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Isoform-specific expression and induction of UDP-glucuronosyltransferase in immunoactivated peritoneal macrophages of the rat

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Abbreviations: UGT, UDP-glucuronosyltransferase; LPS, lipopolysaccharide; MRP, multidrug resistance-associated protein; NO, nitric oxide; 3-OH-B[*a*]P, 3-hydroxybenzo[*a*]pyrene; PAH, polycyclic aromatic hydrocarbon; ANOVA, analysis of variance between groups.

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Abstract

Phase I drug-metabolizing enzymes such as cytochrome P450 in immunocytes are known to play a role in metabolic activation of toxic and immunosuppressive compounds such as polycyclic aromatic hydrocarbon (PAH). UDP-glucuronosyltransferase (UGT), a drug-metabolizing phase II enzyme, accelerates elimination of these compounds. However, there is little information on the expression and function of UGT in immunocytes. In this study, we investigated the expressions of UGT isoforms in rat peritoneal macrophages and the role of UGT in macrophage functions. Expressions of UGT1A1, 1A6 and 1A7 were observed in macrophages by immunohistochemical staining and RT-PCR. When macrophage cells cultured in plates were exposed to 1-naphthol and 3-hydroxybenzo[*a*]pyrene (3-OH-B[*a*]P), these glucuronides increased in the medium, indicating that macrophages glucuronidated the chemicals. Production of the glucuronides of 1-naphthol and 3-OH-B[*a*]P was induced by lipopolysaccharide (LPS) treatment of the cultured macrophage cells. Northern blot analysis revealed that UGT1A7 mRNA was induced by LPS treatment. This result is the first evidence that a drug-metabolizing enzyme is induced by immunoactivation. The results indicated that macrophages can detoxify various toxic and immunosuppressive compounds with UGT and that ability is enhanced by immunoactivation. We propose that macrophages contribute to protection against not only macromolecules as immunocytes but also small molecules such as the immunosuppressive agents PAHs in peripheral blood and interstitial tissues.

Introduction

UDP-glucuronosyltransferase (UGT) is a major phase II drug-metabolizing enzyme that catalyzes glucuronidation of bilirubin and steroids as well as xenobiotic compounds, including carcinogens. UGTs have been classified into two major subfamilies (UGT1 and UGT2) on the basis of amino acid sequence similarities. In UGT1 subfamily members, each mRNA is produced from an individual promoter in a single gene locus followed by alternative splicing, and these transcripts thus contain their own amino-terminal domains followed by an identical carboxy-terminal domain. Isoforms belonging to the UGT1 subfamily primarily conjugate bilirubin and exogenous and endogenous phenolic compounds such as 3-hydroxybenzo[*a*]pyrene (3-OH-B[*a*]P) (Harding et al., 1988; Fournel-Gigleux et al., 1991; Coffman et al., 1995).

Previous studies showed that phase I drug-metabolizing enzymes such as cytochrome P450 (CYP) are expressed in lymphocytes and macrophage populations. These enzymes play central roles in the metabolic activation and detoxification of various xenobiotics, including small molecular compounds that cause immunosuppression and allergic reactions. Macrophage populations include alveolar macrophages and Kupffer cells, splenic adherent cells, peritoneal macrophages and peripheral blood monocytes, which also have CYPs (Germolec et al., 1995). CYPs catalyze metabolic activation of polycyclic aromatic hydrocarbons (PAHs), including B[*a*]P. Metabolically activated B[*a*]P has been reported to show immunosuppressive toxicities

toward immunocytes such as monocytes, macrophages and lymphocytes. B[*a*]P and dimethylbenzo[*a*]anthracene inhibit antibody production from murine B-cells (White et al., 1984). They also suppress mitogenesis of human T lymphocytes (Mudzinski, 1993) and alter B lymphopoiesis through triggering apoptosis of pre-B lymphocytes (Yamaguchi et al., 1997). B[*a*]P also inhibits differentiation of human monocytes into macrophages (Van Grevenynghe et al., 2003). It is well known that B[*a*]P is metabolically activated by phase I drug-metabolizing enzymes such as CYP 1A1 and CYP1B1, resulting in toxicological effects. Since CYPs are expressed in immunocytes, these enzymes, as stated above, play central roles in the metabolic activation of B[*a*]P.

Elimination of metabolically activated B[*a*]P and PAHs is accelerated by phase II drug-metabolizing enzymes such as UGT isoforms. Recent studies have shown that UGTs are expressed in immunocytes such as alveolar macrophages (Willy et al., 1996; Yamashiki et al., 2002) and Kupffer cells (Lafranconi et al., 1986; Oesch et al., 1987 and 1992) and in bone marrow stroma (Ganousis et al., 1992). Additionally, glucuronidation activity against B[*a*]P has been shown in rat lymphocytes, and it has been suggested that lymphocyte UGTs play a role in cytoprotection by eliminating bioactivated B[*a*]P that may covalently bind to DNA (Hu and Wells, 1994 and 2004). These studies suggest that UGTs function to ward off toxicities of metabolically activated PAHs, including B[*a*]P, in immunocytes.

However, little is known about isoform-specific expression of UGT in immunocytes and the

relation to immunofunctions of the cells. In the present study, we investigated expression of UGT isoforms and the ability of glucuronidation of chemicals in rat peritoneal macrophages. We found isoform-specific regulations of UGTs in the immunoactivation phase. The role of UGTs in macrophage functions is discussed in this paper.

Materials and Methods

Materials. Restriction endonucleases, other DNA-modifying enzymes and reagent kits were purchased from Takara Shuzo (Kyoto, *Japan*), Toyobo (Osaka, *Japan*), New England Biolabs (Beverly, MA) and Roche (Mannheim, *Germany*). 1-Naphthol (1-Hydroxynaphthalene) and UDP-glucuronic acid (uridine-5'-diphosphoglucuronic acid trisodium salt) were obtained from Wako Chemicals (Osaka, *Japan*) and Nakarai Chemicals (Kyoto, *Japan*), respectively. Lipopolysaccharide (LPS) was obtained from List Biological Laboratories (Campbell, CA). 3-Hydroxybenzo[*a*]pyrene and benzo[*a*]pyrene-3-β-*D*-glucopyranosuronic acid were obtained from Midwest Research Institute (Kansas City, MO). Other reagents were of the highest grade available. Polyclonal antibodies against phenol UGT were prepared as previously described (Yokota and Yuasa, 1990). The antibodies recognized mainly phenol UGT corresponding to UGT1A6. Peroxidase-conjugated anti-rabbit IgG antibody was obtained from Jackson Immuno Research Laboratories (West Grove, PA).

