. The calculated LD_{50} of sodium arsenite was 16 mg/kg in *mrp*(+/+) mice and 15 mg/kg in *mrp*(−/−) mice (not significant by Student's *t* test). This result was unexpected, because in two separate *mrp* double knockout cell lines, the cytotoxic activity of sodium arsenite was 3-fold greater than that in wild-type ES cells (10). This discrepancy can be reconciled if the organ(s) targets of acute sodium arsenite toxicity do not express significant levels of mrp and/or if other proteins can substitute for the mrp detoxification function.

GSH Levels in mrp-deficient Mice. MRP has been hypothesized to be the GS-X pump, a transporter of GSH *S*-conjugates. Recent evidence from our laboratory and from those of others strongly suggest that MRP is a co-transporter of drug(s) and GSH. In fact, Zaman *et al.* (22) have reported that MRP-transfected cells export two times more GSH into the extracellular medium than parental cells, and we have found, by comparing two MRP knockout cell lines with wild-type parental cells, that baseline MRP expression protects cells from the toxic effects of xenobiotics by effluxing the xenobiotics and GSH from the intracellular compartment into the extracellular medium (11). Tissue levels of GSH in *mrp*(−/−) mice were elevated by 25–90% in most tissues, especially in those tissues that are known to express physiologically high levels of mrp. Thus, tissue levels of GSH in *mrp*(−/−) mice were markedly increased in breast, lung, thymus, heart, colon, kidney, muscle, testes, ovary, and erythrocytes and were unchanged in the small intestine and liver, which are known to express very little if any mrp (Table 2).

To confirm these findings, GSH levels were measured by a different method in suspended blood cells, based on the fact that in murine cells, monochlorobimane binds to GSH via a GSH *S*-transferase-mediated reaction, producing a fluorescent conjugate (15). The fluorescence of GSH-bimane conjugates was higher in *mrp*(−/−) than in

Table 2 *Tissue levels of GSH and γ-GCS in mrp(+/+) and (−/−) mice*[a]

Tissue	GSH (nmol/mg)		γ-GCS activity (nmol NADH/min)	
	mrp(+/+)	*mrp*(−/−)	*mrp*(+/+)	*mrp*(−/−)
Breast	0.31 ± 0.06	0.60 ± 0.08[b]	6.4 ± 0.5	7.1 ± 0.8
Lung	1.74 ± 0.19	2.33 ± 0.16[b]	14.8 ± 1.4	15.7 ± 0.8
Thymus	1.29 ± 0.21	1.73 ± 0.24[b]	12.8 ± 3.2	11.1 ± 1.1
Heart	1.16 ± 0.10	1.69 ± 0.08[b]	12.3 ± 2.4	14.3 ± 1.0
Colon	1.83 ± 0.21	2.38 ± 0.20[b]	12.7 ± 0.9	12.0 ± 2.0
Kidney	2.66 ± 0.31	3.27 ± 0.21[b]	21.3 ± 1.4	18.6 ± 2.2
Muscle	0.80 ± 0.06	1.23 ± 0.09[b]	33 ± 8.4	27.5 ± 6.5
Testes	4.56 ± 0.07	5.46 ± 0.08[b]	ND[c]	ND
Ovary	2.78 ± 0.18	3.21 ± 0.18[b]	14.5 ± 4.6	14.5 ± 4.6
Erythrocytes	1.07 ± 0.05	1.70 ± 0.09[b]	ND	ND
Fat	0.29 ± 0.09	0.35 ± 0.09	1.9 ± 0.5	2.3 ± 0.6
Brain	1.55 ± 0.12	1.74 ± 0.13	2.6 ± 0.3	2.8 ± 0.4
Small intestine	2.92 ± 0.62	2.87 ± 0.33	28.1 ± 2.0	28.6 ± 3.0
Pancreas	1.94 ± 0.17	2.09 ± 0.23	11.5 ± 2.0	12.3 ± 1.5
Liver	6.65 ± 0.53	7.14 ± 0.56	15 ± 1.3	14.2 ± 1.8
Spleen	3.30 ± 0.61	3.52 ± 0.48	9.3 ± 1.0	11.6 ± 3.0
Plasma	0.032 ± 0.003	0.033 ± 0.006	ND	ND

[a] GSH levels and γ-GCS activity were determined as described in "Materials and Methods." Mean ± SE of four to eight animals is shown.
[b] Statistically significant difference from value for *mrp*(+/+) mice ($P < 0.05$ by unpaired Student's t test).
[c] ND, not determined.

mrp(+/+) mice, with ratios of 1.75, 1.91, and 1.67 for blood erythrocytes, blood mononuclear leukocytes, and bone marrow cells, respectively (Fig. 3). The fact that in the absence of exposure to xenobiotics, most tissues of *mrp*(−/−) mice displayed an increased level of GSH is consistent with the abrogation of mrp-mediated GSH export in knockout animals. This observation reinforces the hypothesis that the physiological function of mrp is not to protect the organism from xenobiotics but rather to co-transport GSH and a presently unknown endogenous compound or compounds (metabolites).

