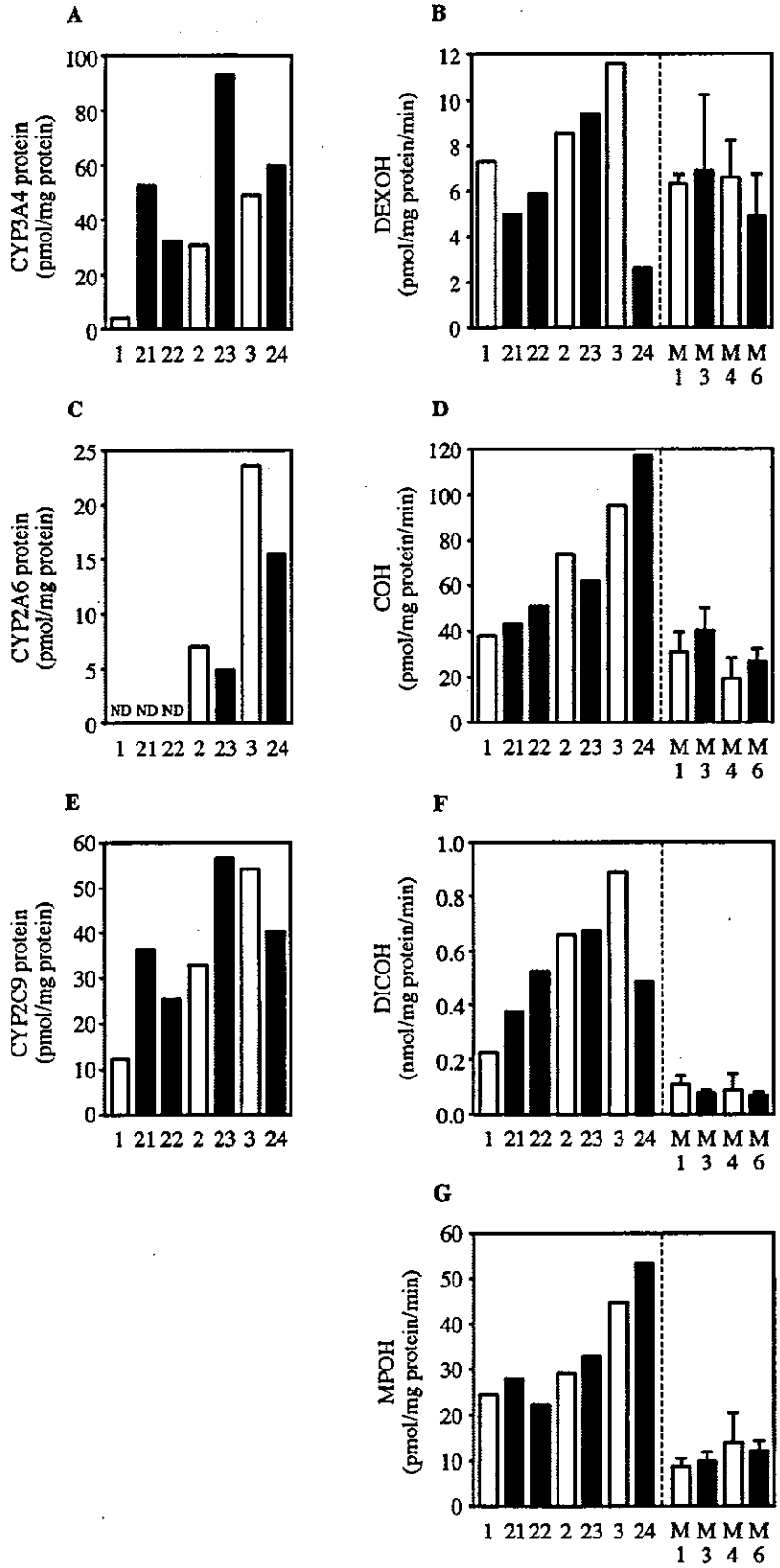

FIG. 8. Changes of human CYP3A4, CYP2A6, CYP2C9, and CYP2C19 expressions in 3-MC-treated chimeric mice. Protein contents of human CYP3A4 (A), CYP2A6 (C), and CYP2C9 (E) in the chimeric mice were measured by Western blot analysis. DEXOH catalyzed by CYP3A4 (B), COH catalyzed by CYP2A6 (D), DICOH catalyzed by CYP2C9 (F), and MPOH catalyzed by CYP2C19 (G) were determined by HPLC. Open and closed columns are the values of the non- and 3-MC-treated chimeric mice, respectively. B, D, F, and G, columns of M1, M3, M4, and M6 represent the mean ± S.D. (n = 3). ND, not detected; M1, nontreated uPA$^{+/-}$/SCID mouse; M3, 3-MC-treated uPA$^{+/-}$/SCID mouse; M4, nontreated uPA$^{-/-}$/SCID mouse; M6, 3-MC-treated uPA$^{-/-}$/SCID mouse.

consistent with previous reports. As we described, it is not always the case that the induction between the mRNA, protein, and enzyme activity can be correlated. This point concerning both constitutive and inducible expression is still controversial. This phenomenon is observed not only in chimeric mice, but also in human hepatocytes.

Rifampicin induced CYP3A4 protein but not CYP3A5 protein in human hepatocytes (Schuetz et al., 1993). Since both donors in this study were genotyped as homozygous for the *CYP3A5*3* allele (Katoh et al., 2004), further studies using chimeric mice generated from various donors are needed to clarify the induction of CYP3A5.

Rifampicin moderately induced murine Cyp3a11 mRNA and Cyp3a protein at a dose of 50 to 100 mg/kg/day for 2 to 4 days (Yanagimoto et al., 1997; Schuetz et al., 2000). TESOH is catalyzed by Cyp3a in mice. TESOH in uPA$^{+/-}$/SCID mice was not changed by rifampicin treatment, whereas it was increased 2.4-fold in uPA$^{-/-}$/SCID compared with the control ($P < 0.01$; data not shown), suggesting that rifampicin weakly induced Cyp3a. In both uPA$^{+/-}$/SCID and uPA$^{-/-}$/SCID mice, rifampicin had no effect on DEXOH, indicating that DEXOH was specific to human CYP3A but not to murine Cyp3a (Tomlinson et al., 1997).

