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Minireview

Shining a Light on Inflammation as a Critical Modulator of Drug Metabolism

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ABSTRACT

Since his graduate studies on alcohol induction of a novel cytochrome P450 (P450) enzyme, through his postdoctoral work on hormonal regulation of sexually differentiated cytochrome P450s (P450s), the author has maintained an interest in the regulation of drug metabolizing enzymes. This article is a recounting of his scientific career and focuses on his laboratory's work on inflammatory regulation of P450 enzymes that formed the basis for the Bernard B. Brodie Award. Key findings and publications are identified and discussed that contributed to the elucidation of some important principles: 1) inflammatory stimuli generally downregulate P450 enzymes, resulting in reduced metabolism of substrate drugs; 2) the main mechanism for this downregulation is transcriptional and involves both the activation of negatively acting transcription factors and the suppression of positive transcription factors; 3) inflammatory cytokines such as interleukin 1, interleukin 6, and tumor necrosis factor α act on hepatocytes to mediate this regulation; 4) these cytokines selectively regulate different P450 enzymes, and therefore different P450s are downregulated in different inflammatory diseases or disease models; 5) nitric oxide formed by inducible nitric oxide synthase 2 reacts with P450s in an enzyme-specific manner to stimulate their proteolytic degradation; and 6) both tyrosine nitration and heme nitrosylation are likely required for this NO-stimulated degradation. Finally, findings from clinical studies are discussed that shine a light on the importance of P450 regulation by inflammation for drug development, clinical practice, and personalized medicine.

SIGNIFICANCE STATEMENT

This article discusses the key publications and findings in the author's laboratory that helped to identify inflammation as an important factor contributing to interindividual variation in drug metabolism.

Introduction

Research in the last half-century has shown that inflammatory prompts can downregulate hepatic cytochrome P450 (P450) enzymes, and that the enzymes affected depend on the nature of the inflammatory stimulus. We now understand that inflammation is an important component of many human diseases, including arthritis, nonalcoholic fatty liver disease, asthma, congestive heart failure, cancer, obesity, metabolic disease, and many neurologic disorders, as well as of infections and responses to tissue and organ damage. Therefore, people with any of these diseases or conditions may have altered metabolism and exposure to drugs and herbal preparations taken not only to treat the disease and its symptoms, but also for other coexisting ailments, with consequences for drug toxicity or efficacy. Clinical studies have borne this out. When my laboratory began research in this area in the mid-1980s, much of the research on drug metabolizing enzyme (DME) regulation was focused on enzyme inhibition, enzyme induction and on the burgeoning field of pharmacogenetics. Each of these factors often have larger effects on drug metabolism than the effects of inflammation that we and others in the field were reporting. For this reason, and possibly also the fact that the contributions of inflammation to human disease were not as well understood, it took time for the significance of inflammatory regulation of P450s, other DMEs and drug transporters to be fully appreciated.

This review will concentrate on the contributions of my own laboratory, but I am indebted to my colleagues in the field for their work (including Drs. Ken Renton, Micheline Piquette-Miller, Patrick Maurel, André Guillouzo, Fakhreddin Jamali, Takashi Ashino, Patrick Du Souich, Steven Shedlofsky, Remi Ghose, Maria Croyle, Jose Castell, Magnus Ingelman-Sundberg, Geoffrey Farrell, Stephen Clarke, Almira Correia, Yoichi Osawa, and Oleg Khatsenko) who contributed greatly to our understanding of this phenomenon. We informed and inspired each other's research, so that gradually the importance of inflammatory regulation of drug metabolism was brought to light.

Because the Bernard B. Brodie Award is bestowed by the Drug Metabolism and Disposition Division of ASPET, and as ASPET has played a huge part in my career, it is pertinent to note that two-thirds of our original research papers in this area were published in ASPET journals, including the most-cited work (Aitken and Morgan, 2007).

From Alcohol and Sex to Inflammation

In my graduate work at the University of Glasgow, I used an alcohol vapor inhalation system to gather evidence that alcohol induces a novel

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ABBREVIATIONS: DME, drug metabolizing enzyme; GH, growth hormone; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; NO, nitric oxide; NOS, nitric oxide synthase; P450, cytochrome P450; *PccAS*, *Plasmodium chabaudi chabaudi AS*; poly I:C, polyriboinosinic acid-polyribocytidylic acid; PXR, pregnane X receptor; TLR, toll-like receptor; TNFα, tumor necrosis factor-α.

form of cytochrome P450 in rat liver (Morgan et al., 1981). At the age of 24, I graduated and moved to Dr. Jud Coon's laboratory at the University of Michigan, where I was younger and less seasoned than the other postdocs in the laboratory. I am grateful to all of them for their encouragement and advice, especially to Dr. Dennis Koop, who showed me not only how to purify P450s but also how to think like a rigorous independent scientist. Through discussions of my doctoral work, Dennis became interested in the ethanol-induced P450. I contacted Dr. Paul Skett in Glasgow, who agreed to expose a rabbit to alcohol in our vapor inhalation chamber and ship its liver to Michigan. Gel electrophoresis of the intoxicated rabbit's liver microsomes convinced Dennis that it was worth trying to purify this novel P450, and together we purified (mainly Dennis) and characterized CYP2E1 (Koop et al., 1982; Morgan et al., 1982). We had a lot of great times on the golf course as well!

After 3 years in Ann Arbor, I accepted an invitation from Dr. Jan-Åke Gustafsson to join his laboratory at the Karolinska Institute as a Visiting Scientist. During my last year in Glasgow, Paul Skett had introduced me to Jan-Åke whose laboratory had worked for years on characterizing sex-dependent steroid and drug-metabolizing activities in rat liver (Einarsson et al., 1973; Gustafsson et al., 1983). Paul, together with Dr. Agneta Mode and others in the Gustafsson group, had demonstrated that these activities were regulated through the hypothalamo-pituitary axis by the sex-specific temporal pattern of growth hormone (GH) secretion (Skett et al., 1980; Mode et al., 1982). The group hypothesized that these sex-dependent activities were due to the presence of sex-specific forms of cytochrome P450. On arriving in Sweden, my remit was to purify a male-specific form catalyzing the 16α-hydroxylation of testosterone and other steroids.

