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Peroxisome Proliferator-Activated Receptor α-Dependent Induction of Cell Surface Antigen Ly-6D Gene in the Mouse Liver

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Spontaneous peroxisome proliferation-related pleiotropic responses occurring in the liver of mice lacking peroxisomal fatty acyl-CoA oxidase ($AOX^{-/-}$) are attributed to sustained activation of peroxisome proliferator-activated receptor α (PPARα) by its putative natural ligands that require AOX for their metabolism. In this study, using a gene expression screen, we show that Ly-6 (lymphocyte antigen 6 complex, locus D; mouse ThB), which belongs to a distinctive family of low molecular weight phosphatidyl inositol anchored cell surface glycoproteins, is upregulated in mouse liver with peroxisome proliferation. Increases in Ly-6D mRNA levels are observed in $AOX^{-/-}$ mouse liver with spontaneous peroxisome proliferation and also in the liver of wild-type mice treated with synthetic peroxisome proliferators. Peroxisome proliferators failed to increase hepatic Ly-6D mRNA levels in mice lacking PPARα ($PPAR\alpha^{-/-}$), suggesting a regulatory role for PPARα in the induction of Ly-6D. These observations suggest that changes in certain cell surface proteins also form part of the pleiotropic responses associated with peroxisome proliferation.

PPARα Ly-6D Fatty acyl-CoA oxidase Peroxisome proliferators

PEROXISOMES, cytoplasmic organelles of about 0.5 μm in diameter, surrounded by a membrane, are widely distributed in most animal and plant cells (7). Although peroxisomes possess more than 60 proteins, it is essential that they contain at least one H_2O_2-generating flavinoxidase together with the H_2O_2-degrading peroxisomal marker enzyme catalase to be designated as peroxisomes (7,31,38). These organelles participate in several metabolic functions, including simple respiration characterized by H_2O_2 production and H_2O_2 degradation, β-oxidation of long-chain and very long-chain fatty acids, metabolism of glyoxalate, degradation of uric acid, and synthesis of ether lipids and cholesterol (7,31,38). In humans, defects in peroxisome biogenesis lead to several peroxisomal disorders, and most of them are lethal during childhood (39). In liver cells of most animals, peroxisomes are few in number but they can be induced to proliferate in response to structurally diverse nonmutagenic chemicals designated as peroxisome proliferators (32,33). These agents form a broad group of compounds of industrial, pharmaceutical, and agricultural importance and include certain phthalate ester plasticizers, leukotriene D4 antagonists, and hypolipidemic drugs, such as clofibrate, ciprofibrate, gemfibrozil, and Wy-14,643, among others (12,32,33). Despite their structural diversity, peroxisome proliferators induce qualitatively predictable immediate and delayed pleiotropic responses including the development of liver cancer in rats and mice (12,26,29,34). The induction of peroxisome proliferation is mediated by peroxisome proliferator-activated receptor α (PPARα), a member of the family of ligand-dependent nuclear transcription factors that regulate the expression of genes associated with lipid metabolism and adipocyte differentiation (8,19). The PPAR

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subfamily of nuclear receptors has three isotypes (α, β or δ, and γ), which exhibit distinct patterns of tissue distribution and differ considerably in their ligand binding domains and ligand specificities, suggesting that they perform different functions in different cell types (8,45). The induction of peroxisome proliferation is associated with PPARα-dependent transcriptional activation of several genes, in particular those encoding for the classical peroxisomal β-oxidation system, and cytochrome P450 CYP 4A isoforms, CYP4A1 and CYP4A3 (16,25,35). For this to occur, PPARα heterodimerizes with retinoid X receptor (RXR) (receptor for 9-*cis*-retinoic acid), and this PPAR–RXR complex binds to PPARα response element (PPRE), a region consisting of a degenerate direct repeat of the canonical AGGTCA sequence separated by one base pair (DR1), present in the 5′-flanking region of target genes (21,27,37,44).

