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Inflammation Research

Decreased expression of P-glycoprotein in interleukin-1β and interleukin-6 treated rat hepatocytes

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Abstract. *Objective and Design:* As acute inflammation is known to cause a reduction in hepatic P-Glycoprotein (PGP) expression and activity in rats, we tested the hypothesis that the pro-inflammatory cytokines interleukin (IL-)1β and IL-6 also mediate reductions in PGP.
Methods: Hepatocytes were incubated with 0–50 ng/ml of cytokine for 24–72 h. PGP/*mdr* expression was examined by immunodetection and quantitative RT-PCR analysis and PGP efflux activity was assayed.
Results: PGP protein was significantly reduced in cells treated for 3 days with IL-1β and 24 h with IL-6 ($p < 0.05$), maximal effects occurring at 5 ng/ml for each cytokine. PGP activity was reduced in both IL-1β and IL-6 treated cells ($p < 0.05$). *mdr1* mRNA was decreased in cells treated with IL-6, but not IL-1β. *spgp* and *mdr2* were not affected.
Conclusions: Our data indicate that IL-6 and IL-1β have suppressive effects on the expression and activity of PGP in cultured hepatocytes, likely occurring through distinct mechanisms. These cytokines may have a potential role in PGP regulation during inflammatory responses.

Key word. Cytokines – Multidrug Resistance – P-Glycoprotein – Gene Regulation – Hepatocytes

Transporters in the liver play a critical role in the elimination of metabolic products and ingested chemicals. The hepatic canalicular membrane contains a 170 kDa ATP-dependent transporter, P-Glycoprotein (PGP), which is involved in the cellular efflux of a broad range of structurally diverse chemicals [1, 2]. PGP, which is encoded by the multidrug resistance (MDR) gene family, MDR1 in humans and *mdr1a* and *mdr1b* in rodents, is normally expressed in the apical epithelium of the liver, intestine, kidney and blood-brain barrier [3]. Although its normal physiological function has yet to be established, PGP overexpression is thought to play an important role in cellular detoxification. Furthermore, PGP has been implicated as a principle mechanism of tumor drug resistance. PGP is frequently overexpressed in tumors arising from epithelial tissues and its substrates include many clinically important antineoplastic agents thereby resulting in reduced intracellular drug concentrations and therapeutic efficacy of these drugs [4–6].

To date, relatively little information on the mechanisms by which PGP is physiologically regulated exists and few studies have examined pathways of downregulation. Recently, we reported a reduction of PGP expression in rat liver in response to turpentine and endotoxin-induced inflammation [7]. Both of these experimental models of inflammation are associated with a characteristic hepatic response resulting from IL-6 and IL-1 induction [8, 9]. As little information exists which could aid in characterizing physiological pathways of PGP downregulation, one of our primary objectives was to ascertain the mechanisms involved in PGP suppression. It is known that the expression of many liver-derived proteins, including several of the cytochrome P-450 drug metabolizing enzymes, is altered or suppressed during an acute phase response [8]. Generally, it is believed that these changes stem from the release of local and systemic inflammatory mediators [8]. Of these, it is thought that the cytokines interleukin (IL) -1β and IL-6 are the principle mediators of the hepatic changes in gene transcription and protein synthesis rates [10]. It is therefore possible that these cytokines could also be involved in the suppression of PGP during an inflammatory response. Thus, in order to investigate the role of these cytokines on PGP regulation, we examined the effects of IL-6 and IL-1β treatment on the expression and activity of PGP in cultured rat hepatocytes. This in vitro model was utilized, as these hepatocytes are responsive to endotoxin and pro-inflammatory cytokines, similar to that seen in vivo [8, 9, 11]. Furthermore much of the knowledge on PGP regulation as well as cytokine-mediated effects on gene regulation has been generated in rat and cultured rat hepatocytes [7, 9, 12–14].

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Methods

Hepatocyte isolation and culture

Animal studies were conducted according to the guidelines set out by the United States National Institutes of Health. Animals were anesthetized using 0.6 ml (65 mg/ml) sodium pentobarbitol. Hepatocytes were isolated from livers of male Sprague Dawley rat (250–300 g) by collagenase perfusion, as earlier described [7, 15]. Briefly, the hepatic portal vein was perfused with Hanks buffer containing 0.045% collagenase followed by separation and sedimentation of the hepatocytes. The hepatocytes were then washed and plated as described previously [12]. Cell viability, assessed with trypan blue staining, was always ≥ 80%. Cells were cultured in "maintenance medium": 1 : 1 DMEM/Waymouth's MB 752/1 medium containing dexamethasone (50 nM), gentamicin (50 μg/ml), bovine serum albumin (1.25 mg/ml) and ITS+ culture supplements (Collaborative Biomedical Products; contains 6.25 μg/ml of insulin, transferrin and selenium and 5.35 μg/ml of linoleic acid). This culture system has been used extensively for examining PGP gene regulation [12]. Cells were kept in a 5% CO_2, 37 °C environment.