I already knew that Schenkman and colleagues had purified a P450 enzyme from male rat livers, RLM5, that possessed this activity (Cheng and Schenkman, 1982), although they had not tested whether RLM5 was sex-specific. I therefore decided to purify RLM5 (also known as P450h, now CYP2C11) using the published method. I prepared specific antibodies to the enzyme and used them to demonstrate that it was expressed specifically in males and that this was imprinted by neonatal androgens (Morgan et al., 1985b). Under the supervision of myself and Dr. Jim Halpert, graduate student Catriona MacGeoch also purified P450i (CYP2C12), showing that it is female-specific and responsible for steroid sulfate 15β-hydroxylase activity in rat liver (MacGeoch et al., 1984). These papers provided conclusive evidence that sex differences in metabolism are due to differential enzyme expression and not modulation of enzyme activity. My growing friendship with Jim Halpert at this time was pivotal, as he became an important influence throughout my career development. At the time of writing, I just learned that Jim died following an accident near his home in Arizona. He was a titan in the P450 field and is sorely missed.

We went on to demonstrate that the sex-specific expression of CYP2C11 and CYP2C12 are regulated via the hypothalamo-pituitary axis and GH (MacGeoch et al., 1985; Morgan et al., 1985a), and with another graduate student, Anders Ström, we cloned the cDNAs for CYP2C11 (Strom et al., 1988) and CYP2C12 (MacGeoch et al., 1987).

While the scientific environment at Karolinska was superb, my wife Leslie and I decided that our futures lay in the United States. After four years at Karolinska, I accepted a position at Emory University as an Assistant Professor of Pharmacology. I knew that I wanted to continue to study regulation of CYP2C11 and CYP2C12, as I was in possession of unique tools to do so, but that I needed to distinguish my research from the Gustafsson laboratory. Waiting for equipment and supplies to fill my mostly empty laboratory, I pondered my first NIH grant submission, wracking my brain for ideas on how to do this.

An idea came when I recalled the work of my colleague Dr. Gunnar Norstedt at Karolinska. In the laboratory of Dr. Richard Palmiter at the University of Washington. He previously found that the mouse Major Urinary Protein, a male-specific hepatic gene product, was regulated by the sexually differentiated pattern of GH secretion (Norstedt and Palmiter, 1984), just as we had discovered for the sex-specific P450s. Interestingly, α2-urinary globulin, a similar male-specific protein synthesized in rat liver, was downregulated during an inflammatory response (Schreiber et al., 1986). Connecting these two findings, I speculated whether regulation by inflammation might be a common property of sex-specific liver proteins.

I then remembered the work of Dr. Ken Renton, first with Dr. Gil Mannering at the University of Minnesota and subsequently in his own laboratory at Dalhousie University, in which they described the effects of interferon inducers including the synthetic double-stranded RNA polyriboinosinic acid-polyribocytidylic acid (poly I:C), or interferons themselves, in causing decreased total cytochrome P450 content and decreased P450-associated drug metabolizing activities in rodent livers and hepatocytes (Renton and Mannering, 1976; Renton et al., 1978; Singh and Renton, 1981). This reinforced my growing ideas, and I included an Aim to investigate the inflammatory regulation of CYP2C11 and CYP2C12 in my grant submission, which was funded in 1988.

Inflammatory Regulation of P450 Expression

Lipopolysaccharide (LPS) Model

For my first venture into the field of inflammation, I chose injection of bacterial endotoxin or lipopolysaccharide (LPS), a recognized model of gram-negative bacterial sepsis that had been shown to decrease total P450 content and drug metabolizing activities in rats (Gorodischer et al., 1976; Renton and Mannering, 1976), as the inflammatory stimulus. As shown in Fig. 1 (Morgan, 1989), intraperitoneal injection of 1 mg/kg LPS caused rapid downregulation of hepatic CYP2C11 (P450h) and CYP2C12 (P450i) mRNAs in male and female rats, respectively, falling to approximately 10% of control within 12 h. The mechanism of downregulation of CYP2C11 was pretranslational, since the protein regulation trailed that of the mRNA. However, CYP2C12 protein downregulation closely parallelled its mRNA, suggesting that posttranslational mechanisms may also contribute.

Dr. Kristina Wright then joined the laboratory on sabbatical leave from Georgia State University and used nuclear run-on assays to demonstrate that CYP2C11 mRNA downregulation by LPS and by turpentine-induced inflammation is due to transcriptional suppression (Wright and Morgan, 1990), which Dr. Po-Yung Cheng later showed occurs within 1 h of LPS injection (Cheng et al., 2003) (Fig. 2). Further experiments with LPS in rats and mice revealed that the majority of P450 gene products studied are downregulated (Morgan, 1993; Sewer and Morgan, 1997; Sewer et al., 1998), and Dr. Madhu Chaluvadi showed that this is dependent on the LPS receptor toll-like receptor (TLR)-4 (Chaluvadi et al., 2009). However, a few enzymes were upregulated, including CYP4As in rat liver (Sewer et al., 1997) and Cyp2d9 and Cyp3a13 in mice (Chaluvadi et al., 2009).

We also determined that the inflammatory downregulation of sex-specific P450s is not due to perturbation of their GH regulation, because the same or even amplified responses occurred in the livers of hypophysectomized rats in which the level of GH was held constant via osmotic minipumps (Morgan, 1993).

Other Models of Inflammation and Infection

Studies in our laboratory and others in the 1990s and 2000s were beginning to show that not only are P450 enzymes regulated by inflammatory cytokines, but also that different P450s were sensitive to different cytokines (see next section). These findings implied that the pattern of P450 regulation was likely to differ depending on the specific disease. Therefore, it was an ongoing interest in the laboratory to study P450