Rifampicin also induces the mRNAs and proteins of CYP2C8, CYP2C9, and CYP2C19 in human hepatocytes (Gerbal-Chaloin et al., 2001; Raucy et al., 2002). On the other hand, it has been reported that there were no changes in CYP2C8 protein (Edwards et al., 2003), CYP2C9 protein (Runge et al., 2000; Edwards et al., 2003), and CYP2C19 protein (Runge et al., 2000) following treatment with rifampicin in human hepatocytes or human liver slices. In addition, Raucy et al. (2002) demonstrated that the induction of CYP2C8 protein exhibited large interindividual variability, and HH954 hepatocytes in their report failed to respond to rifampicin treatment. They also reported that the induction of CYP2C19 protein exhibited large interindividual differences (5.7 ± 5.3-fold) (Raucy et al., 2002). In clinical practice, rifampicin may induce CYP2C-mediated metabolism and thus reduce the plasma concentration of CYP2C9 substrates such as warfarin and sulfonylurea antidiabetic drugs (Niemi et al., 2003). In the present study, rifampicin tended to cause a slight increase in CYP2C8 protein, CYP2C9 protein, DICOH catalyzed by CYP2C9, and MPOH catalyzed by CYP2C19 and showed interindividual differences. In the case of CYP2C19, it was difficult to estimate the induction, because the MPOH in donor B chimeric mice was lower. Donor B chimeric mice were genotyped as *CYP2C19*1/CYP2C19*2* (Katoh et al., 2004), which would lead to a reduction of the enzyme activity (Bramness et al., 2003). In this study, the calculated induction ratio is an apparent value, since the human albumin concentrations are similar but not the same between non- and inducer-treated chimeric mice. Further investigations will be needed to clarify the induction potency by rifampicin of CYP2Cs in chimeric mice.