Cytokine treatment and PGP expression

Plated hepatocytes were incubated with varying concentrations of recombinant IL-1β or IL-6 (Sigma, ON) in maintenance medium. Time- and dose-dependent studies on the effects of recombinant IL-1β and IL-6 on PGP protein expression were performed for incubation periods 24–120 h and doses 0–50 ng/ml cytokine. Further experiments on PGP/*mdr1* function and mdr mRNA expression were conducted using specific incubation periods and cytokine concentrations determined from the preliminary time- and dose-dependence studies: IL-6, 24 h incubation, 5 ng/ml, and IL-1β, 72–120 h incubation, 5-10 ng/ml. Time–dependent studies on the effects of cytokine treatment on mdr mRNA expression were also conducted with 5 ng/ml cytokine and incubation periods as follows: IL-6, 0–24 h; IL-1β, 0–72 h. After the treatment period concluded, the cytokine-containing medium was removed and cells were harvested by trypsinization. As compared to controls (0 ng/ml), cytokine incubations did not significantly affect cell viability (assessed by trypan blue staining).

Influence of dexamethasone

As the media required for primary cultured hepatocytes generally contain low concentrations (50 nM) of the anti-inflammatory agent dexamethasone (DEX) [12, 13], which could potentially influence IL-6 activity, we examined the influence of DEX on PGP expression in IL-6-treated cells. As IL-6 had its greatest effects on PGP protein expression after 24 h incubation, the effects of DEX on IL-6-mediated downregulation of PGP were examined at this time point. Hepatocytes were cultured overnight in maintenance medium containing 0, 50 nM or 250 nM dexamethasone (DEX) and then treated for 24 h with 5 ng/ml IL-6 (IL-6 was replaced by saline in controls). After 24 h, medium was removed and cells were harvested by trypsinization and analyzed for PGP expression. Cells cultured in the absence of DEX generally had a slightly lower viability than cells cultured with DEX, although these differences were not significant.

Western blot analysis

Crude membrane fractions were isolated from cultured hepatocytes and Western blots were performed as previously described [7, 14]. Briefly, 3–5 μg samples were separated on SDS-PAGE electrophoresis gels and transferred to nitrocellulose membranes. After blocking, the blots were washed and incubated in 4 μg/ml C-219 overnight at 4 °C. The bound antibody was visualized using chemiluminescence (ECL, Amersham) and the optical density was quantified using the Kodak Digital Science ID Image Analysis Software (Eastman Kodak).

Rh123 efflux assay

PGP activity was assayed using the fluorescent PGP substrate Rhodamine 123 (Rh123) as previously described [7, 16]. Cells were plated with cytokines and after the appropriate treatment period (1–5 days) the cells were washed and incubated with Rh123 (1 μg/ml) for 15 minutes. The cells were then incubated with Rh123-free buffer, in the absence or presence of verapamil (30 μg/ml). At 0 and 60 minutes after removal of Rh123, the cells were washed three times with ice cold Phosphate Buffered Saline, lysed for 30 minutes using 1N NaOH, neutralized with 1 N HCl and the intracellular fluorescence intensity measured with a PTI Deltascan Spectrophotofluorimeter (excitation 518 nm, emission 532 nm). Optimal incubation times were determined from initial time course studies of RH123 accumulation and efflux (data not shown). Intracellular Rh123 was corrected on the basis of protein content as assessed by the Bio-Rad protein assay kit [16]. % Rh123 Efflux is calculated as:

$$\%\text{Rh123 Efflux} = \frac{\text{Intracellular Rh123 } (t=0) - \text{Intracellular Rh123 } (t=60)}{\text{Intracellular Rh123 } (t=0)} \times 100\%$$

The PGP mediated efflux was estimated as the fraction of efflux over 60 minutes which was inhibited by verapamil: PGP-mediated efflux = $\%\text{Rh123 Efflux}_{\text{(no verapamil)}} - \%\ \text{Rh123 Efflux}_{\text{(+ verapamil)}}$

Quantitative RT-PCR

Total RNA samples were isolated from control and treated cells [17] at various times (0–72 h) after incubation with IL-6 or IL-1β. The mRNA levels of the *mdr* isoforms were determined using a quantitative RT-PCR assay as described previously [18] with slight modifications. Briefly, cDNA was synthesized from 0.5 μg total RNA, according to manufacturer protocol (GeneAmp RNA PCR kit, Perkin Elmer, Norwalk, CT). Serial dilutions (2–16,000 fold) of RT product were used to generate standard curves for PCR and optimal amounts of template were determined from the linear portions of these curves (data not shown). Standard curves for RT-PCR were performed for each set of RNA samples analyzed. RT-PCR standard curves were found to be highly reproducible. Selected RT-PCR results were also confirmed on Northern Blotting. Relative efficiencies of RT-PCR reactions, as determined from the standard curves, were approximately 30% for *mdr1a, mdr1b, spgp* and GAPDH. PCR was performed using 2.5 U Taq polymerase (Gibco BRL), 50 pmol sense and antisense primers (DNA Synthesis Centre, University of Toronto, ON) and RT product (100 ng for *mdr1a*, 50 ng for *mdr1b* and 6.25 ng for GAPDH) for 30 cycles (94 °C for 45 sec, 55 °C for 30 sec, and 72 °C for 75 sec) on a GeneAmp 2400 PCR System (Perkin-Elmer). Primer sequences for *mdr1a*, *mdr1b, mdr2, spgp* and GAPDH have been reported elsewhere [19]. To test for IL-6 induction in IL-1β treated cells, total RNA was isolated from cells that were incubated for 72 h with 5 ng/ml IL-1β and RT-PCR was run as outlined above, using the IL-6 primers reported by Siegling et al. [20]. Water was used as a negative control for all PCR procedures. PCR products were visualized on a 2.0% agarose gel using SYBR Gold stain (Molecular Probes, Eugene, OR) and quantitated with Kodak Digital Science 1D Image Analysis software. Optical intensities of the *mdr* genes were normalized to GAPDH.