Animlas and preparation of peritoneal macrophages. Male Sprague Dawley rats (9-12 weeks old) were used in this study. The rats were housed under standard conditions and given food and water *ad libitum*. The rats were killed under anesthesia with 60% urethane (0.5 ml/kg) by exsanguination via carotid arteries. Peritoneal cells were collected and suspended in Hank's buffer. The cells were cultured in Eagle's minimal essential medium (MEM) (GIBCO Invitrogen, Carlsbad) supplemented with 10% fetal calf serum (FCS; GIBCO Invitorogen) and

100 U/ml penicillin - 100 µg/ml streptomycin (Sigma Chemical Co., St Louis, MO) and incubated at 37˚C in a CO_2 incubator (5% CO_2, 95% room air). After 3-hour incubation, nonadhering cells were washed out and adhering cells were then collected by scraping and used as peritoneal macrophages. Macrophages were identified by means of α-naphtyl acetate esterase (nonspecific esterase) staining, and cell viability (over 95%) was determined by a Trypan blue exclusion test (Eichner and Smeaton, 1983; Melnicoff et al., 1989).

Preparation of microsomes and enzyme assay. Macrophages and liver were homogenized with 4 volumes of 0.15 M KCl solution containing 1 mM EDTA. The homogenate was centrifuged at 9,000 x g for 15 min, and the supernatant fraction was further centrifuged at 105,000 x g for 60 min to obtain microsomes. Approximately 30 – 50 µg of microsomal proteins from macrophages were obtained per animal. UGT activities were determined toward various substrates in microsomes, which were activated with 0.05% sodium cholate, in 200 µl of 50 mM Tris-HCl buffer (pH 7.4), 3 mM UDP-glucuronic acid, and 0.5 mM $MgCl_2$ containing phenolic substrates such as 0.05 mM 1-naphthol, 10 mM 4-methylumbelliferone and 0.005 mM 3-OH-B[*a*]P at 37°C. Approximately 50 µg of microsomes of macrophages and liver were used for a single assay. The enzymatic activity was determined by HPLC analysis as described previously (Yokota et al., 1999). Fluorescence of 1-naphthol glucuronide and B[*a*]P glucuronide were detected with excitation/emission wavelengths of 296/336 nm (1-naphthol glucuronide) and 300/421 nm (B[*a*]P glucuronide), respectively

(Yokota et al., 1999; Singh et al., 1979). A peak derived from each glucuronide in the HPLC analysis was confirmed by disappearance of the peak in a sample pretreated with β-glucuronidase. Protein concentration was determined by the method of Lowry (Lowry et al., 1951) using bovine serum albumin as a standard. Kinetic parameters were estimated by determining enzymatic activities with various concentrations of 1-naphthol (0.00625-0.125 mM). Apparent *Km* values were estimated by the Lineweaver-Burk plot of the data.

Immunohistochemical staining. A cell suspension of peritoneal macrophages was smeared on a coverslip coated with 3-aminopropyltriethoxy silane (Sigma) and fixed for 1 hour in 4% paraformaldehyde/phosphate-buffered saline (PBS). After being treated with cold methanol for 10 min, semi-dried specimens were immersed in PBS with 0.25% bovine serum albumin and 0.1% Triton X-100 (PBS-BT) buffer for 30 min at room temperature. Immunostaining of smeared cells was carried out as follows. After incubation in normal goat serum (Sigma) for 30 min, anti-UGT antibodies/PBS-BT (1:100 dilution) were applied on the coverslip for 2 hours at room temperature. After washing three times with PBS/BT, the specimens were treated with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobulin (MP Biomedicals, Irvine, CA) / PBS-BT (1:500 dilution) -containing propidium iodide (Sigma) for 1 hour at room temperature. The coverslip was mounted on a slide with 70% glycerol containing 5% *n*-propyl gallate. In control studies for specific immunofluorescence, the specimens were incubated with PBS. Sections of stained cells were examined under a confocal laser scanning microscope

(Fluoview, Olympus, *Japan*).

Immunoblot analysis. Microsomal protein samples (10 μg) were subjected to SDS-polyacrylamide slab gel electrophoresis. The polypeptide bands thus separated were transferred to a nitrocellulose membrane, and immunoreactive bands were detected using polyclonal antibodies against UGT1A6 (Yokota and Yuasa, 1990).

RT-PCR. Total RNA was isolated from peritoneal macrophages by using an RNeasy mini kit (QIAGEN, Heidelbelg, *Germany*). DNase digestion was performed in all RNA preparations before RT-PCR. cDNA was synthesized from total RNA with Superscript II (Invitrogen) reverse transcriptase. The coding regions of the respective cDNA species were amplified by PCR with oligonucleotide primers that were designed by reference to the sequences of rat UGT1A family members (Grams et al., 2000). A UGT1A1 sense primer, 5'-TGGTGTGCCGGAGCTCATGTTCG-3'; UGT1A2 sense primer, 5'-GGAAGAATATCAGCGGGAAATACTGGGC-3'; UGT1A3 sense primer, 5'-ATTTTCTCTGAAGTTAGTTCTACAG-3'; UGT1A5 sense primer, 5'-GTGGTCTTTGAAACAGGCAACTATGTG-3'; UGT1A6 sense primer, 5'-CCTCAGTGAACGCGGACACGAC-3'; UGT1A7 sense primer, 5'-CAGTTGGCAGCTGGGAAAACCA-3'; and UGT1A8 sense primer, 5'-GGCACATGG-GAAAGTCGTTGA- 3' were designed from their isoform-specific regions. An antisense primer, 5'-CTGGAATCTCTGAGACCATGGATC-3', was designed from the UGT1A family

common region. A UGT2B family sense primer, 5'-GGAAGAATTTGTTCAGAGC-3', and an antisense primer, 5'-AACAGCTGCTCCTTTGGC-3', were designed from the common region of the family. An MRP1 sense primer, 5'-ACCTGCGCTTCAAGATCACC-3'; MRP1 anitisense primer, 5'-GCCAGAGATCAGTTCACACC–3'; MRP2 sense primer, 5'-CCAGACATGGTGAGATCCAG-3'; MRP2 antisense primer, 5'-CACTGGGAGAACTCCTTTCG–3'; MRP3 sense primer, 5'-CCATGACCTGCGTTCACAAC–3'; and MRP3 antisense primer, 5'-AGGCGAGTCCTGCATCTTTG–3', were synthesized for amplification of MRPs cDNA. All cDNA bands amplified by PCR were sequenced by a Model 310 sequencer (Applied Biosystems, Foster City, CA).

Detection of UGT activity in cells. Isolated peritoneal macrophages were plated on dishes in a fresh medium. After cell culturing for 12 hours, 1-naphthol was added to the medium to a final concentration of 0.04 mM. Medium samples were periodically collected, and 1-naphthol glucuronide concentration in the medium was measured by HPLC (Yokota et al., 1999). LPS (0.1 and 1 μg/ml of culture medium) was added to the culture medium for investigation of immunostimulation and pre-incubated for 24 hours. After pre-incubation with or without LPS, 1-naphthol (final concentration: 0.04 mM) or 3-OH-B[*a*]P (final concentration: 2.5 μM) was added to the culture medium. 3-OH-B[*a*]P was dissolved by DMSO. The final concentration of DMSO in the culture medium did not exceed 0.05% in these assays. After 24 hours, collected culture

media were assayed for 1-naphthol glucuronide and 3-OH-B[*a*]P glucuronide by HPLC, and NO was assayed using a Grass Reagent System (Promega, Madison, WI).

