Functional Modulation of the Glucocorticoid Receptor and Suppression of NF-κB-dependent Transcription by Ursodeoxycholic Acid

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Ursodeoxycholic acid (UDCA) is the current mainstay of treatment for various liver diseases including primary biliary cirrhosis. UDCA acts as a bile secretagogue, cytoprotective agent, immunomodulator, and inhibitor of cellular apoptosis. Despite this cumulative evidence of the cytoprotective and immunosuppressive effects of UDCA, both the target molecule and pathway of UDCA action remain unknown. We previously described that, in the absence of glucocorticoid ligand, UDCA activates the glucocorticoid receptor (GR) into DNA binding species but does not elicit its transactivation function in a transient transfection assay. Here we further studied the molecular mechanism of UDCA action and revealed that the ligand binding domain of the GR is responsible for UDCA-dependent nuclear translocation of the GR. Indeed, we demonstrated that UDCA acts on the distinct region of the ligand binding domain when compared with the classical GR agonist dexamethasone, resulting in loss of coactivator recruitment and differential regulation of gene expression by the GR. Our data clearly indicated that UDCA, at least in part via activation of the GR, suppresses NF-κB-dependent transcription through the intervention of GR-p65 interaction. Together with the established clinical safety of UDCA, we may propose that UDCA could be a prototypical compound for development of a novel and selective GR modifier.

Ursodeoxycholic acid (UDCA) is the current mainstay of treatment for primary biliary cirrhosis, which is a chronic cholestatic liver disease characterized by the destruction of biliary epithelial cells (i.e. cholangiocytes), presumably by autoimmune mechanism(s) (1–3). This hydrophilic bile acid is reported to induce biochemical, histological, and prognostic improvement in patients with primary biliary cirrhosis in the virtual absence of adverse reactions (3). UDCA acts as a bile secretagogue and cytoprotective agent (1) and exerts diverse immunomodulatory actions in vitro: suppression of immunoglobulin, interleukin-2, interleukin-4, and interferon-γ production from lymphocytes; attenuation of major histocompatibility complex expression on hepatocytes and cholangiocytes; increase in natural killer cell activity; and inhibition of eosinophil degranulation (1, 4–9). Recently, it has been shown that UDCA inhibits cellular apoptosis via stabilization of the mitochondria membrane (10, 11). Despite this cumulative evidence of the cytoprotective and immunosuppressive effects of UDCA, both the target molecule and pathway of UDCA action remain unknown.