Among the potential consequences of increased tissue levels of GSH are resistance to drugs and natural toxins that are detoxified by GSH *S*-conjugation. Our laboratory is currently investigating whether the sensitivity of *mrp*(−/−) mice to alkylating agents or to aflatoxin B-induced carcinogenicity is altered. In preliminary experiments, we have found that the acute toxicity of cisplatin, injected i.p. as a single dose, is equivalent in *mrp*(+/+) and *mrp*(−/−) mice, with LD_{50} values being about 18 and 20 mg/kg, respectively.

Elevated levels of GSH may be due to the increased expression of γ-GCS, the rate-limiting enzyme in the synthesis of GSH (23). Recently, a coordinated overexpression of MRP and γ-GCS activity was reported in several cell lines as well as in human tumor specimens (24). However, no significant differences in γ-GCS activity were found in any of the tissues analyzed between *mrp*(+/+) and *mrp*(−/−) mice (Table 2). These findings imply that the increase in tissue GSH in *mrp*(−/−) mice does not derive from an increased rate of GSH synthesis, thereby strengthening the possibility that a deficiency in mrp-mediated export of GSH is responsible for the increased levels of GSH in tissues of *mrp*(−/−) mice.

The hypothesis that mrp co-transports GSH and etoposide is reinforced by our findings *in vitro* that the export of GSH from wild-type ES cells but not from *mrp* double knockout clones increased in the presence of etoposide and that the depletion of intracellular GSH by D,L-buthionine sulfoximine increased the intracellular accumulation of radiolabeled etoposide in parental ES cells up to levels present in the two *mrp* knockout clones but did not change the levels of etoposide in the *mrp* knockout clones (11).

The discovery of compounds that inhibit MRP activity may have application in the reversal of MRP-mediated MDR in human cancers. One potential complication to such an approach is the unknown effects of MRP inhibitors on the natural function(s) of endogenous MRP present in normal cells and tissues. Our findings suggest that it

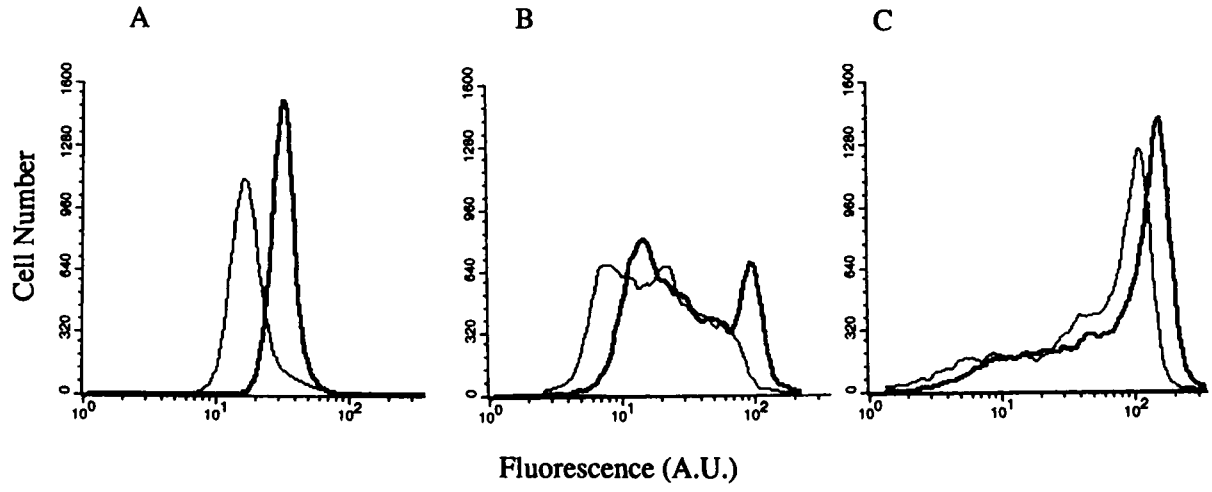

Fig. 3. Fluorescence of GSH-bimane conjugates in blood erythrocytes, blood mononuclear leukocytes, and bone marrow cells from *mrp*(+/+) and *mrp*(−/−) mice. Cells were incubated with 40 μM monochlorobimane for 15 min at 37°C, and the intracellular levels of fluorescence, which were directly proportional to the intracellular levels of GSH, were measured by flow cytometry as described in "Materials and Methods." *Light line*, *mrp*(+/+) mice; *heavy line*, *mrp*(−/−) mice. *A*, blood erythrocytes; *B*, blood mononuclear leukocytes; *C*, bone marrow cells.

may be possible to completely block MRP activity in humans, at least transiently, without affecting vital biological functions. However, greater host toxicity exerted by some chemotherapeutic agents, *e.g.*, etoposide, particularly at the level of the bone marrow, should be expected.

In conclusion, we have found that mrp is dispensable for development and viability of mice and that mrp has the potential to physiologically protect mice from the detrimental effects of toxins and antitumor drugs, such as etoposide. Parental and MRP knockout mice can be used to identify compounds that can reverse MRP-mediated MDR in human malignancies and to ascertain whether the addition of such reversing agents to preexisting chemotherapy regimens results in changes in the pharmacokinetics and toxicity of anticancer agents.

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