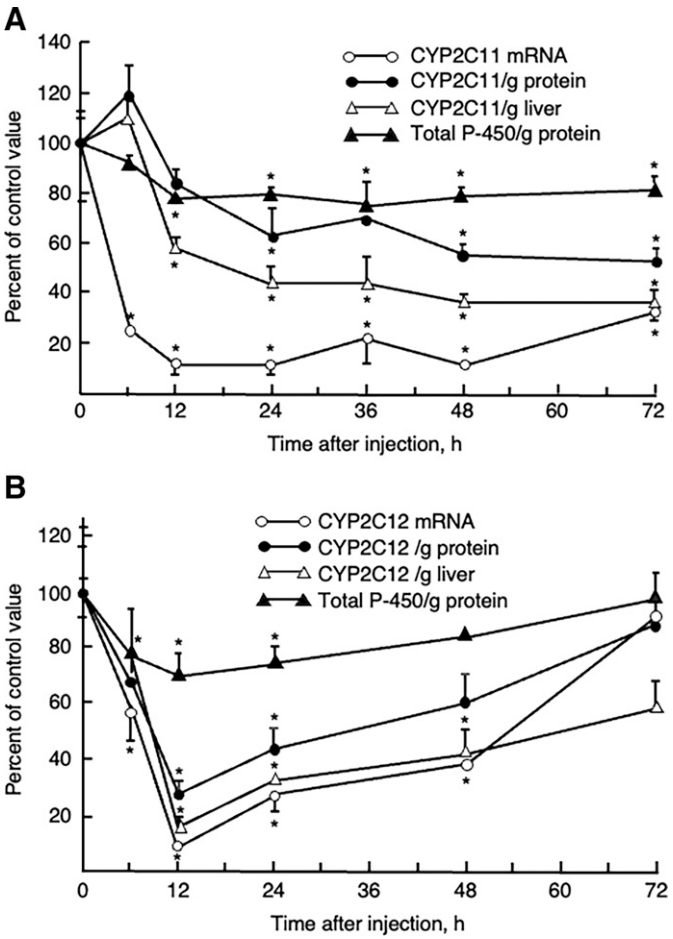

Fig. 1. Time course of downregulation of CYP2C11 and CYP2C12 in LPS-treated rats. Adult male (A) and female (B) rats were given a single intraperitoneal injection of 1 mg/kg LPS and killed at the times indicated. Relative hepatic levels of P-450h (CYP2C11, n = 7–8) and P450i (CYP2C12, n = 4) proteins were determined by Western blotting. Relative levels of the respective mRNAs were measured by slot blotting, normalized to the hepatic content of β-actin. All values are given as percentages of the mean values for the control group. * Significantly different from control, $P < 0.05$. Redrawn and edited from Figs. 2 and 6 in Morgan (1989).

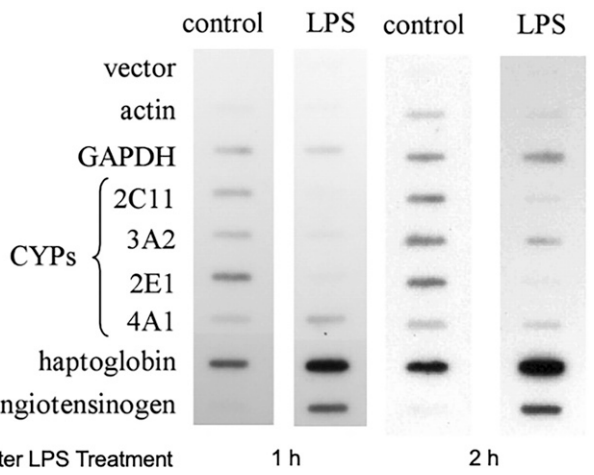

Fig. 2. Transcriptional suppression of P450 genes following LPS treatment. Male Fischer F344 rats were injected intraperitoneally with 1 mg/kg Escherichia coli LPS and killed at the indicated times. Nuclear run-on assays were performed by hybridizing nuclear RNA to linearized plasmids containing the respective cDNAs, and the signals on the blots were quantified. Redrawn and edited from Fig. 1 in Cheng et al. (2003).

regulation in different disease models. Some of our most informative work is described here. Taken together, these studies suggest that in models or diseases where there is a high degree of liver involvement (LPS, parasitic infections), there tends to be a more global downregulation of P450s and other DMEs than if the inflammation originates extrahepatically (e.g., intestinal inflammation, sterile irritants).

Interferon Inducers. In their pioneering work, Renton and Mannering had shown that interferon-inducing agents, including poly I:C, cause suppression of total P450 and selected drug metabolizing activities in rat liver (Renton and Mannering, 1976). We now know that double-stranded RNAs such as poly I:C act via TLR2 (Alexopoulou et al., 2001). An undergraduate student, Catherine Norman, and I together found that, as for LPS, poly I:C injection causes pretranslational downregulation of CYP2C11 in male rats and CYP2C12 in females (Morgan and Norman, 1990; Morgan, 1991).

Sterile Irritants. Dr. Marion Sewer was a graduate student in my laboratory who published five first-author papers and four coauthored articles from her doctoral work. Her major efforts were in delineating the contribution of nitric oxide (NO) to inflammatory P450 regulation (see below). She also found that three different particulate irritants, such as LPS, each downregulated CYP2C11 and upregulated CYP4As in rat livers (Sewer et al., 1997). However, unlike LPS the irritants did not downregulate CYP3A2 or CYP2E1, nor did they induce nitric oxide synthase (NOS)2 (Sewer and Morgan, 1998), supporting the hypothesis that P450s are regulated differently according to disease state.

***Citrobacter Rodentium* Infection.** In the mid-2000s, I met Dr. Dan Kalman from the Department of Pathology at a graduate program mixer. I was intrigued by his studies of the murine pathogen *C. rodentium*, that causes colonic inflammation and pathology indistinguishable from those elicited by enteropathogenic *Escherichia coli* in human food poisoning. Notably, infected mice show only minor clinical symptoms of infection, and so I decided that *C. rodentium* would be an excellent holistic model in which to study the impact of a human-relevant, nonfatal infectious disease.

Dr. Terrilyn Richardson took charge of this project, collaborating with the Kalman laboratory and also Dr. Henry Strobel's laboratory at the University of Texas Medical School in Houston to delineate the effects of infection on hepatic and renal P450s (Richardson et al., 2006a) and UDP-glucuronosyltransferases (Richardson et al., 2006b) expression. *C. rodentium* infection caused a more enzyme-selective hepatic P450 downregulation than LPS, with some P450s unaffected or induced (Richardson et al., 2006a). These effects were largely independent of TLR4 (and therefore of LPS) because the effects were the same in wild-type and TLR4-deficient C3/HeOuJ mice (Fig. 3). Notably, Cyp4a enzymes were profoundly downregulated to less than 5% of control in infected mice. Therefore, we sought to ask why P450s are downregulated in inflammation by performing studies on mice that were null for Cyp4a10 or 4a14. Absence of either gene reduced the hepatic inflammatory response to *C. rodentium* infection, suggesting roles for Cyp4a enzymes in regulating inflammation (Nyagode et al., 2014b). This concept has since been validated by at least three other groups (Zhang et al., 2017; Xuan et al., 2018; Gao et al., 2020).