Northern blot analysis. Adherent peritoneal macrophages were incubated with 1 μg/ml LPS. After 24-hour incubation, macrophage cells were harvested from a culture plate, and total RNA was prepared. Total RNA was subjected to electrophoresis (5 μg/lane loaded) after denaturation by formamide and heating. The total RNA species were then transferred to a nylon membrane. Digoxigenin (DIG)-labeled exon fragments of UGT1A1, UGT1A6, UGT1A7, and glyseraldehyde-3-phosphate dehydrogenase (GAPDH) were used to detect individual mRNA as cRNA probes. A DIG-UTP-labeled antisense cRNA probe was prepared by using a DIG RNA labeling kit (Roche). The amounts of UGT mRNA and 18S rRNA were quantified by the software NIH image, and the amount of each mRNA was normalized with the 18S rRNA amount.

Statistical analysis. Statistical analysis of data in Figure 5 was performed by using analysis of variance between groups (ANOVA) with the statistical software Kaleida Graph (ver. 3.6, Synergy Software, Reading, PA). When statistically significant differences were found, the data were further analyzed by Bonferroni's multiple comparison test. Statistical analysis of data in Figure 6 was performed by using Student's *t* test. In each analysis, values of $p < 0.05$ were considered to be statistically significant.

Results

Rat peritoneal macrophages were immunostained with polyclonal antibodies against rat phenol UGT corresponding to UGT1A6 isoforms (Yokota and Yuasa, 1990). The results are shown in Fig. 1A (peritoneal macrophages), B (alveolar macrophages) and C (whole blood cells). Rat alveolar macrophages were immunostained with UGT antibodies (Fig. 1B) as previously reported for bovine alveolar macrophages (Yamashiki et al., 2002). Rat peritoneal macrophages and blood monocytes were also immunostained with UGT antibodies as shown in Fig. 1A and 1C. Western blot analysis showed the UGT contents in microsomes of liver and peritoneal macrophages. Since the polyclonal antibodies used in this study recognized mainly phenol UGTs corresponding to UGT1A6, two close bands in peritoneal macrophages must be UGT isoforms expressed (Fig. 2) (Yokota and Yuasa, 1990). In the control experiment under absence of anti-UGT1A6 antibody (only second antibody), all positive bands were not detected (data not shown).

In addition, UGT activities in the microsomal fractions of rat peritoneal macrophages were detected (Table 1). UGT activities were detected in the microsomal fractions of rat peritoneal macrophages toward 1-naphthol, 4-metylumbelliferon and 3-OH-B[*a*]P, which are glucuronidated by phenol UGTs such as UGT1A5, 1A6 and 1A7 (Table 1). The levels of UGT activities toward these substrates in peritoneal macrophages were lower than these in the liver. These results correspond to the results shown in Figure 2. Furthermore, the *Km* value of

microsomal UGT activities against 1-naphthol in peritoneal macrophages was smaller than that in hepatic microsomes (peritoneal macrophages, 0.0224 ± 0.0023; liver, 0.0508 ± 0.001).

Peritoneal macrophages were prepared from rats and cultured in the medium described in Materials and Methods. 1-Naphthol was added to the culture medium and the metabolites in the medium were analyzed by HPLC. An HPLC peak of 1-naphthol glucuronide was observed in the culture medium 48 hours after addition of the substrate to the culture plate of peritoneal macrophages as shown in Fig. 3A. The glucuronide concentrations in the media increased with increase in incubation time and were proportional to the amounts of cells (Fig. 3B). These results indicate that the peritoneal macrophages on the plate can take up 1-naphthol from the medium and glucuronidate the xenobiotics, and the resultant glucuronide was excreted into the culture medium.

cDNAs of various UGT isoforms were amplified by RT-PCR using specific primers described in Materials and Methods. The amplification of cDNAs is shown in Fig. 4 A (peritoneal macrophages) and B (liver). Four UGT1 isoforms, UGT1A1, UGT1A5, UGT1A6 and UGT1A7, and UGT2B family members were expressed in the rat liver, whereas peritoneal macrophages expressed only three isoforms, UGT1A1, UGT1A6 and UGT1A7 (Fig. 4A and 4B). To investigate the significance of UGT1A6 and UGT1A7 conjugation in macrophages, excretion of 1-naphthol glucuronide from the cells was studied as follows. Multidrug resistance-associated proteins (MRPs) MRP1 (Nunoya et al., 2003), MRP2 (Ito et al., 1998) and MRP3

(Hirohashi et al., 1999) have been reported to transport xenobiotic-glucuronide conjugate through cell membranes. These transporter mRNAs were amplified using specific primers described in Materials and Methods, and the results are shown in Fig. 4C. These three transporter mRNAs were observed by RT-PCR in the rat liver, and MRP1 and MRP3 were also detected in rat peritoneal macrophages (Fig. 4C), suggesting that these MRPs participate in the excretion of 1-naphthol glucuronide from the macrophages.

We also investigated regulation of UGT in macrophages by immunostimulation. UGT activities toward 1-naphthol, 3-OH-B[*a*]P and NO production in the macrophage cells were assayed after LPS (final concentrations of 0.1 and 1 μg/ml) had been added to the culture medium. The results are shown in Fig. 5. 1-Naphthol glucuronide, 3-OH-B[*a*]P glucuronide and NO production levels were increased with increase in the amount of LPS. NO has been used as an indicator of immunoactivation, because NO production from immunocytes is increased by immunoactivation. mRNA expressions of the UGT isoforms UGT1A1, UGT1A6 and UGT1A7 were analyzed by Northern blotting using specific probes described in Materials and Methods. In LPS-stimulated macrophage cells, UGT1A7 mRNA was specifically induced by 1.7 fold, whereas UGT1A1 was only slightly induced. In contrast, UGT1A6 was reduced in the cells (Fig. 6). These results suggested that immunoactivation of macrophages alter the expression of these UGT isoforms in an isoform-specific manner. Interestingly, the expression levels of the UGT isoforms in macrophages differed greatly from those in normal liver. Expression levels of

UGT1A6 and 1A7 in normal and immunoactivated macrophages were remarkably higher then those in the liver. UGT2B family members were not expressed in immunoactivated macrophages (data not shown).