The glucocorticoid receptor (GR) is a member of the nuclear receptors and an important transcriptional regulator involved in widely diverse physiological functions such as control of embryonic development, cell differentiation, and metabolic homeostasis (12, 13). Moreover, therapeutic activities of glucocorticoids are believed to be mediated by the GR (14). The nuclear receptors share several structural features (e.g. the ligand binding domain (LBD), DNA binding domain (DBD), and several transactivation domains (15)). Concerning the GR, the NH2-terminal domain activation function-1 contains sequences responsible for activation of target genes and presumably interacts with the components of the basal transcription machinery and/or with cofactors and other transcription factors, largely in a cell- or tissue-specific context. The central part of the receptor constitutes the DBD, which participates in receptor dimerization, nuclear translocation, and transactivation. The structural motif of the DBD is two zinc fingers formed by the coordination of four cysteines to one zinc atom. Adjacent to the second zinc finger, the amino acids responsible for the nuclear localization, the nuclear localization signal, exist. The carboxyl-terminal portion of the receptor includes the LBD and the sequences for heat shock protein 90 (hsp90) binding, nuclear translocation, dimerization, and transactivation. The COOH-terminal transcriptional activation domain is hormone-dependent and termed activation function-2. The very COOH-terminal portion of the receptor, activation function-2 core, serves as a molecular switch that recruits coactivator proteins and activates the transcription of target genes when flipped into the active conformation by hormone binding (12, 16–18).
On the other hand, the GR can also mutually interfere with other signaling pathways such as those mediated by the transcription factor NF-κB (19), which is an inducible transcription factor that regulates expression of various genes involved in inflammation and immune responses (20–22). NF-κB consists of a dimer from five related proteins, most typically a heterodimer composed of p65/RelA and p50 subunits. The regulation of NF-κB is achieved through interaction with an inhibitory protein known as IκB that binds to NF-κB and sequesters it in the cytoplasm. Once cells are stimulated with inducers of a dimer from five related proteins, most typically a heterodimer composed of p65/RelA and p50 subunits. The regulatory protein known as IκB binds to NF-κB and sequesters it in the cytoplasm. Once cells are stimulated with inducers such as proinflammatory cytokines (e.g., tumor necrosis factor α and interleukin-1), two serine residues of the IκB protein are phosphorylated by IκB kinases. Phosphorylation of IκB targets it for ubiquitination and subsequent degradation by the 26 S proteasome and renders the nuclear localization signal of NF-κB unmasked. Then NF-κB translocates from the cytoplasm into the nucleus and regulates the transcription of target genes (23, 24). In addition to this “classical” milieu, recent reports have suggested that several alternative pathways lead not only to activation but also to repression of NF-κB (25–27). Inhibition of NF-κB by glucocorticoids has been well documented, which may constitute a plausible mechanism of anti-inflammatory and immunosuppression by glucocorticoids (19). Although several possibilities have been proposed as an inhibitory mechanism, involvement of the GR appears to be consistent (28–32). Despite possible therapeutic antagonism of NF-κB by the GR in inflammatory disorders, however, side effects such as hypothyroidism-arthritis-renal axis insufficiency, diabetes, altered lipid metabolism, osteoporosis, steroid myopathy, and infectious and neuropsychiatric complications limit the therapeutic use of the classical glucocorticoid agonists (14). In this line, dissociation of glucocorticoid-dependent transactivation and transrepression may lead to the development of better tolerated drugs (20). Already several compounds have been reported to exhibit strong inhibition of NF-κB but weak induction of the GRE-dependent reporter gene; however, clinical application of those compounds is still pending (33–36).

We previously described that UDCA, without direct binding to the GR, activates the GR into DNA binding species but does not elicit its transactivational function in a transient transfection assay (37). Moreover, we predicted that the target domain in the GR of UDCA might be the LBD (37). Here we further studied the molecular mechanism of UDCA action and revealed that the LBD is responsible for UDCA-dependent translocation of the GR. Indeed, it is suggested that UDCA interacts with the distinct region of the LBD when compared with a classical GR agonist dexamethasone, resulting in differential regulation of gene expression by the GR. Our data clearly indicated that UDCA-activated GR suppresses NF-κB-dependent transcription via interaction with the p65 subunit. Taking into consideration the established clinical safety of UDCA, we propose that UDCA could be a prototypical compound for the development of a novel and selective GR modifier.

**EXPERIMENTAL PROCEDURES**

**Reagents**—UDCA was donated by Mitsubishi-Tokyo Pharmaceutical Co., Tokyo, Japan. Dexamethasone was purchased from Sigma, and other chemicals were purchased from Wako Pure Chemical (Osaka, Japan) unless otherwise specified. Antibodies against the GR (PA1–512) and hsp90 (3B6 and 3G3) were purchased from Affinity Bioreagents (Golden, CO), and those against TIF2 (sc-6264), p65 (sc-372), p50 (sc-1190), and IκBα (sc-71) were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antibodies against phosphorylated IκBα and paxillin were from New England Biolabs (Beverly, MA) and Transduction Laboratories (Lexington, KY), respectively.