The classical peroxisomal β-oxidation system, induced by synthetic peroxisome proliferators, utilizes straight chain saturated fatty acyl-CoAs as substrates, and it consists of fatty acyl-CoA oxidase (AOX), the first and rate-limiting enzyme, which dehydrogenates acyl-CoA esters to their corresponding *trans*-2-enoyl-CoAs (17). The second and third reactions, hydration and dehydrogenation of enoyl-CoA esters to 3-ketoacyl-CoAs, are carried out by a single enzyme, enoyl-CoA hydratase/L-3-hyadroxyacyl-CoA dehydrogenase [L-bifunctional enzyme (L-PBE)] (17,31). The third enzyme of this classical system, 3-ketoacyl-CoA thiolase (PTL), cleaves 3-ketoacyl-CoAs to acetyl-CoA, and an acyl-CoA that is two carbon atoms shorter than the original molecule, which can reenter the β-oxidation spiral (17,31). Mice lacking AOX ($AOX^{-/-}$) exhibit sustained activation of PPARα, resulting in profound spontaneous peroxisome proliferation in liver cells and induction of genes that are regulated by PPARα (9,10). These observations implied that AOX is responsible for the metabolic degradation of PPARα ligands and that substrates of AOX serve as endogenous or biological ligands for PPARα, leading to an enzyme–receptor crosstalk because AOX gene is transcriptionally regulated by this receptor (10,43). The magnitude of peroxisome proliferation occurring spontaneously in the liver of $AOX^{-/-}$ mice is comparable with that induced in the liver of wild-type mice by exogenous peroxisome proliferators (10).

In an effort to identify novel genes expressed in livers with sustained activation of PPARα we used a "gene expression screen," which is a PCR-based cDNA subtraction method that identifies differentially expressed genes by comparing two populations of closely related mRNA (40,41). Comparison of liver mRNA populations between wild-type and $AOX^{-/-}$ mouse identified Ly-6D [lymphocyte antigen 6 complex, locus D; also called mouse thymocyte B cell antigen (ThB)] (3,5,13–15,22,36), as a novel upregulated gene in liver with peroxisome proliferation. Murine Ly-6 family of molecules constitutes a distinct set of low molecular weight (12–20 kDa) phosphatidyl inositol-anchored cell surface glycoproteins, which are encoded by a multiple gene complex on mouse chromosome 15 and are known to be expressed predominantly in leukocytes (14,15,22,23). We now report that Ly-6D mRNA level in liver is markedly increased in $AOX^{-/-}$ mouse and in the wild-type mouse treated with synthetic peroxisome proliferators and the induction is dependent on PPARα. This study also identified major mouse urinary protein II (MUP II) (36), as a downregulated gene in livers with peroxisome proliferation.

MATERIALS AND METHODS

PCR-Based cDNA Subtraction

Poly(A)$^+$ RNA was selected from the total RNA using an oligo(dT) column (Pharmacia, type 7) for cDNA. Double-stranded cDNA was synthesized using the Copy Kit (Invitrogen). cDNA subtraction hybridization was performed according to the procedures described previously (40,41). Briefly, the amplified PCR products were the starting materials for subtractive hybridization. PCR-amplified driver cDNA was photobiotinylated using a 175-W sunlamp from a 7.5-cm distance for 15 min. A 1:20 nonbiotinylated tracer to biotinylated driver ratio was established in our subtraction. After subtractive hybridization, the removal of driver was carried out by adding 200 μg streptavidin followed by three extractions with phenol/chloroform and one with chloroform extraction. After three cycles of PCR-based subtraction, the highly enriched cDNA fragments were cloned into pBluescript (Stratagene) and transformed into competent *E. coli* DH5 cells for screening.

Sequencing Analysis

Distinct cDNA fragments identified from the PCR-based subtraction library were sequenced using an ABI automated sequencing machine with fluorescent dye terminators and the sequence homology was searched in the GenBank database (1,3).

Animals

Wild-type (C57BL/6J), AOX-null ($AOX^{-/-}$) (9), and PPARα-null ($PPAR\alpha^{-/-}$) (24) male mice were housed in a controlled environment with a 12-h light/dark cycle with free access to water and standard laboratory chow. Hypolipidemic peroxisome proliferators, Wy-14,643 (0.125%, w/w), nafenopin (0.125%,

w/w), or ciprofibrate (0.025%, w/w) were administered to wild-type and PPARα$^{-/-}$ mice in powdered diet ad libitum for 2 weeks. For dose–response studies, wild-type and PPARα$^{-/-}$ mice were given a dose (2, 10, 50, or 250 mg/kg body weight) of ciprofibrate or Wy-14,643 by gavage and killed 24 h later. For time course of induction, a single dose of Wy-14,643 (250 mg/kg body weight) was administered by gavage to wild-type and PPARα$^{-/-}$ mice and they were killed 0, 8, 16, or 24 h after dosing. Control animals received 0.15 ml of dimethyl sulfoxide by gavage, which was used as solvent for ciprofibrate and Wy-14,643. All animal procedures used in this study were reviewed and approved by the Institutional Review Board for Animal Research of Northwestern University.