Discussion

In this study, we found the expression of UGT isoforms, UGT1A1, UGT1A6 and UGT1A7, in macrophage cells by determination of enzymatic activities (Table 1), immunohistochemical study (Fig. 1), Western blot analysis (Fig. 2) and RT-PCR (Fig. 4). When 1-naphthol, a substrate of UGT1A6 (Burchell et al., 1989), and 3-OH-B[*a*]P, a substrate of UGT1A7 (Grove et al., 1997), were added to culture media of rat peritoneal macrophages, both glucuronides were observed in the media (Figs. 3 and 5). MRP1 and MRP3, which are members of a large family of ATP-binding cassette proteins, transport many anionic conjugates, such as estradiol glucuronide (Nunoya et al., 2003; Hirohashi et al., 1999), across the cell membrane. MRP1 and MRP3 have been shown to be expressed in various tissues (Langmann et al., 2003). In blood cells, MRP1 has been reported to be expressed in erythrocytes (Flens et al., 1996). We also found the expression of these MRP mRNAs in rat peritoneal macrophages (Fig. 4C). These results suggested that many xenobiotics were oxidized by CYP and glucuronidated by UGT isoforms in macrophages and that he resultant glucuronides were excreted into the medium by the MRPs. These results imply that macrophages play a role not only as scavenger cells for macromolecules such as proteins but also in metabolism of small molecules in blood.

It is known that activities of drug-metabolizing enzymes are suppressed by immunoactivation. Expression of an isoform of hepatic cytochrome P450 was reduced by LPS or cytokines such as IL-1β and TNFα in *in vivo* and *in vitro* assays (Barker et al., 1992; Abdel-Razzak et al., 1993;

Muntane-Relat et al., 1995). Ke et al. reported that TNFα- and LPS-induced down-regulation of CYP1A1 expression was mediated by nuclear factor-*κ*B (NF-*κ*B) and aryl hydrocarbon receptor (AhR) interaction (Ke et al., 2001). A UGT isoform was also reduced by IL-1β treatment in the liver (Ferrari et al., 1993). It has been reported that CYPs in macrophages were also reduced by immunoactivations using LPS (Nakamura et al., 1998). However, there is little information on drug-metabolizing enzymes in immunocytes. We first showed the specific expression of UGT1A1, 1A6 and 1A7 in macrophages. In these UGT isoforms, mRNA of UGT1A7 in rat peritoneal macrophages was specifically increased by LPS treatment (Fig. 6C). In addition, glucuronidation of 3-OH-B[*a*]P, which is mainly catalyzed by UGT1A7 (Grove et al., 1997), was also increased by LPS treatment (Fig. 5). These results showed that UGT1A7 was induced by immunoactivation of macrophages. This is the first report of a drug-metabolizing enzyme being induced by immunoactivation in immunocytes. We also revealed that UGT1A1, 1A6 and 1A7 were differently regulated in the phase of immunoactivation (Fig. 6). These isoform-specific regulations may indicate that an individual isoform of UGTs in macrophage cells plays an independent role. In the experiment for which results are shown in Figure 5, we measured NO production from macrophages for confirmation of immunoactivation. Although NO production was increased by only LPS treatment, it was further increased by the addition of a UGT substrate, 1-naphthol or 3-OH-B[*a*]P (Fig. 5C). A previous study also showed that 17β-estradiol increased NO production through induction of inducible NO synthase (iNOS) (You et al., 2003). These

results clearly indicate that UGT activities in macrophages are induced by immunoactivation of macrophages, which is synergistically enhanced by substrates.

3-OH-B[*a*]P, which we used for measurement of UGT activities, is a well-known chemical causing immunosuppression in immunocytes (White et al., 1984; Mudzinski, 1993; Yamaguchi et al., 1997; Van Grevenynghe et al., 2003). Many studies have suggested that UGTs play a role in cell protection against PAHs. Hu and Wells showed by using lymphocytes from UGT1A-deficient rats (Gunn rats) that UGTs contribute to reduction of covalent binding of B[*a*]P to DNA (Hu and Wells, 1992). Our experiment showed that the ability of glucuronidation against 3-OH-B[*a*]P was increased by activation of immunity (Fig. 5). This response would be a protective mechanism against immunosuppressive chemicals in macrophages. Therefore, our results suggest that regulation of metabolic enzymes and metabolism of chemicals in macrophages are of importance in the study of immunosuppressive chemicals.

Exogenous chemicals such as drugs and endocrine disrupters taken into a living subject and endogenous materials such as hormones are mainly metabolized in the liver and the digestive tract. However, drug-metabolizing enzymes are expressed in many organs, including immunocytes, and it is thought that they contribute to metabolism of xenobiotics at the local sites. However, functions of drug-metabolizing enzymes in immunocytes such as macrophages are not well known. In this study, we demonstrated the isoform-specific expressions of UGTs and their glucuronidation activities in macrophage cells. Although the number of macrophage cells was

small, UGT1A6 and UGT1A7 mRNA were expressed in normal peritoneal macrophages at levels 5-fold and 10-fold higher than those in a normal liver, respectively (Figs. 6B and 6C). This finding may indicate differences in stabilities of these mRNAs in each organ. However, the differences between the expressions of these mRNA in the liver and peritoneal macrophages indicate that macrophage UGTs may play a role different from that of liver UGTs. For example, some hormones contribute to regulation of cell functions in immunocytes. Macrophage UGTs may be related to the regulation of cell functions through hormone metabolisms, because UGT1A family members are known to glucuronidate several hormones.

In summary, we showed detailed expression profiles of UGT and glucuronidation activities in rat peritoneal macrophage cells. We also showed immunoinducibility of the UGT1A7 gene for the first time. We conclude that macrophages contribute to protection against not only macromolecules as immunocytes but also small molecules such as immunosuppressive agents in peripheral blood and interstitial tissues. We propose here that regulation of drug-metabolizing enzymes is an important factor in the study of immunosuppressive agents such as PAH in immunocytes.

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Footnotes

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Legends

Fig. 1. Immunohistochemical staining of UGTs in rat peritoneal macrophages.

Immunohistochemical staining of rat peritoneal macrophage cells was performed as described in Materials and Methods using polyclonal antibodies against a UGT isoform corresponding to UGT1A6. Confocal laser scanning microscopic images of peritoneal macrophages (A, D), alveolar macrophages (B, E) and monocyte cells in whole blood cells (C, F) are shown. The red signal of propidium iodide shows cell nuclei. The green signal indicates the existence of UGT. No signals were detected in the cells stained only with the second antibody (D, E, F). Bar indicates 10 μm.

Fig. 2. Western blot analysis for peritoneal macrophages. The contents of UGT protein in microsome fractions of the liver and peritoneal macrophages were compared. The three lanes on the left were loaded with liver microsomes, and the three lanes on the right were loaded with peritoneal macrophage microsomes. Each lane was loaded with 10 μg protein. Open delta symbol indicates a positive band corresponding to UGT protein.