Parasitic Infections. In 2012, Sylvie Mimche, MSc began work as a volunteer in my laboratory. Sylvie approached me about working on two parasitic infections common in her native country (Cameroon): malaria and schistosomiasis. Prompted by Sylvie's enthusiasm and persistence, I initiated a collaboration with Dr. Rick Cummings's laboratory in Biochemistry, and we obtained livers from *Schistosoma mansoni*-infected mice to study their DMEs. Sylvie and Dr. Betty Nyagode found that at the height of infection, most P450, UDP-glucuronosyltransferase,

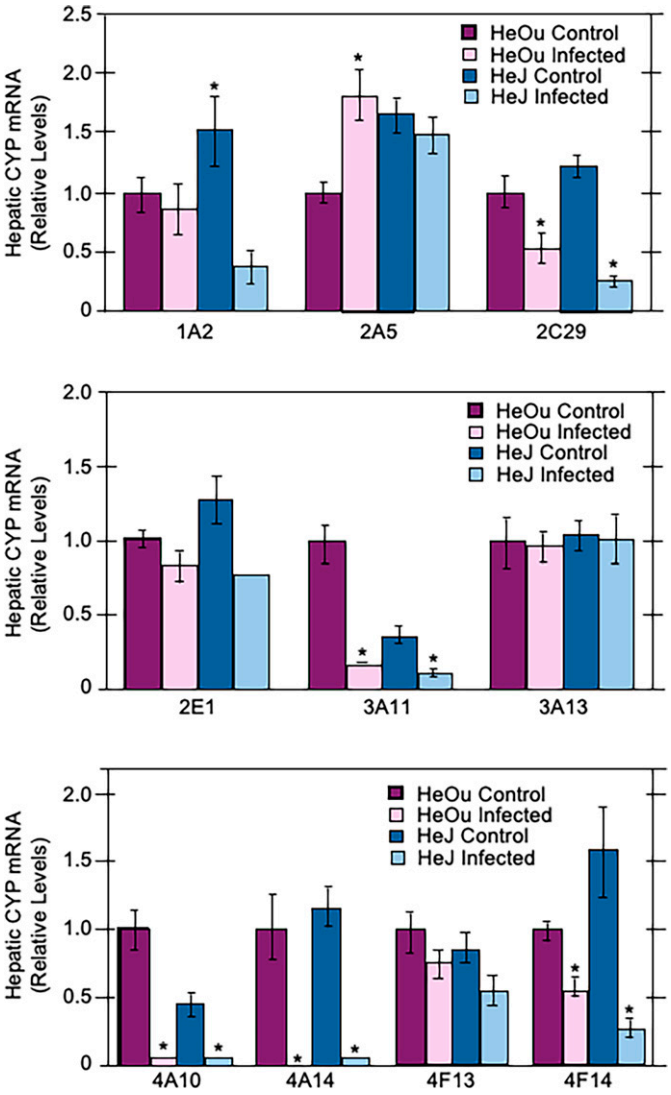

Fig. 3. Effect of *C. rodentium* infection on mRNA expression of hepatic P450 enzymes in C3/HeOu and C3/HeJ mice. Mice were treated with either saline or *C. rodentium* by oral gavage, and hepatic mRNA expression was determined after 6 days. Values are expressed as relative levels of mRNA expression after normalization to glyceraldehyde 3-phosphate dehydrogenase, and represent means ± S.E.M. for each group ($n = 4$ or 6). Designations denote significant differences ($P < 0.05$) from respective control groups (*). Redrawn and edited from Figs. 1 and 2 in Richardson et al. (2006a).

sulfotransferase, and nuclear receptor (NR) mRNAs and proteins measured (Mimche et al., 2014) were markedly downregulated.

Sylvie also spearheaded a collaboration with Dr. Tracey Lamb's laboratory in the Microbiology and Immunology Department to study DMEs in malarial infection. Tracey was working with the rodent malarial pathogen *Plasmodium chabaudi chabaudi AS*(*PccAS*). *PccAS* has many attributes in common with human *Plasmodium* parasites and, in contrast to the more commonly used *Plasmodium berghei*, is not a lethal infection. Mice were infected with *PccAS* in the Lamb laboratory, and Sylvie measured the relative hepatic mRNA levels of ten P450s, four UDP-glucuronosyltransferases, five sulfotransferases, six nuclear receptors, flavin monooxygenase-3 and NAD(P)H-quinone oxidoreductase-1 at 6, 8, and 12 days postinfection. We found that almost all these mRNAs were downregulated at 8 days, the peak of the infection (Mimche et al., 2019).

Given the substantial declines seen in several P450 mRNAs, we teamed with Ken Liu, then a graduate student in Dr. Dean Jones's laboratory in the Department of Medicine, to measure the functional consequences. Mice at the peak of *PccAS* infection were administered a cocktail of prototypic substrates of human P450s by intravenous injection, and plasma levels of drugs were measured on a high-resolution mass spectrometry metabolomics platform. As seen in Fig. 4, infection significantly increased exposure to caffeine, bupropion, tolbutamide and midazolam, substrates of human CYPs 1A2, 2B6, 2C9, and 3A4/5, respectively, and this corresponded to 60–70% decreases in the clearances of these drugs. These findings indicate that people with malaria are likely to experience increased exposure to not only antimalarial drugs, but also to comedications that are metabolized by P450 enzymes.

Roles of Cytokines and Interferons

In the late eighties, Ghezzi et al. and Shedlofsky et al. demonstrated that administration of the inflammatory cytokines tumor necrosis factor alpha (TNFα) (Ghezzi et al., 1986a) or interleukin (IL)1 (Ghezzi et al., 1986b; Shedlofsky et al., 1987) caused suppression of total hepatic P450 levels as well as of P450-dependent microsomal activities. Therefore, we began to investigate cytokines as likely mediators of the downregulation of P450 mRNAs and proteins. Kris Wright reported that in vivo administration of IL1α, but not IL6, downregulated CYP2C12 expression in female rats (Wright and Morgan, 1991), and we could reproduce this selectivity in cultured rat hepatocytes (Morgan et al., 1994). In male rats CYP2C11 was downregulated by either IL1 or IL6 (Morgan et al., 1994). In contrast, CYP3A2 was sensitive to IL-1 but not IL6, and CYP2E1 was sensitive to IL6 but not IL1 alone. Dr. Jin-Qiang Chen recapitulated the downregulation of CYP2C11 by IL1β, IL6, and TNFα in primary rat hepatocyte cultures (Fig. 5). These findings, as well as contemporary work in other laboratories (Craig et al., 1990; Chen et al., 1992), clearly showed that rodent P450s are differentially responsive to cytokines (Chen et al., 1995).