Statistical analysis

In each study, treated and control samples were obtained from 3 to7 individual culture plates that were obtained from at least two separate experiments. Student's t-tests (unpaired, two-sample, unequal variance with two-tailed distributions) were run on all results using Microsoft Excel 97 and Sigma Plot 4.0/2000 software with $p < 0.05$ taken as the significance level.

Results

Influence of culture times on expression of the mdr gene family

Similar to previous reports [12, 21], we observed an increase in PGP expression with culture time. PGP protein expression was increased by 1.5 fold after 24 h and by 3 fold after 72 h in cultured rat hepatocytes (data not shown). Functional activity was similarly increased by 3 fold between 24 h and 72 h in culture (illustrated in Fig. 1). This was accompanied by a 2.4 ± 0.6 fold increase in *mdr1a* and a 1.9 ± 0.4 fold increase in *mdr1b* mRNA mRNA levels over the first 24 h of culture; further increases in mRNA levels were not evident at 72 h. As shown in Figure 2, while *mdr1a* and the bile salt transporter, *spgp,* are expressed to a moderate extent, *mdr2* was not found to be significantly expressed in 24 or 72 h cultures; GAPDH is present as a positive control.

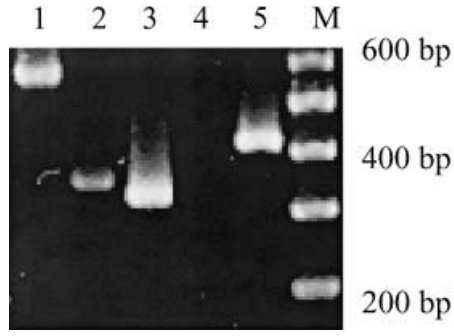

Fig. 2. Representative RT-PCR gel depicting relative *mdr* gene expression. As described in methods, total RNA was isolated from 24 h cultured hepatocytes, RT-PCR was performed, PCR products were separated on 2% agarose gels, stained with SYBR Gold stain and visualized with Kodak Digital Science Image Analysis Software. Lane #1 contains GAPDH, #2 *mdr1a*, #3 *mdr1b*, #4 *mdr2* and #5 *spgp*. Lane #6 GeneRuler™ 100 bp DNA ladder.

Effects of IL-1β on PGP expression and activity

PGP protein expression

Incubation of hepatocytes for 3–5 days with IL-1β appeared to influence the C-219 immunodetectable expression of PGP. As compared to controls, significant reductions of 32–75% ($p < 0.05$) were seen in cells treated with 1–25 ng/ml IL-1β after 3 days, with maximal suppression occurring at a concentration of 5 ng/ml (Fig. 3). Expression of PGP also tended to be lower in cells treated with 50 ng/ml IL-1β, however this difference did not reach statistical significance. Significant reductions in PGP expression were still maintained after 5 days of IL-1β incubation with 1 ng/ml (64 ± 9.1% decrease from control values, $n = 5$, $p < 0.05$) to 10 ng/ml (17 ± 5.5% reduction, $n = 7$, $p < 0.05$). No significant effects on PGP expression were seen after 24 h of IL-1β incubation. Furthermore, cells cultured in the presence or absence of DEX did not exhibit significant differences in IL-1β mediated down-regulation of PGP protein expression (data not shown).

PGP functional activity

The influence of IL-1β on PGP mediated transport activity was initially examined in hepatocytes treated with 10 ng/ml IL-1β. Relative to controls, significant reductions in the PGP-mediated efflux of RH123 were seen after 3 and 5 days of IL-1β treatment (Fig. 1). Furthermore, significantly higher (34% greater than controls, $p < 0.05$) initial accumulations of Rh123, indicative of diminished PGP function, were also observed in IL-1β treated cells.