Fig. 3. Glucuronidation of 1-naphthol and excretion of its glucuronide from rat peritoneal macrophages. Peritoneal macrophage cells were prepared from the rat as described in Materials and Methods. Macrophages were cultured on plates and exposed to 1-naphthol (final

concentration: 0.04 mM). The culture media after 6, 12, 24 and 48 hours were analyzed by HPLC as described in Materials and Methods. The HPLC chromatogram shown in A indicates the production of 1-naphthol glucuronide in the culture medium. The glucuronide was confirmed by deconjugation using β-glucuronidase. The product concentrations in the medium are shown in B (6×10^6 cells/plate: open squares, 3×10^6 cells/ plate: closed squares). Data are shown as means ± S.E. of three independent experiments.

Fig. 4. Detection of UGT1A, UGT2B and MRP transcripts in rat liver and peritoneal macrophages by RT-PCR. Total RNA was prepared from peritoneal macrophages and liver of the rat. RT-PCR was performed as described in Materials and Methods. The transcripts of parts of UGT isoforms such as UGT1A1, UGT1A2, UGT1A3, UGT1A5, UGT1A6, UGT1A7, UGT1A8 and UGT2B family members as well as glyceraldehydes-3-phosphate dehydrogenase (GAPDH) are shown in respective lanes in the peritoneal macrophages (A) and liver (B). The three major MRP transcripts were also amplified from total RNA of rat peritoneal macrophages and liver (C).

Fig. 5. Effect of LPS on UGT activities in rat peritoneal macrophages. Adherent peritoneal macrophages were prepared and cultured on plates, and LPS (0.1 μg/ml and 1 μg/ml) was added to the culture medium. After 24-hour pre-incubation, the culture medium was changed to a fresh

medium containing LPS and 1-naphthol (final concentration: 0.04 mM) or 3-OH-B[*a*]P (final concentration: 2.5 μM). After 24-hour incubation, 1-naphthol glucuronide (A) and 3-OH- B[*a*]P glucuronide (B) and NO (C) in the medium were assayed as described in Materials and Methods. Each value is the mean ± S.E. of three independent experiments. *$p < 0.05$, **$p < 0.01$ with respect to the control (saline). $^{\dagger\dagger}p < 0.01$ with respect to no substrate.

Fig. 6. Effect of LPS on mRNA expression of UGT isoforms in rat peritoneal marophages. mRNAs of major UGT isoforms were analyzed by Northern blotting as described in Materials and Methods. Total RNAs prepared from rat peritoneal macrophages cultured with 1 μg/ml LPS for 24 hours were electrophoresed, blotted, and hybridized with probes UGT1A1 (A), UGT1A6 (B), UGT1A7 (C) and GAPDH (D). Upper panels show typical results of Northern blotting analysis, and lower panels show data obtained from densitometric analysis of Northern blotting experiments by NIH image. The amount of mRNA was normalized with 18S rRNA amounts. Each value is the mean ± S.E. of three independent experiments. *$p < 0.05$, with respect to the control (saline).

Table 1. Microsomal UGT activities in rat peritoneal macrophages. Microsomal fractions were prepared from rat liver and peritoneal macrophage cells and activated with 0.05% sodium cholate. UGT activities toward 1-naphthol, 4-methylumbelliferone, bisphenol A, Testosterone and 3-OH-B[*a*]P were assayed as described in Materials and Methods. Data are shown as mean ± S.E. of three independent experiments.

	UGT activities (nmol/min/mg)	
	Liver	Peritoneal Macrophages
1-Naphthol [a]	16.4 ± 1.0	0.508 ± 0.018
4-Methylumbelliferone [a]	6.8 ± 1.1	0.276 ± 0.009
Bisphenol A [b]	22.9 ± 2.4	N.D. [b]
Testosterone [b]	5.2 ± 1.7	N.D. [b]
3-OH-B[*a*]P	0.05 ± 0.005	0.000148± 0.0000396

[a] 1-Naphthol and 4-methylumbelliferone are glucuronidated mainly by phenol UGTs such as UGT1A5, UGT1A6 and UGT1A7. [b] N.D., not detectable

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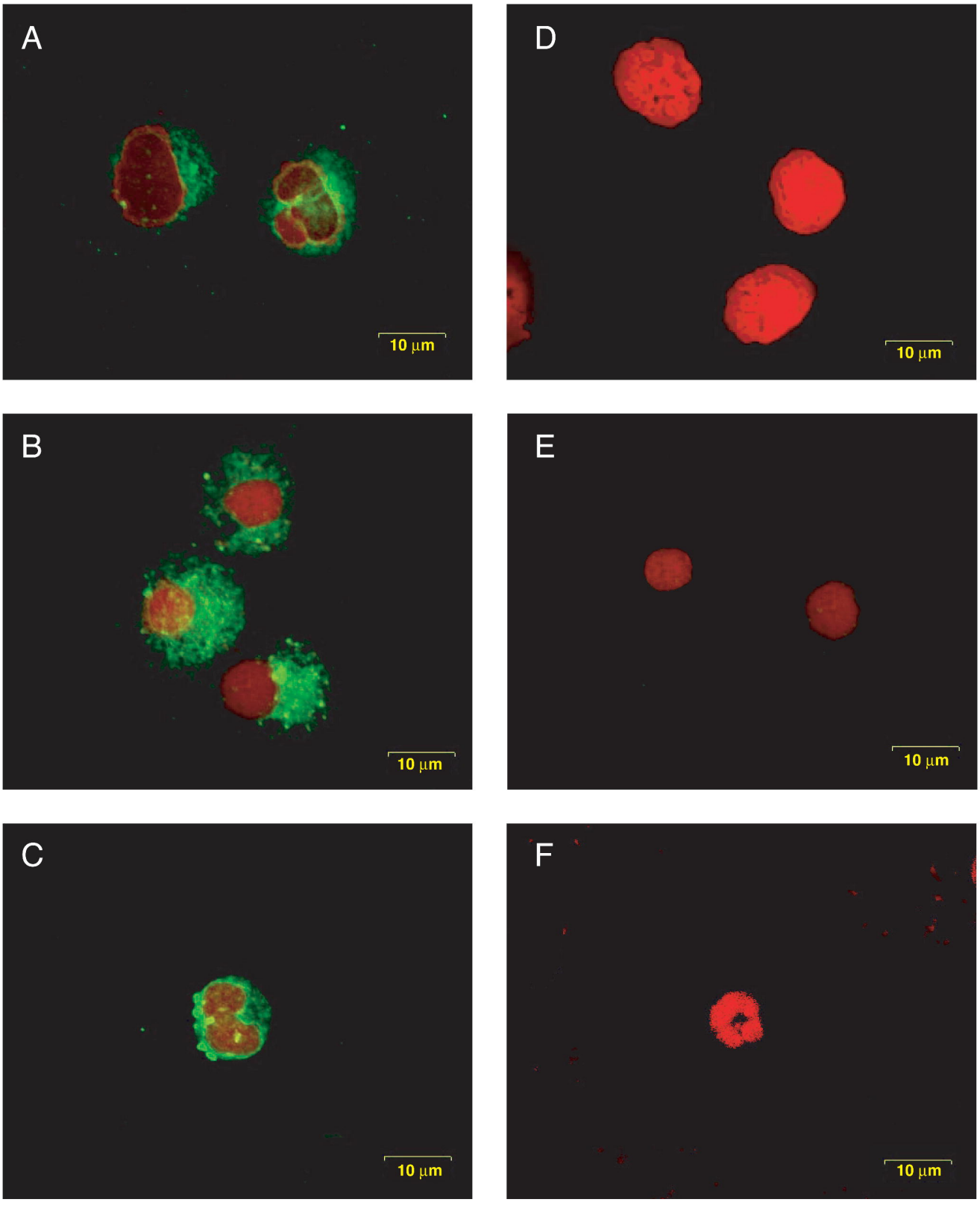