RNA Isolation and Analysis

Total RNA was isolated using the guanidinium/CsCl gradient method (6). RNA was glyoxylated, electrophoresed, transferred to a nylon membrane, and then hybridized at 42°C in 50% formamide hybridization solution using ^{32}P-labeled cDNA probes (35). Changes in mRNA levels were estimated by densitometric scanning of autoradiograms.

In Situ Hybridization

For in situ hybridization, small pieces of tissue were fixed in 4% paraformaldehyde for 16–20 h at 4°C and processed as described previously. Sections, 5 μm thick, were cut under ribonuclease-free conditions. The sense and antisense riboprobes were derived from Ly-6D cDNAs (nucleotides 25–535) (13). Each pBluescript recombinant plasmid containing either Ly-6D cDNA fragment was linearized to generate [^{35}S]UTP-labeled sense and antisense riboprobes. In situ hybridization, washing, coating with NTB2 photographic emulsion (Eastman Kodak, Rochester, NY), and developing conditions were followed as described (2,42). The samples were exposed typically for 10 days and Hoechst 33258 (Boehringer Mannheim) was used as the counterstain for nuclei. Sections were stained with hematoxylin and eosin for routine histological evaluation. All images were visualized on the Leica DMRE microscope and acquired digitally with the Spot2 camera (Diagnostic Instruments). Images were prepared using the Photoshop 5.0 program (Adobe, Mountain View, CA).

RESULTS AND DISCUSSION

Identification of Ly-6D as an Upregulated Gene in AOX$^{-/-}$ Mouse Liver With Spontaneous Peroxisome Proliferation

We have previously shown that AOX$^{-/-}$ mice exhibit spontaneous induction of PPARα-mediated pleiotropic responses, including profound hepatic peroxisome proliferation and development of liver tumors, reminiscent of those encountered in rats and mice maintained on synthetic peroxisome proliferators, such as ciprofibrate and Wy-14,643 (9,10). In the liver of AOX$^{-/-}$ mice, there is clear evidence of enhanced transcriptional activity of PPARα on PPARα-regulated genes (10). Spontaneous peroxisome proliferation in hepatocytes and increases in mRNA levels in the livers of these AOX$^{-/-}$ mice of L-PBE, CYP4A1, and CYP4A3 among others were comparable with those observed in the livers of wild-type mice exposed to synthetic peroxisome proliferators (9,10). These observations established the concept that the endogenous/biological ligands of PPARα are substrates for peroxisomal AOX and a normal AOX gene is indispensable for the physiological regulation of PPARα (10,43). Very long-chain fatty acids and other biological molecules such as eicosanoids function as PPARα ligands under in vitro transactivation conditions, but they fail to induce robust peroxisome proliferation under in vivo conditions, implying that fatty acids are not effective inducers of PPARα because a functional AOX is highly effective in metabolizing these PPARα ligands (8,10,11).

In an effort to identify genes whose expression is altered during sustained spontaneous activation of PPARα by natural ligands in AOX$^{-/-}$ mouse liver, we utilized "gene expression screen," a PCR-based cDNA subtractive hybridization method (40). The occurrence of spontaneous peroxisome proliferation in AOX$^{-/-}$ mouse livers has been very well documented in our earlier studies (10,18,43). We have constructed two subtracted cDNA libraries enriched for cDNA sequences that are up- or downregulated in the liver of a 3-month-old male AOX$^{-/-}$ mouse. These enriched cDNAs did not hybridize to each other, indicating that the subtractive enrichment was effective. The enriched PCR-based cDNA libraries were converted into plasmid libraries by inserting cDNA fragments into pBluescript (Stratagene). This analysis generated 200–600 bases of sequence information for each of the fragments, which enables the identification of up- and downregulated genes. Analysis thus far has enabled us to identify upregulated genes that were not previously known to be upregulated in livers with peroxisome proliferation, in addition to the identification of several genes previously known to be upregulated in these livers such as L-PBE, PTL, CYP4A1, and CYP4A3 genes that possess PPRE-containing promoters (21,25,30,37). One gene that was not previously known to be upregulated in AOX$^{-/-}$ mouse liver or in the liver of wild-type mice treated with synthetic peroxisome proliferators encodes lymphocyte antigen Ly-6D (3,22). The upregulated Ly-6D