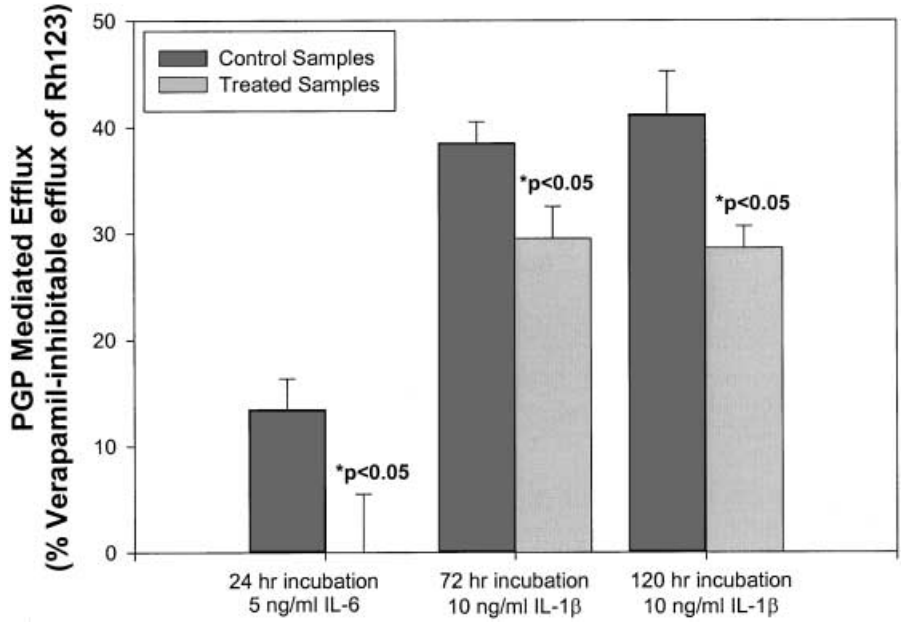

Fig. 1. Effect of cytokine treatment on PGP functional activity. Cells were treated with IL-6 (5 ng/ml, 1 day) or IL-1β (10 ng/ml, 3–5 days) and PGP functional activity was examined using a Rhodamine 123 Efflux Assay, as described in the methods. Values are reported as % (mean ± SEM) of the 60 minute verapamil-inhibitable Rh123 efflux ($n = 4$/group), $*p < 0.05$.

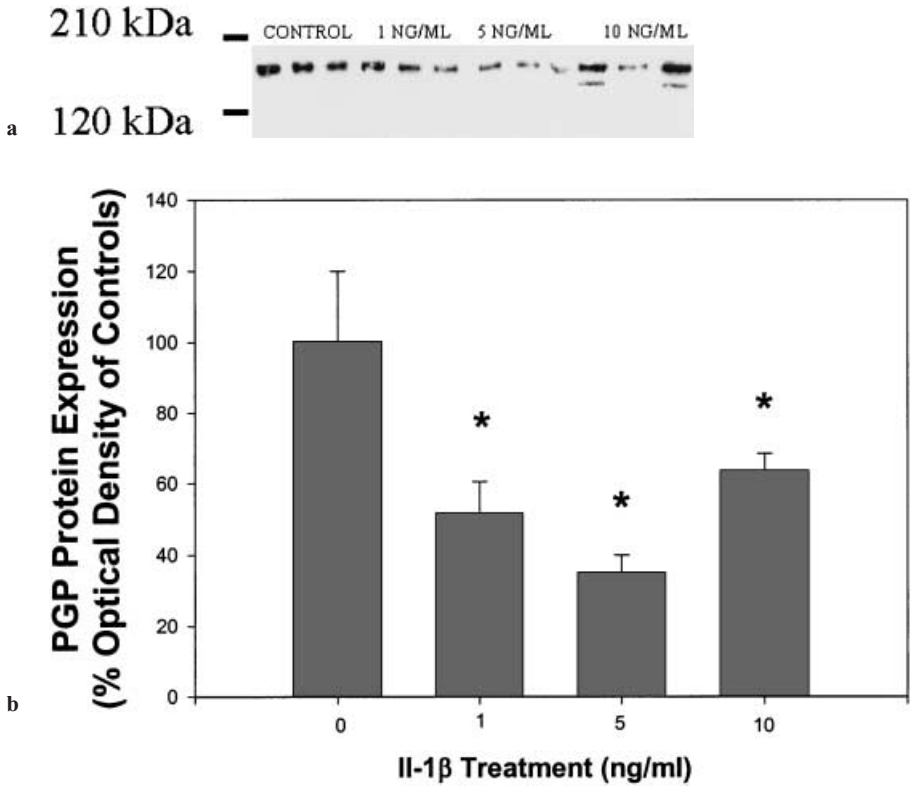

Fig. 3. Effects of IL-1β on PGP Expression. **A**. Representative Western blot, performed as described in the methods. Three each for 0, 1, 5 and 10 ng/ml IL-1β treated samples were run alongside a molecular weight marker. **B.** Plated hepatocytes were treated with reported concentrations of IL-1β for 3 days. PGP expression was measured by immunodetection as described in methods. Values are reported as % of the optical density of controls (mean ± SEM, n = 5/group), *$p < 0.05$.