Fig. 1

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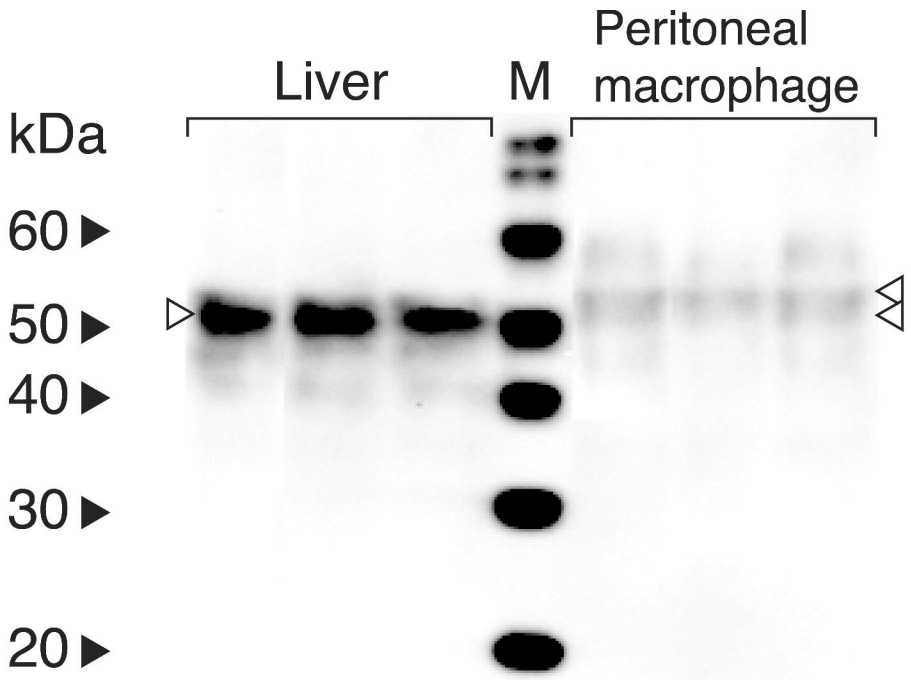

Fig. 2

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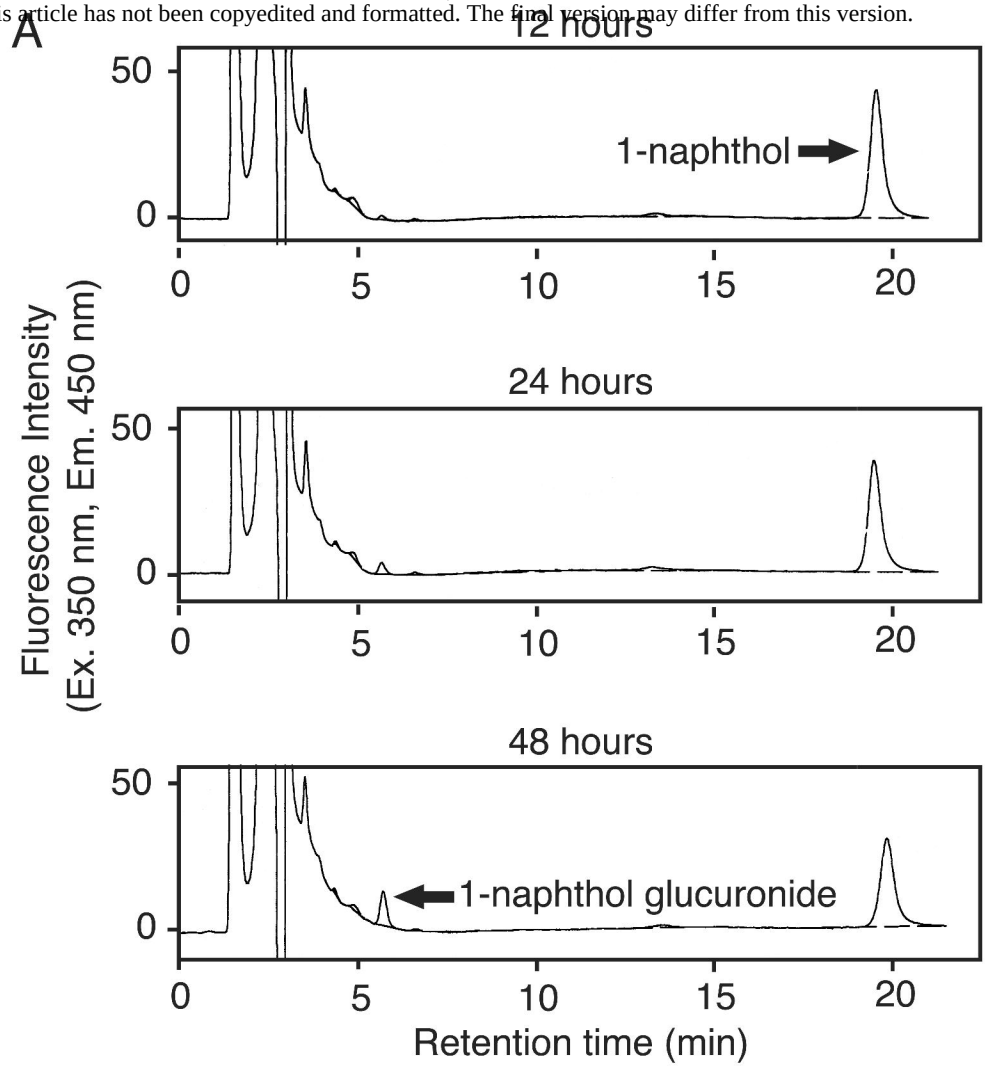