Possibly our most impactful publication was Dr. Allison Aitken's work (Aitken and Morgan, 2007) demonstrating that the selective cytokine regulation observed in rodents is also relevant to humans. We found that IL1β, TNFα, IL6, interferon γ (IFNγ), transforming growth factor-β, and LPS differentially downregulated six different human P450 enzymes in primary human hepatocytes (Fig. 6). This paper brought attention to cytokines, and IL6 in particular, as important regulators of human P450s and showed CYP3A4 to be especially sensitive to inflammatory stimuli. It also advanced the concept that P450 enzymes were likely to be differently affected in different human diseases, depending on the temporal patterns and magnitudes of cytokine release.

Drs. Betty Nyagode and Ryan Kinloch then addressed which cytokines are relevant in inflammatory diseases in vivo using the *C. rodentium* model of gastrointestinal bacterial infection. The responses of knockout mice for IL6 or IFNγ (Nyagode et al., 2010), or the TNFα receptor (Kinloch et al., 2011), as well as mice injected with a dominant-negative form of TNFα (Nyagode et al., 2014a), confirmed that different cytokines regulate different P450s in this infection model.

While the above work focused on cytokines that are proinflammatory, two pieces of evidence suggest that T-cell cytokines or mediators may be important in certain disease states. We found that the downregulation of hepatic P450s in *Schistosoma mansoni*-infected mice occurs in the presence of a T helper cell cytokine response and a weaker proinflammatory response (Mimche et al., 2014). We further documented that the downregulations of some P450s during *C. rodentium* infection were attenuated in mice with the severe combined immunodeficiency mutation, even as bacterial translocation to the liver was increased (Nyagode et al., 2012). Further work on regulation of P450s by T cell cytokines is needed.

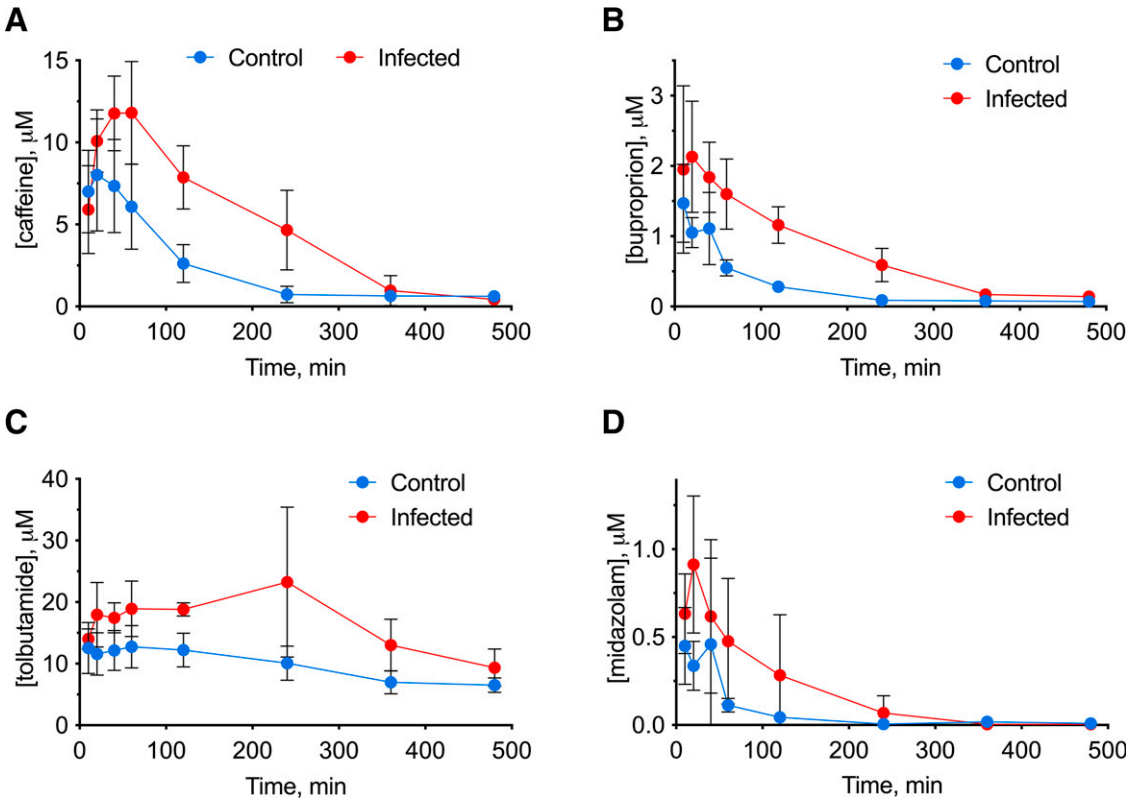

Fig. 4. Pharmacokinetic profiles in naïve and infected mice after cassette drug dosing in *PccAS*-infected mice. Eight days after *PccAS* infection, five naïve and five infected mice were administered a cocktail of five drugs and blood was collected at the indicated times. Drug concentrations in the blood were measured by high-performance liquid chromatography–high-resolution mass spectrometry. The plots show the mean blood concentrations ± S.D. in each group at each time point. A, caffeine; B, buproprion; C, tolbutamide; D, midazolam. Results for bufuralol are not shown due to a mass spectrometry artifact, but can be viewed in the original article. Reproduced from Mimche et al. (2019).

Transcriptional Mechanisms

Because P450s are differentially sensitive to cytokines, the mechanisms of transcriptional downregulation are gene- and cytokine-dependent. Using a chloramphenicol acetyltransferase reporter gene in primary rat hepatocytes, Jin-Qiang Chen demonstrated that CYP2C11 downregulation by IL1, IL6, and TNFα is transcriptional (Chen et al., 1995) (Fig. 5). The same study established that the sequences supporting the suppression of CYP2C11 by IL1 and IL6 lie within the first 200 base pairs of the proximal promoter. Later, Dr. Heinrich Iber demonstrated that CYP2C11 gene suppression by IL1 (but not IL6) was due to binding of the transcription factor nuclear factor-κB to a negative response element that spans the transcription start site of the gene (Iber et al., 2000).

Downregulation can occur either by a direct suppressive effect as described above, or by the reduced influence of positive transcription factors. In line with the latter idea, Po-Yung Cheng documented that the transcriptional suppression of multiple P450 genes within 1 hour of LPS treatment of rats was accompanied by reduced DNA binding activity of hepatocyte nuclear factors 1α, 3β, and 4α, basal transcription factors known to regulate P450 gene expression (Cheng et al., 2003).