cDNA fragment we isolated is 511 bases, which corresponds to nucleotides 25–535 of the mouse ThB cDNA sequence reported by Gumley and coworkers [(13,15); GenBank accession number Y63782]. Ly-6 family of molecules are characterized as small 12- to 20-kDa glycosyl-phosphatidylinositol-anchored membrane proteins expressed on lymphocytes (22,23). The Ly-6D cDNA we identified in this gene expression screen is the mouse homologue of human E48 antigen (4,5) expressed in squamous epithelium and squamous cell carcinomas and appears to be involved in keratinocyte adhesion (5,20,28). We have also identified Ly-6D as an upregulated gene in cDNA microarrray screening of mRNA samples obtained from $AOX^{-/-}$ mouse liver and from the liver of wild-type mouse treated with Wy-14,643 (unpublished). Also of note is that both gene expression screen (this study) and cDNA microarray procedures (unpublished) identified mouse major urinary protein II (MUP II) (36), as a downregulated gene in the liver of $AOX^{-/-}$ mouse. Downregulation of rat α_{2u}-globulin, a homologue of MUP, was observed previously in rat liver with peroxisome proliferation induced by synthetic peroxisome proliferators (2).

Confirmation of Inducibility of Ly-6D mRNA in AOX Mouse Liver

To confirm the results obtained by gene expression screening, we isolated total RNA from the livers of wild-type and $AOX^{-/-}$ mice and performed Northern blot analysis (Fig. 1). For comparison, RNA isolated from the livers of wild-type mice that were fed for 2 weeks a diet containing ciprofibrate, a peroxisome proliferator, was also used (Fig. 1). It is evident that Ly-6D mRNA levels increased >25-fold in the livers of wild-type mice treated with ciprofibrate for 2 weeks (Fig. 1, lanes 1–3), and in $AOX^{-/-}$ mice maintained on normal chow (Fig. 1, lanes 7–9). In control wild-type liver, Ly-6D mRNA was barely detectable. As expected, the mRNA levels of L-PBE and CYP4A10 genes, identified in our gene screen as upregulated, and that are well known to be upregulated in the livers with peroxisome proliferation (16,35), increased markedly in the livers of $AOX^{-/-}$ mice as well as in ciprofibrate-treated wild-type mice (Fig. 1). Furthermore, the hepatic mRNA level of MUP, identified as a downregulated gene in our gene expression screen, decreased somewhat in both $AOX^{-/-}$ on control diet and wild-type mouse exposed to ciprofibrate (Fig. 1). The reduction of MUP mRNA level is qualitatively more pronounced in the liver of $AOX^{-/-}$ than that seen in wild-type mouse exposed to ciprofibrate for 2 weeks.

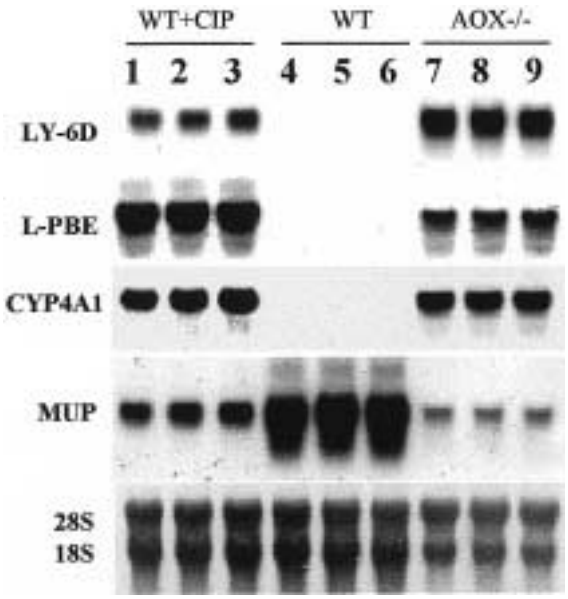

Figure 1. Confirmation of the gene screen results by Northern blot analysis. Representative Northern blots of Ly-6D, L-PBE, CYP4A, and MUP are included with total RNA (20 μg/lane) extracted from the liver of $AOX^{-/-}$ mice on control diet ($AOX^{-/-}$; lanes 7–9), and wild-type mice fed either control diet (WT; lanes 4–7) or ciprofibrate-containing diet (WT + CIP; lanes 1–3) for 2 weeks. The bottom blot, stained with methylene blue to assess sample loading, indicates the positions of 28S and 18S RNA.