Expression of the *mdr* genes

As compared to controls, IL-1β treatment (72 h, 5 ng/ml) did not elicit significant effects on mRNA of *mdr1a* or *mdr1b.* Furthermore, IL-1β did not significantly alter levels of *spgp* mRNA (optical densities of RT-PCR products were 93 ± 10% that of controls). Similar to untreated cells, *mdr2* mRNA expression was not detected. Furthermore, RT-PCR analysis did not detect differences in IL-6 mRNA levels between control and IL-1β-treated cells (5 ng/ml, 72 h).

Effect of IL-6 on PGP/mdr expression and activity

PGP protein expression

As compared to controls, immunodetectable levels of PGP were significantly reduced in 1–10 ng/ml IL-6 treated cells after 24 h of incubation. A 20–38% reduction in PGP protein levels was observed in IL-6 treated cells, with a maximal suppression occurring at a concentration of 5 ng/ml (Fig. 4). This suppression was significant over the concentrations of 1 to 10 ng/ml, however diminished levels of PGP in 25 or 50 ng/ml IL-6-treated cells did not reach significance.

PGP functional activity

As compared to controls, the verapamil-inhibitable efflux of Rh123, which is representative of PGP transport activity, was substantially reduced in IL-6 treated cells (Fig. 1). Indeed RH123 efflux was often below the limit of detection in the cells treated with 5 ng/ml IL-6.

Expression of the mdr genes

As compared to controls, significant reductions in *mdr1b* mRNA levels were seen at 24 h in 5 ng/ml IL-6 treated cells (Fig. 4). Detectable levels of *mdr1a* also demonstrated a downward trend in the treated cells, however this decrease did not reach significance (Fig. 5). On the other hand, mRNA levels of *spgp* were not significantly different between control and treated cells (Fig. 4).

Influence of IL-1β and dexamethasone on IL-6-treated cells

The influence of IL-1β on IL-6 mediated effects was examined in hepatocytes co-incubated (24 h) with 5 ng/ml of each cytokine. As compared to controls or to cells that were treated with IL-1β or IL-6 alone, a further reduction in the immunodetectable levels of PGP was seen in cells treated with both cytokines (Fig. 6). After 24 h, we saw a 55% reduction in PGP protein levels in cells co-incubated with both IL-6 and IL-1β which was substantially larger than the 36% reduction seen with IL-6 only. Significant effects of IL-1β on PGP expression were not seen at 24 h. The reductions in protein expression did not yield corresponding changes in mRNA as cells co-incubated with IL-1β and IL-6 did not display significant changes in mRNA levels of *mdr1a, mdr1b* or *spgp* (Fig. 4).

The influence of DEX inclusion on IL-6 mediated changes in PGP expression was examined in IL-6 and control