In relation to human CYP2A6, some in vitro reports using human liver slices and human hepatocytes exhibited the induction of CYP2A6 protein and COH by rifampicin treatment, respectively (Edwards et al., 2003; Madan et al., 2003); however, another study that used human hepatocytes showed no change in CYP2A6 mRNA and COH (Donato et al., 2000). Ethoxyresorufin *O*-dealkylase activity catalyzed by CYP1A2 was increased by treatment with rifampicin in human hepatocytes (Madan et al., 2003). In CYP1A2 and CYP2A6, rifampicin increased the mRNA, protein content, and enzyme activity 3-fold at most compared with the control (Edwards et al., 2003; Madan et al., 2003). Therefore, these results did not contradict those of the present study.

Following the exposure of 3-MC, human CYP1A2 protein and ethoxyresorufin *O*-dealkylase activities were increased in human hepatocytes, leading to the induction of CYP1A2 in humans (Donato et al., 1995; Runge et al., 2000). In the present study, human CYP1A2 mRNA and protein were induced by treatment with 3-MC in the chimeric mice, which was consistent with previous reports (Donato et al., 1995; Runge et al., 2000). 3-MC significantly increased the expression levels of human CYP1A1 mRNA in the present study. The CYP1A1 antibodies used in this study were very sensitive and could detect 25 fmol of the recombinant human CYP1A1, but the pooled human liver microsomes from BD Gentest did not show the band (data not shown). CYP1A1 is known as an isoform with low expression in normal human liver (Turesky et al., 1998). CYP1A1 protein in both non- and 3-MC-treated chimeric mice could not be detected. CYP1A1 protein in the liver of donor A may have been very low; therefore, human CYP1A1 proteins in donor A chimeric mice could not be detected, although human CYP1A1 mRNA could be detected.

There seemed to be some variability in the responses to these inducers in terms of the mRNA, protein, and enzyme activity. The difference is thought to be due to the interindividual variability of the chimeric mice, but the reasons are still unclear. We think that such variability could be overcome by increasing the number of chimeric mice, because it was also observed in studies using human hepatocytes.

As described above, it was demonstrated that P450 enzymes were induced in the chimeric mice with humanized liver. The expression of each murine P450 mRNA in chimeric mouse 3, which exhibited the highest hAlb concentration in this study, was no more than 5% compared with that in uPA$^{+/-}$/SCID mice (data not shown). It was surmised that human P450s in the chimeric mice were induced by rifampicin or 3-MC treatment, but further study is needed to clarify the expression of human nuclear receptors and the transcriptional regulation mechanism in the chimeric mice.

In conclusion, human P450s expressed in chimeric mice with humanized liver respond to induction via treatment with rifampicin and 3-MC. At present, human hepatocytes are still a better model for investigating the induction of P450s, but the number of human hepatocytes that can be obtained from one donor may not be sufficient for the experimental purposes, and frequently none can be obtained at all. Using these chimeric mice, human hepatocytes could be made to proliferate easily at low cost. In some countries, including Japan, large amounts of human organ materials such as hepatocytes and microsomes are very difficult to obtain. In such cases, the lack of a stable supply of human liver is a serious problem. One of the advantages of the chimeric mice is that they could be used to proliferate human hepatocytes. In addition, this chimeric mouse line would be a better tool than any other experimental animal for estimating the in vivo induction potency in humans. It would be of interest to measure the pharmacokinetics of drugs in chimeric mice treated with a typical P450 inducer. This chimeric mouse line could be more useful than human hepatocytes for estimating the pharmacokinetics and drug metabolism in humans. We hope that this study will greatly contribute to future advances in studies of drug metabolism as well as drug development.

Acknowledgments. We acknowledge Brent Bell for reviewing the manuscript.

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