Inducibility of Ly-6D mRNA in Liver by Peroxisome Proliferators

$AOX^{-/-}$ mice exhibit spontaneous peroxisome proliferation in liver due to activation of PPARα by natural ligands (10). As shown in Figure 1, Ly-6D mRNA levels increased in both $AOX^{-/-}$ mouse liver and in livers of wild-type mice treated with ciprofibrate, a synthetic peroxisome proliferator. To assess the inducibility of Ly-6D mRNA by various other peroxisome proliferators, we performed Northern blot analysis on total RNA extracted from livers of wild-type mice that were fed a powdered diet containing Wy-14,643 (0.125%, w/w), nafenopin (0.125%, w/w), or ciprofibrate (0.025%, w/w) ad libitum for 2 weeks (Fig. 2). Ly-6D mRNA level is markedly induced by all three structurally diverse hypolipidemic peroxisome proliferators (Fig. 2). All three hypolipidemic peroxisome proliferators markedly induced Ly-6D mRNA in liver compared with controls and the induction appeared as pronounced as that seen in AOX-null liver with spontaneous peroxisome proliferation. These observations suggest that expression of Ly-6D is under the control of PPARα. It is important to note that feeding of diets containing these peroxisome proliferators for 2 weeks results in significant peroxisome proliferation in mouse liver (32,33).

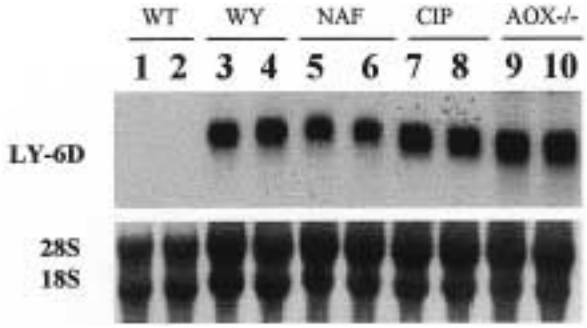

Figure 2. Three structurally diverse peroxisome proliferators induce Ly-6D gene expression in liver in wild-type mouse liver. Northern blot analysis of total RNA (20 μg/lane) from the liver of wild-type mice fed either normal chow (WT; lanes 1 and 2) or a diet containing a peroxisome proliferator Wy-14,643 (WY; lanes 3 and 4); nafenopin (NAF; lanes 5 and 6); or ciprofibrate (CIP; lanes 7 and 8) for 2 weeks. Lanes 9 and 10 represent liver RNA from $AOX^{-/-}$ mice on normal chow. The bottom blot, stained with methylene blue to assess sample loading, indicates the positions of 28S and 18S RNA.

Thus, increases in Ly-6D mRNA occurred in wild-type mice with peroxisome proliferation occurring as a result of exposure to synthetic peroxisome proliferators (32,33), and in AOX-null mice exhibiting spontaneous peroxisome proliferation. Because these morphological changes have been well documented before, we chose not to include information of peroxisome proliferation in this report.

Dose Responsiveness of Induction of Ly-6D mRNA

To determine the dose responsiveness of Ly-6D induction, we performed Northern blot analysis on total RNA extracted from livers of wild-type male mice 24 h after they were administered a single intragastric dose of ciprofibrate (Fig. 3). At 24 h, the mRNA level of L-PBE, a well-recognized PPARα-target gene (16,35), showed some increase in wild-type mice given ciprofibrate dose at 2 mg/kg body weight, and the increase became more prominent with higher doses (Fig. 3). Although no perceptible increase in hepatic Ly-6D mRNA level was seen in mice given ciprofibrate at 2 mg/kg body weight, increases in Ly-6D mRNA levels were readily evident in mice given this compound at 10, 50, or 250 mg/kg body weight (Fig. 3). These results suggest that does response of Ly-6D induction is somewhat different from that of L-PBE induction or that basal levels of Ly-6D are considerably lower for the detection sensitivity. Figure 3 also illustrates the MUP mRNA levels in wild-type mouse liver following a single dose of ciprofibrate. No appreciable decreases in mRNA levels of this downregulated gene were noted 24 h following a single dose.