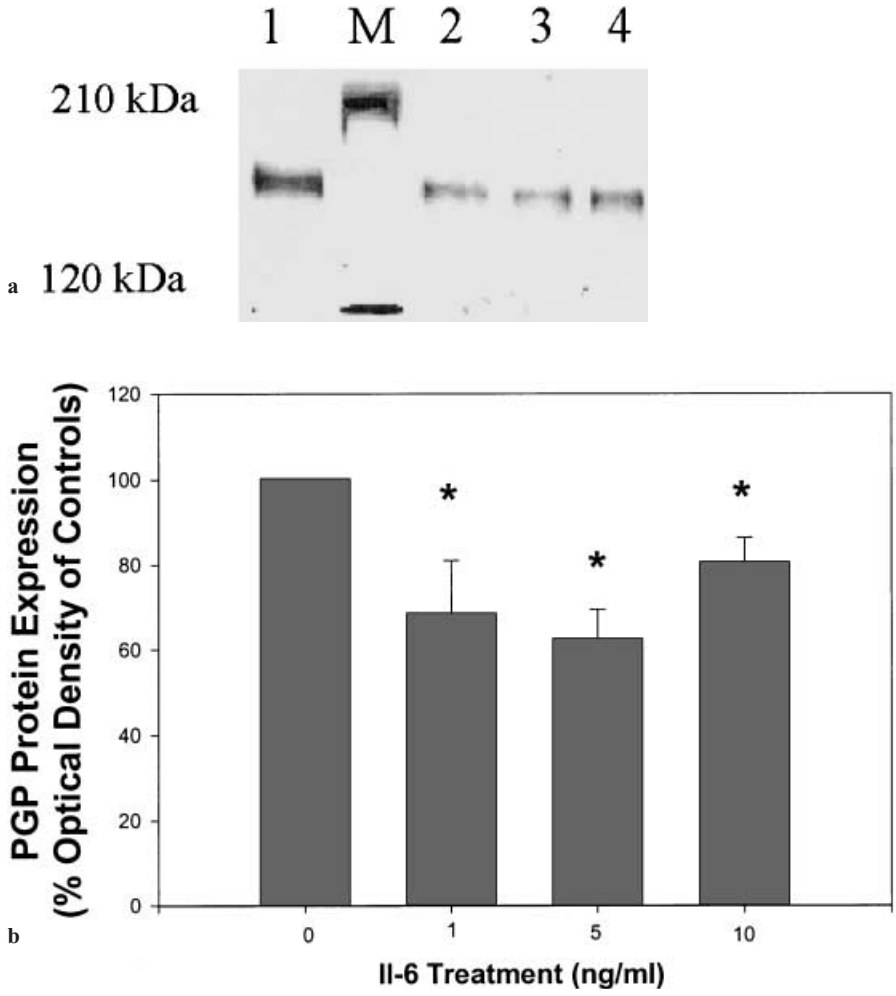

Fig. 4. Effects of IL-6 on PGP Expression. **A.** Representative Western blot, performed as described in the methods. Lane identities are as follows: Lane #1 0 ng/ml IL-6, lane #2 1 ng/ml, lane #3 5 ng/ml and lane #4 10 ng/ml. **B.** Plated hepatocytes were treated with reported concentrations of IL-6 for 24 h. PGP expression was measured by immunodetection as described in methods. Values are reported as % of the optical density of controls (mean ± SEM, n = 5/group), *p < 0.05.

hepatocytes. We observed significant reductions in the immunodetectable levels of PGP in IL-6 treated cells that were cultured with both DEX-free or standard media, however the effect of IL-6 on PGP expression was diminished in cells cultured in DEX free media. Relative to controls, IL-6 treatment in DEX free media resulted in a 20% decrease in PGP levels whereas a 40% reduction was apparent in IL-6 treated cells cultured in standard media containing 50 nM DEX. Furthermore, the suppressive effect of IL-6 on PGP expression was abolished (significant effects not seen) in high DEX media which contained 250 nM DEX.

Discussion

The findings from this study, which demonstrate a reduction in PGP expression and activity as well as significantly reduced *mdr1b* mRNA levels in IL-6 treated hepatocytes, indicate a regulatory effect of IL-6 on PGP. Our laboratory and others have observed an increased PGP/*mdr1b* expression in hepatocytes with culture time [21, 22]. As we saw a 1.5 fold induction in PGP expression in non-treated cells over the 24-h treatment period, results from these experiments may stem from an IL-6 mediated suppression of PGP induction rather than a reduction in the basal expression of PGP [22–24]. Although increasing PGP expression is an inherent problem with the primary hepatocyte model, many rat hepatoma cell lines have limited responsiveness to cytokine-mediated acute inflammatory stimuli [11, 25, 26] and thus are not practical for examining the effects of cytokines and inflammatory stimuli on gene regulation. Nevertheless, a downregulation in the basal expression and activity of PGP/*mdr1* has been previously observed in the livers of turpentine and endotoxin-treated rats [7] and mice [27]. As these animal models of inflammation are associated with elevated concentrations of IL-6 and IL-1β_ this may imply involvement of IL-6 and/or IL-1β in down-regulation of PGP expression in vivo. Indeed, recent in vivo studies have demonstrated an IL-6-mediated suppression in the hepatic expression of the *mdr1*/PGP isoforms in IL-6 treated mice [27].

The reduced protein expression of PGP in IL-6 treated cells was associated with a corresponding decrease in *mdr1b*

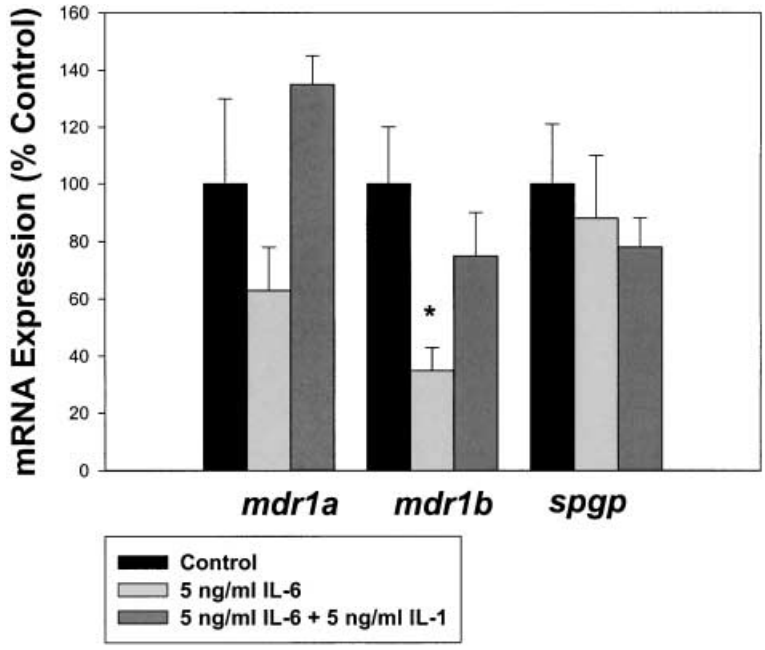

Fig. 5. Effect of cytokines on *mdr* and *spgp* mRNA expression. Hepatocytes were incubated for 24 h in the presence or absence of IL-6 (5 ng/ml), or IL-6 (5 ng/ml) + IL-1β (5 ng/ml), and quantitative RT-PCR was run on the isolated total RNA samples as described in the methods. Values (mean ± SEM, n = 6/group) are reported as % of controls; raw intensities of the *mdr1a, mdr1b* and *spgp* genes were normalized with respect to GAPDH, * $p < 0.05$.