Several groups had observed that the pregnane X receptor (PXR) was downregulated in parallel with many P450 enzymes, and therefore it had been proposed that PXR suppression was the cause of P450 downregulation. Terrilyn Richardson demonstrated that P450 downregulation after LPS injection was largely unaffected in PXR-null mice (Richardson and Morgan, 2005), supporting that P450 downregulation in the LPS model occurs independently of PXR. Conversely, graduate student Thom Barclay found that mice deficient in peroxisome proliferator activated receptor-α had attenuated downregulations of Cyps 2a5, 2c29, and 3a11 (Barclay et al., 1999), and (in collaboration with Henry Strobel's laboratory) Cyps 4f15 and 4f16 (Cui et al., 2001), suggesting a role of peroxisome proliferator activated receptor-α in the suppressive mechanism.

Post-Transcriptional Regulation – Nitric Oxide

Inflammation causes the induction of NOS2 in many cells, including the liver. In the mid-1990s, two laboratories reported that NOS inhibitors blocked or attenuated the downregulation of drug-induced CYP2B (Khatsenko et al., 1993) and CYP1A enzymes (Stadler et al., 1994) by inflammatory stimuli. We therefore investigated whether NO might be involved in the downregulation of constitutively expressed P450s. Through studies in rat hepatocytes and in vivo, Marion Sewer demonstrated that downregulation of the major constitutive enzyme CYP2C11 was independent of NO (Sewer and Morgan, 1997; Sewer and Morgan, 1998). We also confirmed that NO inhibitors administered to rats, or genetic knockout of NOS2 in mice did not affect the downregulation of other constitutively expressed P450s (Sewer et al., 1998; Sewer and Morgan, 1998).

In 1995, Dr. Luc Ferrari visited the laboratory on sabbatical from the University of Nancy, and we decided to revisit the question of whether/how CYP2B enzymes are NO-regulated. In doing so, we uncovered an entirely novel mode of P450 regulation. Dr. Ferrari found that phenobarbital-induced CYP2B1 protein was rapidly downregulated by LPS treatment of hepatocytes, and that this was abrogated by inhibition of NOS2 (Ferrari et al., 2001) (Fig. 7). CYP2B1 mRNA was also downregulated, but this was slower and NO-independent.

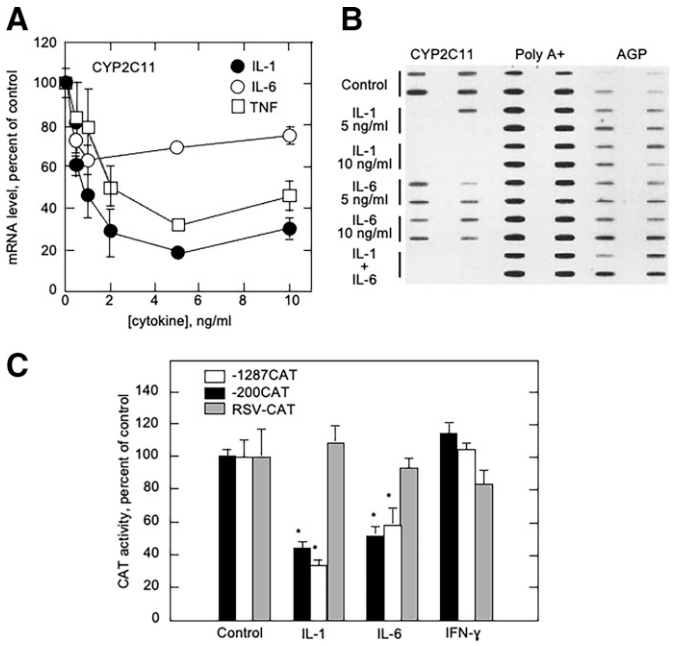

Fig. 5. Cytokine-selective transcriptional downregulation of CYP2C11 in primary rat hepatocyte cultures. A. Concentration dependence of the effects of IL1, lL6, and TNFα on the expression of CYP2C11 mRNA. Hepatocytes were cultured on Matrigel for 5 days and then treated for 24 h as shown. B. Representative RNA slot-blot showing the effects of IL1 and lL-6 on CYP2C11 and α1-acid glycoprotein (AGP) mRNA levels. Hepatocytes were treated for 24 hours with IL-1, IL-6, or both, at the indicated concentrations. C. Effects of IL1 and lL6 on the expression of CYP2C11-chloramphenicol acetyltransferase (CAT) reporter constructs in transfected primary hepatocytes. Hepatocytes were transfected with CYP2C11 -1287CAT, -200CAT or RSV-CAT (bearing the Rous sarcoma virus promoter). All cells were cotransfected with a luciferase vector control. After 24 h of culture, cells were treated with IL1 (5 ng/ml), lL-6 (10 ng/ml), or IFNɤ for 24 h and then harvested for assay of CAT and luciferase activities. CAT activities of each sample were normalized to luciferase activities, and data are expressed as percentages of the activity in untreated cells bearing the same plasmid. The data represent the mean ± S.E. of three to five samples for each group. *, Significantly different from untreated control group mean, $P < 0.05$. Redrawn and edited from Figs. 1, 2, and 8 in Chen et al. (1995).

Allison Aitken then showed that human CYP2B6 was also regulated by NO in cultured human hepatocytes (Aitken et al., 2008), and Dr. Choon Lee demonstrated that the degradation of rat CYP2B1 (Lee et al., 2008) and human CYP2B6 (Lee et al., 2017) occurred via NO-evoked, ubiquitin-dependent proteasomal degradation. Choon's comprehensive mechanistic studies (Lee et al., 2020) found that mutation of Y190, Y317, or Y380 in CYP2B6 each partially attenuated the ubiquitination and downregulation of the enzyme by cellular NO produced by doxycycline-induced NOS2 (Fig. 8), or by NO donor chemicals. Molecular dynamics simulations performed by Dr. Ross Wilderman at the University of Connecticut found that nitrations of these tyrosines were likely to destabilize the protein (Lee et al., 2020). We also found that inhibitors of CYP2B6 (Lee et al., 2020), CYP2J2 (Park et al., 2018) and CYP2A6 (Cerrone Jr et al., 2020) inhibited their downregulation by NO, leading us to conclude that both cumulative tyrosine nitration and heme nitrosylation are necessary triggers for CYP2B6 degradation (Lee et al., 2020).