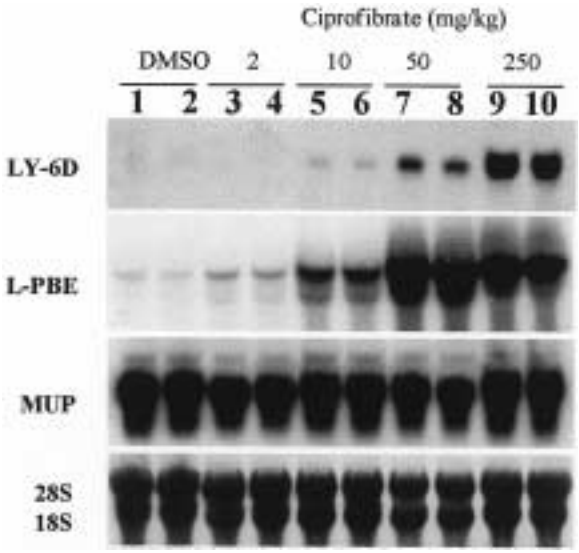

Figure 3. Dose responsiveness of induction of Ly-6D mRNA by ciprofibrate in wild-type mouse liver. Ciprofibrate was administered at a single dose of 2 (lanes 3 and 4), 10 (lanes 5 and 6), 50 (lanes 7 and 8), or 250 (lanes 9 and 10) mg/kg body weight by gavage and RNA (20 μg/lane) extracted from liver 24 h after dosing was analyzed for Ly-6D, L-PBE, and MUP mRNA content. Mice given the solvent dimethyl sulfoxide (DMSO; lanes 1and 2) served as controls. The bottom blot, stained with methylene blue to assess sample loading, indicates the positions of 28S and 18S RNA.

Time Course of Induction of Ly-6D Gene

To determine whether the induction of Ly-6D is rapid, we analyzed total RNA extracted from livers of wild-type male mice, which were administered a single intragastric injection of Wy-14,643 (250 mg/kg body weight) and killed at 8, 16, and 24 h after dosing. Increases in Ly-6D and L-PBE mRNA levels in liver were noted at 8 h after dosing and the levels continued to increase reaching maximum between 16 and 24 h (Fig. 4). The hepatic mRNA levels of both Ly-6D and L-PBE after a single dose of Wy-14,643 were similar to the levels observed in wild-type mice fed this compound for 2 weeks (see Fig. 4). Rapid and coordinate increases in mRNA levels of peroxisomal β-oxidation system genes and members of CYP4A family are the result of increased transcriptional activity (16,35). Parallel increases in Ly-6D and L-PBE mRNA levels suggest that increase in Ly-6D mRNA in liver is most likely due to increased transcription (35).

Induction of Ly-6D mRNA Is Mediated by PPARα

Northern blot analysis presented above clearly indicates that Ly-6D mRNA level is increased in the livers with peroxisome proliferation induced in wild-

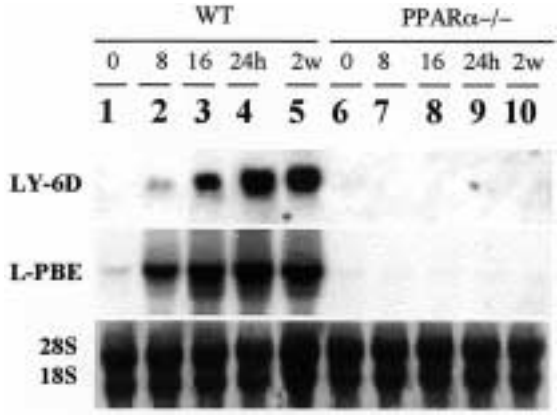

Figure 4. Time course of Ly-6D induction and PPARα dependency. Wy-14,643 was administered to wild-type (WT) and PPARα$^{-/-}$ mice in a single intragastric dose (250 mg/kg body weight) and animals were killed at 0 (lanes 1 and 6), 8 (lanes 2 and 7), 16 (lanes 3 and 8), and 24 (lanes 4 and 9) h later. Lanes 5 and 10 represent wild-type and PPARα$^{-/-}$ mice, respectively, fed Wy-14,643 (0.125% w/w) for 2 weeks. Wild-type mice given Wy-14,643 reveal time course and steady-state induction of Ly6-D and L-PBE in liver. No induction occurred in PPARα$^{-/-}$ mouse liver. The bottom blot, stained with methylene blue to assess sample loading, indicates the positions of 28S and 18S RNA.