(and, to a lesser extent, *mdr1a*) mRNA levels. It is likely that PGP expression is altered by IL-6 at the level of transcription. Modulation of acute-phase gene expression by IL-6 has been shown to be mostly at pretranslational level and IL-6 mediated downregulation in gene transcription of these proteins results in rapid decreases to both protein and mRNA levels [28, 29]. The transcription of CYP gene family members is regulated during an inflammatory response or by inflammatory mediators such as IL-6 [28–33]. It has been established that these changes occur primarily through an IL-6 mediated induction of the CCAAT/Enhancer Binding Protein transcription factors. Reportedly, the *mdr1* regulatory sequence also possesses binding sites for a number of these transcription factors, including CCAAT/Enhancer Binding Protein β (NF-IL-6) [34], NF-κB [35], and AP-1 [36]. Moreover, it is possible that the cytochrome P450 and *mdr* gene families may have similarities in their regulation particularly as many similarities in substrates, inducers, and inhibitors are found between these families [37]. Furthermore, other pro-inflammatory cytokines, namely tumor necrosis factor and interferon, have been reported to downregulate both *mdr1* [38, 39] and cytochrome P450 gene transcription [28], supporting potential parallelism in their regulation.

Interestingly, IL-6 mediated a significant suppression of PGP over the range of 1–10 ng/ml (20–200 Units/ml), bioactive concentrations comparable to those observed in patients with severe inflammatory states [40]. Although non-significant reductions in PGP expression were seen at higher concentrations, it is likely that these concentrations exceeded normal activities and could be toxic to the cells. Similar to our findings, it has been reported that IL-6-maximally suppresses taurocholate uptake in cultured rat hepatocytes at a concentration of 100 U/ml [41]. Dose-response experiments examining IL-6 effects on Factor XII production also indicate that downregulation of Factor XII reaches a plateau at concentrations of 5 ng/ml [42]. Interestingly, Citarella et al. [42] demonstrated a 25–37% decrease in protein levels of Factor XII and the negative acute phase protein, transthyretin after 24 h of 5 ng/ml IL-6 stimulation, similar to the suppression of PGP which we detected with IL-6 treatments.

It is also conceivable that changes in C-219 immunodetectable levels could be reflective of changes in *spgp* and *mdr2* expression, in addition to *mdr1a* and *mdr1b*. However, RT-PCR analysis demonstrated an extremely low expression of *mdr2*, and IL-6 did not alter the expression of *spgp*. Thus our results are consistent with an IL-6-mediated reduction in *mdr1* expression. Furthermore, as Rh123 is transported by gene products of *mdr1* but not of *mdr2* and *spgp*, the decreased PGP-mediated (verapamil-inhibitable) efflux of Rh123 we observed indicates a reduction in the functional activity of the *mdr1* gene products.

Studies examining the effects of various stimuli on PGP expression have reported a substantially greater capability for induction of *mdr1b* than *mdr1a* [43]. Furthermore, *mdr1b* expression increases with time in culture [21]. Interestingly we saw increases in both *mdr1a* and *mdr1b* mRNA levels over the first 24 h in culture, along with an increased PGP protein expression and *mdr1* functional activity. This contradicts previous reports suggesting that *mdr1a* is not induced in culture [44]; however, as that work was performed in hepatocytes isolated from a different strain of rat, strain differences may be responsible for this discrepancy. While increased mRNA stability is thought to be responsible for the induction of *mdr1b* [21], further investigation is necessary in order to characterize the mechanism of *mdr1a* induction observed herein.