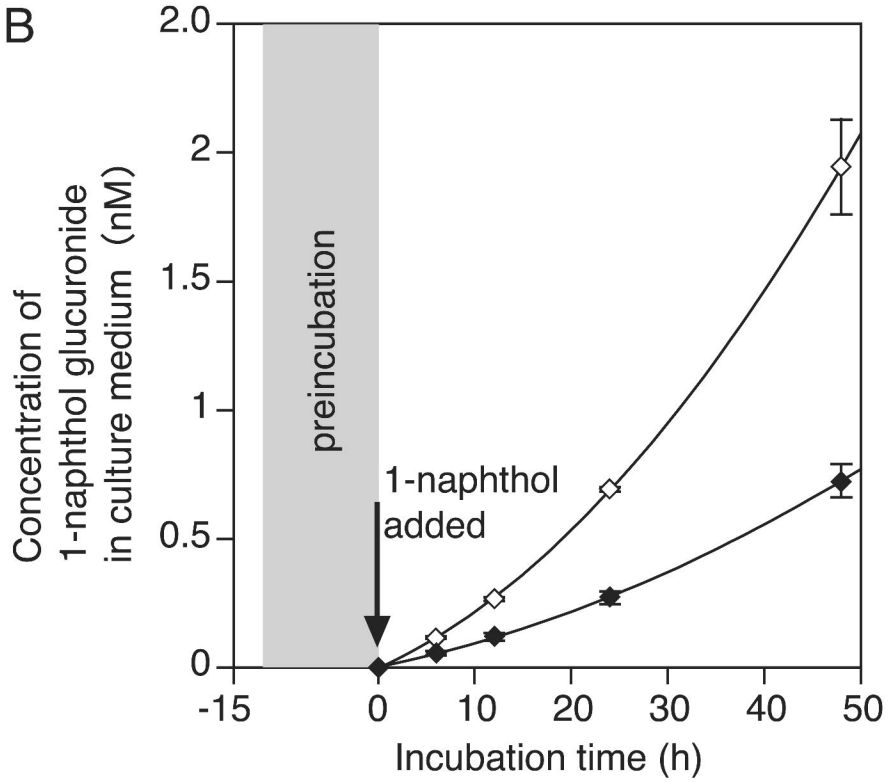

Fig. 3

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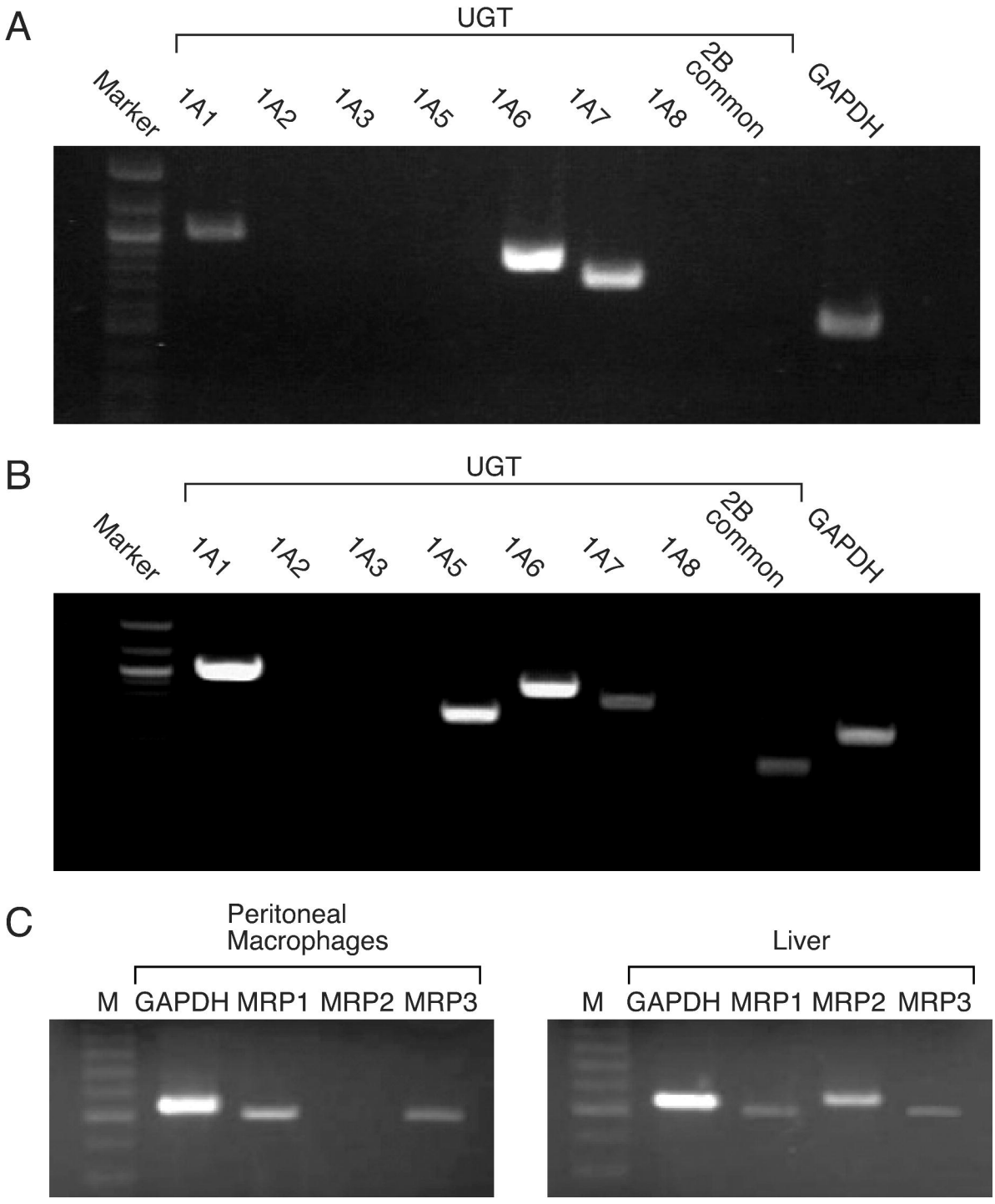