type mice by a synthetic peroxisome proliferator, or occurring spontaneously in the liver of AOX$^{-/-}$ mouse (Figs. 1 and 2). These observations strongly suggested a possible role for PPARα in the induction of Ly-6D. To directly examine the role of PPARα in the induction of Ly-6D, we performed Northern blot analysis on total RNA extracted from the livers of wild-type or PPARα$^{-/-}$male mice that were killed at 8, 16, and 24 h after they were given a single intragastric injection of Wy-14,643. As shown in Figure 4, Ly-6D mRNA levels increased in the liver of wild-type mice given Wy-14,643 in a time-dependent manner and this induction was abrogated completely in PPARα$^{-/-}$ mice. No increase in Ly-6D mRNA content was observed in PPARα$^{-/-}$ mice fed a peroxisome proliferator for 2 weeks in the diet (Fig. 4). As expected, the induction of L-PBE gene, which is known to be regulated by PPARα (44), is also abolished in the liver of PPARα$^{-/-}$ mice when exposed to Wy-14,643 (Fig. 4). Furthermore, mice deficient in both PPARα and AOX (18) failed to show an increase in hepatic Ly-6D mRNA level when exposed to peroxisome proliferators (not shown). These data imply that the upregulation of Ly-6D gene, like other well-characterized PPARα response genes, is mediated by PPARα.

Cellular Localization of Ly-6D Gene Expression in Liver

It has been reported that under normal physiologic conditions, the expression of Ly-6D gene in the adult mouse liver is undetectable by Northern analysis (4). Our results essentially confirm these observations (Figs. 1 and 2). Because this Ly-6D gene is markedly upregulated in the liver of AOX$^{-/-}$ mouse and in wild-type mice treated with peroxisome proliferators (Figs. 1 and 2), we performed in situ hybridization to ascertain the hepatocellular localization of transcripts in the liver. Ly-6D signal appeared intense in the liver parenchyma of AOX$^{-/-}$ mouse (Fig. 5A, B). The expression of this gene was comparable to that of CYP4A1 (Fig. 5C) and L-PBE (Fig. 5D) observed in AOX$^{-/-}$ mouse liver. In wild-type mouse treated with a peroxisome proliferator for 2 weeks, there was in situ hybridization evidence of induction in liver of Ly-6D (Fig. 5E, F), CYP4A1 (Fig. 5G), and L-PBE (Fig. 5H). No appreciable in situ hybridization signal was detected for Ly-6D in the liver of wild-type control mouse (not illustrated).

In summary, the results presented in this report show that Ly-6D gene, which was not hitherto known to be induced in the liver of rats and mice with peroxisome proliferation, is markedly upregulated in AOX$^{-/-}$ mice with spontaneous peroxisome proliferation and in wild-type mice exposed to synthetic peroxisome proliferators. These studies further show that Ly-6D induction in liver is PPARα dependent. Ly-6D, also known as ThB antigen, belongs to the larger Ly-6 family of small glycosyl-phosphatidylinositol (GPI)-anchored membrane proteins (22), and it is homologous to human E48 antigen, a GPI-anchored protein present in desmosomal preparation (4,5). The gene encoding E48 is a single copy gene assigned to human chromosome 8 in the q24-qter region, which is syntenic with mouse chromosome 15, the location of Ly-6 multigene family (23). In mouse, Ly-6D antigen is expressed on the outer surface of lymphocytes, and in the epithelium lining the tongue, tail skin, and ear skin, suggesting that this antigen is of critical importance in keratinocytes (4). The homologue E48 is also expressed in squamous epithelium (4), and in squamous carcinoma cells, transitional epithelial cells, and keratinocyte of stratified squamous epithelia and may function in cell–cell adhesion (4,20). Junctional complexes containing adhesion molecules are also involved in signal transduction processes implicated in alterations of cell density and loss of matrix adherence, and in this way can influence cell behavior and morphology (4,5,14). In this article, we have shown that Ly-6D, an adhesion molecule that is minimally expressed in normal liver, is markedly induced by peroxisome proliferators in the liver of wild-type, in a time- and dose-dependent manner. This gene is also overexpressed in liver during sustained activation of PPARα in the liver of AOX$^{-/-}$ mouse. This upregulation is mediated by PPARα and