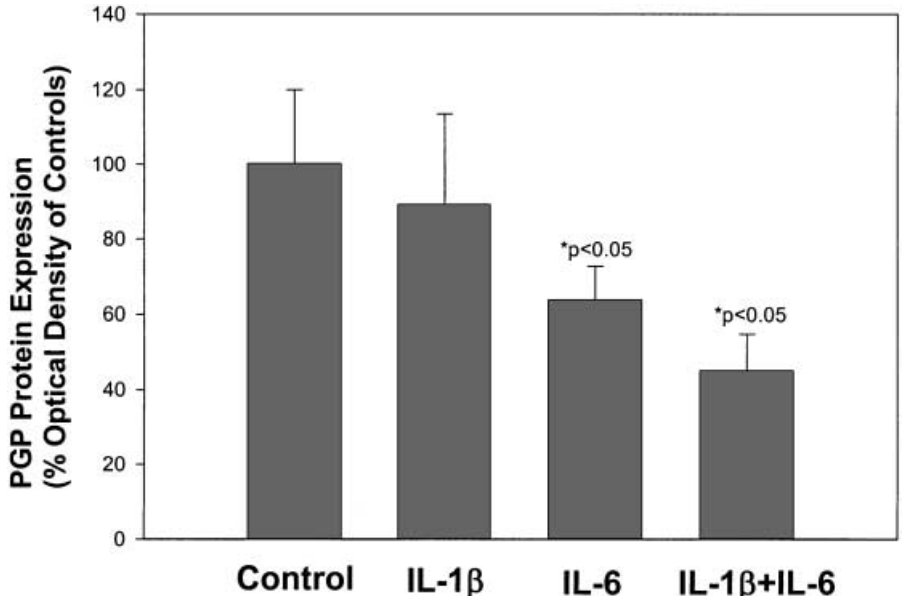

Fig. 6. Effect of combined IL-1β and IL-6 on PGP expression. Plated hepatocytes were treated for 24 h with 5 ng/ml of IL-6 and IL-1β. PGP expression was measured by immunodetection as described in methods. Values are reported as % of the optical density of controls (mean ± SEM, n = 7/group), * $p < 0.05$.

Significant decreases in the expression and functional activity of PGP were also observed in IL-1β treated cells. However as significant effects on PGP were seen only after three to five days of treatment with IL-1β, but within 24 h of IL-6 treatment, this suggests that these cytokines exert their actions on PGP through alternate pathways. Furthermore, in contrast to effects observed with IL-6, *mdr* and *spgp* mRNA levels were not significantly affected by IL-1β treatment. This may indicate that IL-1β-mediated changes in PGP expression occur through post-translational processes, such as decreased protein stability or translation rates. It has been demonstrated that the half-life of PGP is dramatically prolonged (from 17 h to 72 h) [45, 46] in hepatocytes maintained in serum-deprived media, as was utilized in our studies. If changes occur at the level of protein expression, relatively lengthy incubation times would be required before effects become measurable, consistent with our findings. Although cell death could also account for diminished protein levels, IL-1β treatments did not significantly affect cell viability. On the other hand, as IL-1β has been reported to induce the expression of IL-6 in vivo [47] and in vitro [45, 48], it could be hypothesized that effects of IL-1β occur through IL-6. However, this hypothesis is not plausible as RT-PCR analysis indicated a lack of IL-6 mRNA induction in the IL-1β treated cells. We therefore hypothesize two separate mechanisms: a rapid IL-6-mediated reduction in *mdr1* gene transcription and an IL-1β-mediated decrease in PGP protein synthesis and/or stability.

The inflammatory response leads to the production and release of multiple cytokines, which exert a complex, concentration dependent network of biological activities. Evidence of IL-1β exhibiting synergistic or inhibitory influences on IL-6 mediated effects on gene expression has been seen with a number of proteins including α1-acid glycoprotein and fibrinogen [49]. Likewise, IL-1β appeared to influence IL-6-mediated effects on PGP expression. A greater reduction of PGP expression was seen in cells co-incubated for 24 h with both IL-6 and IL-1β as compared to IL-1β or IL-6 alone. In fact, effects of IL-1β on PGP protein levels were not seen before 72 h of treatment. This may indicate additive effects between IL-1β and IL-6 on PGP expression. However, changes in mRNA expression did not correspond to that seen with protein: *mdr1b* levels were slightly, but not significantly, decreased in the co-incubated cells. As mRNA levels were significantly decreased in cells treated with IL-6 alone, this may suggest an inhibitory interaction, diminishing the influence of IL-6 on *mdr1b* gene regulation.

It is known that DEX elicits changes in PGP expression in vitro [50] and in vivo [51]. However, the inclusion of DEX in primary culture media is considered necessary as its absence results in reduction of cell viability and attachment to culture dishes [52]. Moreover, addition of glucocorticoids to cultured rat hepatocytes is generally required for cytokine-mediated activities [53]. Similarly, we observed that while IL-6 mediated a suppression of PGP in both the absence and presence of low concentrations of DEX (50 nM) effects were substantially diminished in the absence of DEX. Furthermore, as expected, PGP suppression was not apparent at 250 nM DEX, anti-inflammatory concentrations associated with the inhibition of cytokine-mediated activities. Thus it appears that inclusion of 50 nM DEX in our culture media did not interfere with, but rather was required to examine IL-6 mediated effects on PGP.

In conclusion, our data suggests that IL-1β and IL-6 are involved in PGP regulation. Changes in the expression and activity of PGP in cultured hepatocytes likely occur through a suppressive effect on PGP induction. Notable differences in time courses of PGP suppression suggest that IL-1β and IL-6 affect PGP expression through different mechanisms. IL-6 appears to affect PGP expression at the level of mRNA, while

IL-1β acts at the level of protein. This supports the hypothesis that IL-1β and IL-6 are involved in the in vivo suppression of PGP observed in experimental models of inflammation [7]. It is therefore possible that IL-1β and IL-6 may work in concert in vivo, resulting in possible additive effects on PGP expression. Future studies will attempt to identify the transcription factors involved in PGP downregulation. Clinically, these findings are important in that they may aid us in understanding the diversity of PGP expression in healthy and diseased tissues. Secondly, elucidating mechanisms of PGP downregulation may ultimately be useful in the development of therapeutic strategies to control PGP-mediated drug resistance.

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