We have conducted extensive studies on the enzyme specificity of NO-evoked P450 downregulation, and the reader is referred to a recent

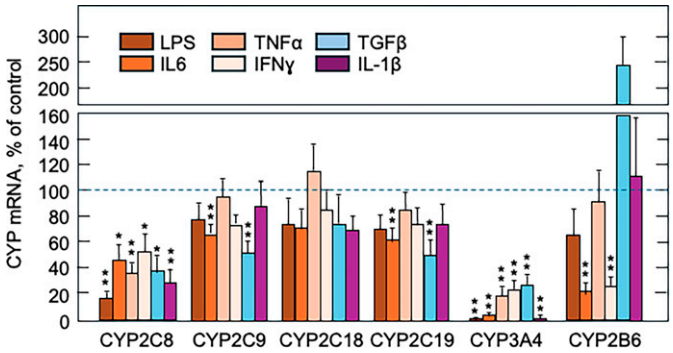

Fig. 6. Effects of inflammatory agents on human P450 mRNAs. Cultured human hepatocytes were treated with phosphate-buffered saline (Control), LPS (10 ng/ml), IL6 (10 ng/ml)), TNFα (10 ng/ml), IFNɤ (10 ng/ml), transforming growth factorβ (TGFβ, 10 ng/ml), or IL1 (5 ng/ml) for 24 h. Relative levels of P450 mRNAs were measured by reverse transcriptase-real time polymerase chain reaction. All treatments were carried out in triplicate, and the results are the means ± S.E.M. of nine human subjects in each group. Significant differences compared with control: *, $P < 0.05$; **, $P < 0.005$. Redrawn from Fig. 1 in Aitken and Morgan (2007).

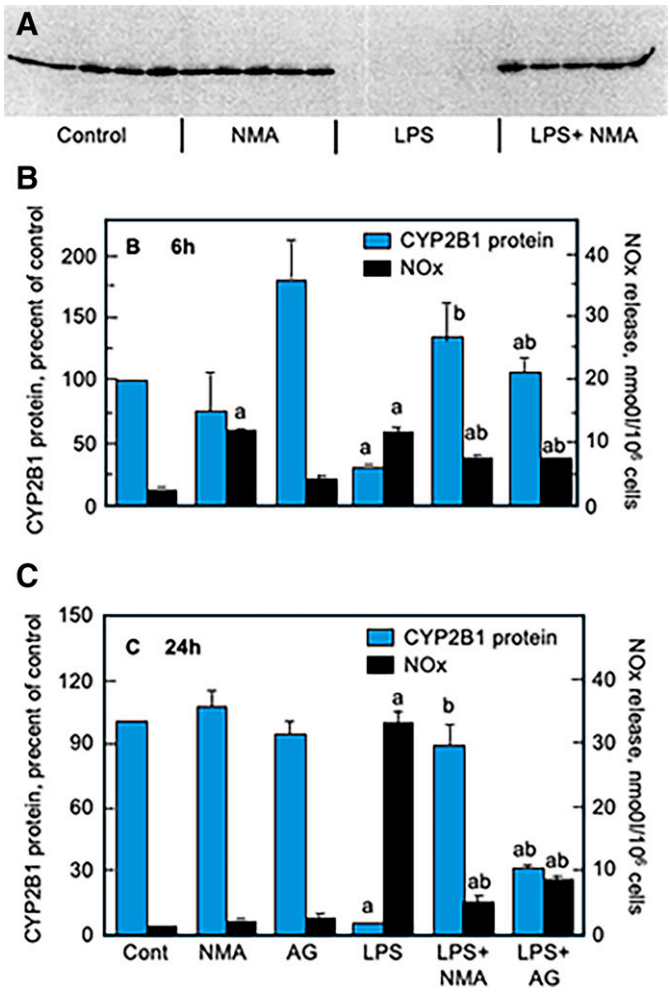

Fig. 7. NOS inhibitors prevent CYP2B1 protein suppression by LPS treatment. After 48 h treatment with 1 mM phenobarbital, cultured rat hepatocytes were treated with fresh medium containing phenobarbital ± 10 μg/ml LPS in the presence or absence of 300 μM aminoguanidine (AG) or N^{ω}-monomethylarginine (NMA). Cells were harvested 6 or 24 hours later, and microsomal CYP2B1 protein levels were assayed by Western blotting. Nitrate+nitrite (NOx) in the media was measured via the Griess reaction. A, Western blot showing inhibition of CYP2B1 protein suppression by NMA, 24 hours after LPS addition. Each band represents the pooled microsomes from two cell culture plates. B and C, quantitative analyses of the effects of NMA and AG on LPS suppression of CYP2B1 protein at 6 and 24 hours, respectively. Data represent the means ± S.E. of five independent samples for each group. The data in B and C are expressed as a percentage of the mean of the control group treated with medium (no LPS). A, significantly different from control; B, significantly different from LPS-treated group, $P < 0.05$. Redrawn from Fig. 6 in Ferrari et al. (2001).