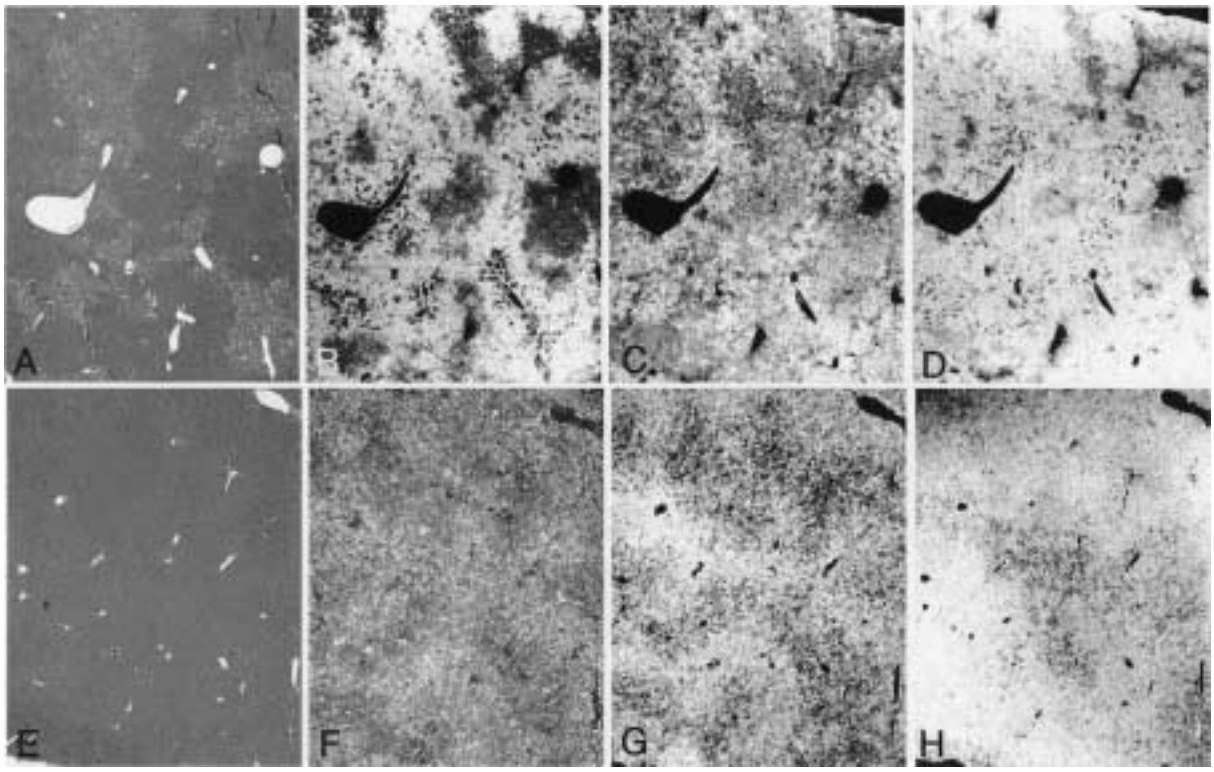

Figure 5. In situ hybridization analysis of Ly6-D gene expression in liver. Mouse liver sections were hybridized with ^{35}S-labeled riboprobes (Ly6-D, CYP4A10, and L-PBE) as described under Materials and Methods. All sections were probed with both antisense and sense probes (not shown). Sense probes gave no appreciable background. The nuclei were counterstained using Hoechst 33258. (A and E) Bright field microscopy of liver of AOX$^{-/-}$ on control diet and wild-type mouse on Wy-14,643 diet for 2 weeks, respectively. (B–D) Liver sections of AOX$^{-/-}$ mouse hybridized with antisense Ly6-D (B), CYP4A10 (C), and L-PBE (D). (F–H) Sections of Wy-14,643-treated wild-type mouse liver hybridized with Ly-6D (F), CYP4A10 (G), and L-PBE (H).

implicates the importance of this receptor in cell–cell adhesion and signal transduction processes. A putative PPRE is found in the upstream noncoding region of mouse Ly-6 gene (16). Further studies are necessary to characterize the promoter region of this gene to establish that it is transcriptionally regulated by PPARa. In conclusion, this is the first observation that a cell surface molecule not expressed in normal liver forms part of the peroxisome proliferator-induced pleiotropic responses in liver. In addition, other novel genes identified as upregulated in the gene expression screen used in this study, and found in the cDNA microarray analysis of AOX$^{-/-}$- and Wy-14,643-treated mouse liver, are currently being evaluated and should provide further insights into the mechanism by which peroxisome proliferators and PPARα induce the predictable pleiotropic responses.

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