Fig. 4

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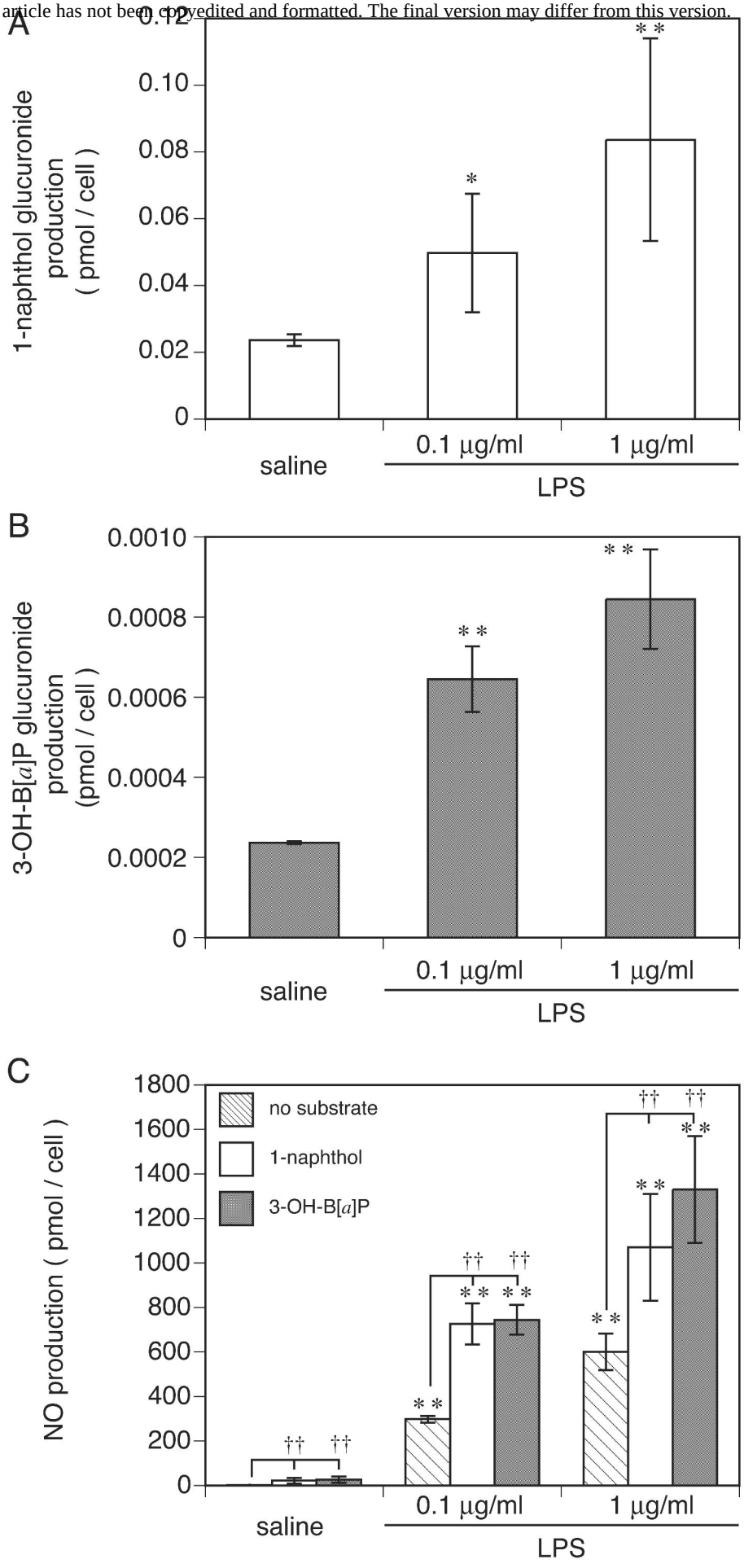

Fig. 5

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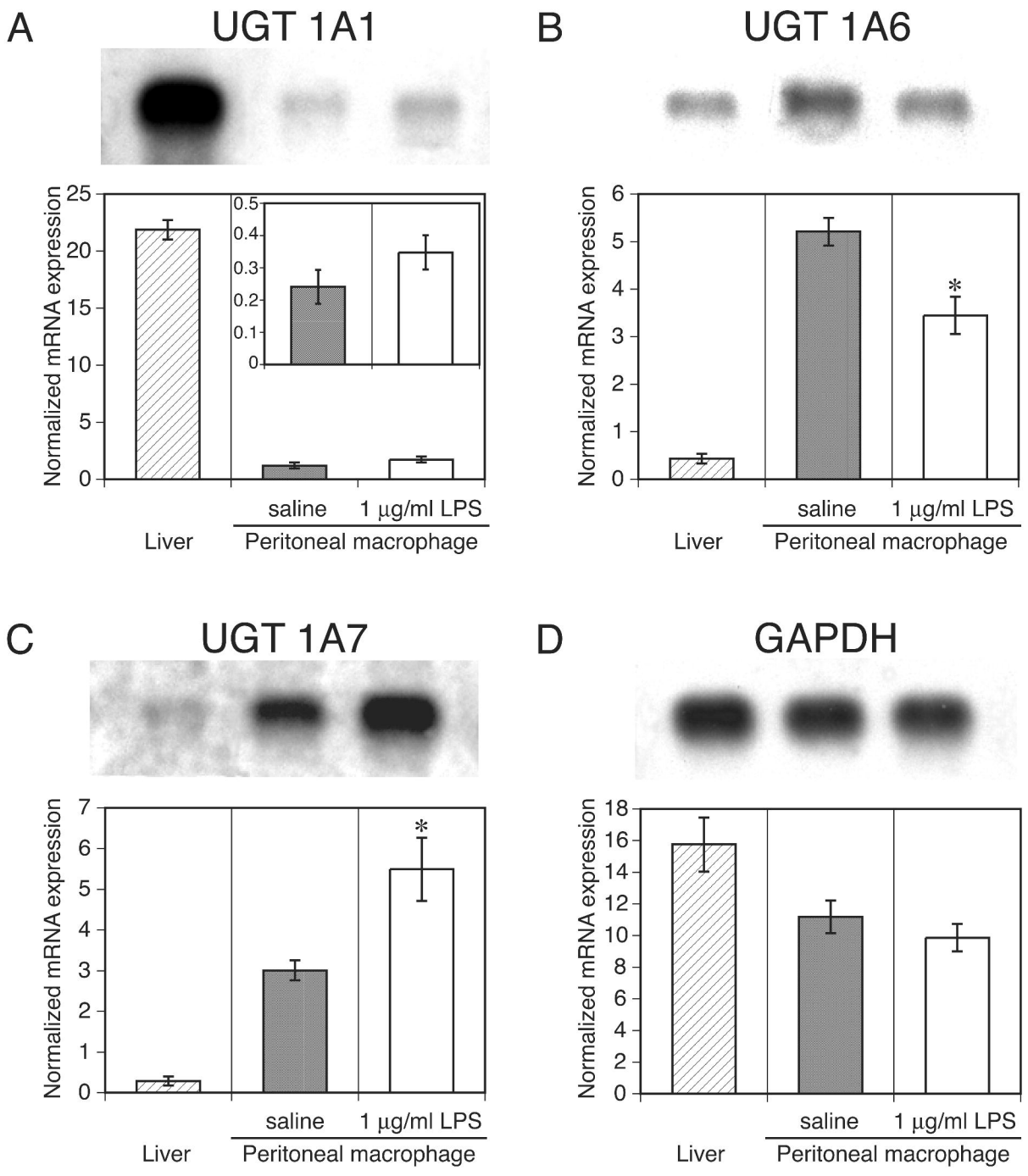

Fig. 6