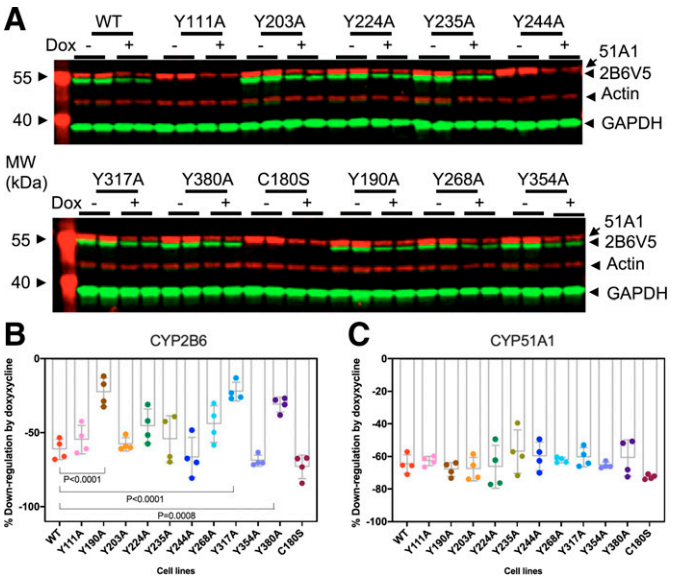

Fig. 8. Downregulation of CYP2B6V5 and its tyrosine mutants in HeLa cells expressing human NOS2 under control of doxycycline. HeLa-hNOS2 cells were transduced with pLX304-2B6V5 or mutant lentiviruses, and CYP2B6V5-expressing cells were selected with puromycin and blasticidin to generate the HeLahNOS2-2B6V5 cell lines. After treatment with doxycycline (10 mg/ml) to induce NOS2 for 18 h total cell lysates were used for immunoblotting with antibodies to the V5 tag, glyceraldehyde 3-phosphate dehydrogenase, CYP51A1, and actin. (A) Representative image showing NO-stimulated CYP2B6V5 downregulation. Images of Y111A, Y244A, and C189S were viewed at a higher gain and cut and pasted on the original image for visualization. (B and C) Quantitation of data for CYP2B6 and CYP51A1, respectively. The percent downregulation by doxycycline was calculated for each cell line, in which no downregulation = -100 and complete downregulation = 0. Values are the means and 95% confidence intervals of four independent experiments. Differences in response among the groups were tested by ordinary one-way ANOVA. Differences between the responses of mutant and wild-type enzymes were determined by Dunnett's test, with significant differences shown. CYP51A1 was used as a positive control and was not analyzed for significant differences. MW, molecular weight. Reproduced from Lee et al. (2020).

review for more details (Morgan et al., 2020). We used proteomic analyses to identify rat CYP3A1 (Lee et al., 2009) and CYP2C22 (Lee et al., 2014) as NO-regulated enzymes. We used lentivirally transduced Huh7 and HeLa cells to show that human CYP2A6 (Park et al., 2018) as well as the physiologically relevant P450s CYP51A1 (Park et al., 2017) and CYP2J2 (Park et al., 2018) are also NO-susceptible, whereas CYP3A4 and CYP3A5 are not (Lee et al., 2020). The mechanisms of degradation were found to be different depending on the enzyme. For example, CYP51A1 degradation is partially proteasome-dependent and may also involve calpains, whereas CYP2J2 undergoes ubiquitin-independent proteasomal degradation (Morgan et al., 2020).

NO-dependent P450 protein degradation has proven difficult to demonstrate in vivo. This may be because NO-independent mRNA downregulation and NO-dependent protein degradation are occurring simultaneously. The downregulation of mRNA eventually results in NO-independent reductions in the protein due to reduced synthesis, as demonstrated in Luc Ferrari's work (Ferrari et al., 2001). This could possibly explain some of the discrepant observations among in vivo studies in different laboratories. There may also be species differences: graduate student Tong Li-Masters found no evidence for NO-dependent regulation of Cyp2b proteins in Nos2 knockout mice (Li-Masters and Morgan, 2002).

Conclusion: Shining the Light

In parallel with ongoing basic, preclinical research in our laboratory and others, clinical evidence gradually emerged showing that moderate to severe inflammation can downregulate human cytochrome P450 enzymes with resultant reductions in the clearance of their drug substrates. Coutant and Hall recently published an excellent review documenting how inflammation associated with influenza infection, surgery, human immunodeficiency virus infection, rheumatoid arthritis, Crohn's disease, cancer, hepatitis C, and congestive heart failure causes significantly increased drug exposure in humans (Coutant and Hall, 2018). Villemure et al. reviewed studies that assessed the status of CYP3A4 in Sars-CoV-2 infected patients (Villemure et al., 2023). Most results predicted 20–50% increases in drug exposure in patients with mild to moderate disease; however, the effect may increase with disease severity. Also, a genome-wide association study identified acute-phase response genes to be negatively correlated with CYP1A2, 2C8, 2C9, and 2C19 activities, as well as with the gene expression of 45 different P450 genes in a correlation network of 466 Caucasian human liver samples (Yang et al., 2010).

The discovery that finally shone the light on the importance of this phenomenon for the broader community, was the discovery of a disease-dependent drug–drug interaction between simvastatin and the anti-IL6 receptor antibody drug tocilizumab, developed for treatment of rheumatoid arthritis. Prior to this finding, it was generally thought that there was little risk of metabolism-based interaction between small molecule drugs and biologics because they are metabolized by entirely different mechanisms and biologics do not bind to the nuclear receptors (e.g., PXR) that mediate induction of drug metabolizing enzymes.

This landmark study (Schmitt et al., 2011) found that administration of simvastatin to rheumatoid arthritis patients was associated with higher plasma concentrations of simvastatin than had been reported in healthy subjects, consistent with inflammatory downregulation of CYP3A4, the enzyme responsible for simvastatin metabolism. One week after tocilizumab treatment, the patients' exposure to simvastatin was reduced by 57%. This effect was still observed 5 weeks after tocilizumab injection, the magnitude of the effect correlating with the inflammatory marker C-reactive protein. The authors concluded that tocilizumab reversed the IL6-dependent downregulation of CYP3A4 these patients. Note that this drug–drug interaction is disease-dependent because it can only happen if the P450 is downregulated. These significant clinical findings were recognized by the Food and Drug Administration (Lee et al., 2010), leading to the publication of a Guidance for Industry for preclinical and clinical assessment of this and other therapeutic protein interactions (FDA, 2023).

The disease-dependent drug–drug interaction phenomenon was actually predicted by prior studies in animal and cell models: In a mouse model of rheumatoid arthritis, Ashino found that treatment with a monoclonal antibody to IL6 reversed the downregulation of Cyp3a11 mRNA and protein (Ashino et al., 2007); the TNFα antibody drug infliximab reversed the downregulations of CYP1A1/2 and CYP3A1/2 proteins in a preadjuvant arthritis model in rats (Ling and Jamali, 2009); and we demonstrated that the natural IL1 receptor antagonist protein reversed downregulation of CYP2C11 in cultured rat hepatocytes (Chen et al., 1995). It is worth noting that neither the two animal studies nor the clinical one with tocilizumab prove that the cytokine targeted by the antibody was responsible for the downregulation. It is possible that the effects of the antibodies were secondary to the relief of inflammation per se, and if so, one might predict that nonsteroidal anti-inflammatory treatment could have a similar effect.

Finally, because inflammation mostly causes a downregulation of P450 expression and activity, this can cause a phenoconversion that can result in a lack of phenotype–genotype concordance in pharmacogenetic studies (Shah and Smith, 2015). Thus, inflammatory status may need to be considered alongside genotype in clinical outcomes research and personalized medicine. In sum, inflammation is now established as an important factor influencing P450 expression and function (phenotype),

which must be considered in the context of other influences, such as hormones, age, diet, and genetic background when assessing likely outcomes for drug exposure, toxicity and therapeutic effects.

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Data Availability

This review article contains no datasets generated or analyzed during the present study.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Morgan.

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