**Plasmids**—The expression plasmids for the chimeric protein of GFP and the human GR, mineralocorticoid receptor (MR), progesterone receptor (PR), and androgen receptor (AR) were kindly provided by Drs. H. Ogawa (Kyoto University) (for GR and MR), G. Hager (National Institutes of Health) (for PR), and J. Palvimo (Helsinki University) (for AR). The expression plasmid for TIF2, pS5G-TIF2, was a generous gift from Dr. P. Chambon (Institut de Génétique et de Biologie Moleculaire et Cellulaire, Strasbourg, France). The expression plasmid for the functional protein of the Ga4 Domain and the LBD of the Ga4–GR LBD that for the VP16 activation domain (VP16AD), pcMX-VP16AD, and the Ga4-driven luciferase reporter plasmid, ptk-GALpx3-LUC, were from Dr. Kazuhiko Umesono (Kyoto University, Kyoto, Japan). To construct the chimeric plasmids for NF-κB p65 and the DBD of Ga4 (amino acids 1–147), the fragments containing cDNA encoding either wild-type (amino acids 1–549), the NH2-terminal half (residues 1–286), or the COOH-terminal half (residues 286–549) of mouse p65 were generated by polymerase chain reaction using pCAGGS-p65 (a gift from Dr. H. Handa, Tokyo Institute of Technology, Yokohama, Japan) as a template. Those fragments were then cloned into EcoRI and EcoRV sites of Ga4 DBD expression plasmid pcMX-Ga4 (a gift from K. Umesono) in frame, resulting in pCMX-Ga4-p65S (1–549), pCMX-Ga4-p65S/1–285, and pCMX-Ga4-p65S/286–549, respectively. To construct an expression plasmid for the chimeric protein of VP16AD and nuclear receptor interaction domain (NID) of TIF2, the DNA fragment encoding 173 amino acids (glutamic acid 594 to leucine 766) of the human TIF2 was amplified by polymerase chain reaction using pS5G-TIF2 as a template, and this fragment was inserted into the parent pCMX-VP16AD plasmid, resulting in VP16AD-TIF2/NID. All plasmids constructed as above were verified by sequencing. The glucocorticoid-responsive reporter plasmid pGRE-Luc was described elsewhere (38).

**Cell Culture—** COS7, CV-1 and HeLa cells were obtained from the RIKEN Cell Bank (Tsukuba Science City, Japan) and maintained in Dulbecco’s modified Eagle’s medium (Iwaki Glass, Chiba, Japan). CHO-K1 cells were obtained from the RIKEN Cell Bank and maintained in Ham’s F-12 medium (Iwaki Glass). All media used in this study were phenol red-free and supplemented with 10% fetal calf serum (FCS) and antibiotics. Serum steroids were stripped from FCS with dextran-coated charcoal, and cells were cultured in a humidified atmosphere at 37 °C with 5% CO2 unless otherwise specified.

**Immunocytochemical Analysis—** Cells grown on eight-chambered sterile glass slides (Nippon Becton & Dickinson, Tokyo, Japan) were fixed for immunostaining using a freshly prepared solution of 4% paraformaldehyde (w/v) in phosphate-buffered saline (PBS) overnight at 4 °C. Immunocytochemistry was carried out as described previously (37) with small modification. Briefly, cells were washed with PBS at room temperature and incubated with appropriate antibodies at 2 μg/ml in PBS containing 0.1% Triton X-100 for 9 h at 4 °C. The cells were then washed and incubated with biotinylated secondary antibody from donkeys (Amersham Pharmacia Biotech) at a dilution of 1:200 in PBS containing 0.1% Triton X-100 for 1 h at room temperature, and then the cells were washed and incubated with fluorescein isothiocyanate (FITC)-conjugated streptavidin at a dilution of 1:100 in PBS containing 0.1% Triton X-100 for 1 h at room temperature. Finally, the cells were mounted with GELMOUNT® (Biomeda Co., Ltd., Foster, CA) and then examined by an Olympus Fluoview microscope (Olympus, Tokyo, Japan) equipped with an FITC filter set.

**Transfection and Reporter Gene Assay—** Before transfection, cell culture medium was replaced with OPTI-MEM medium lacking phenol red (Life Technologies, Inc.). Plasmid mixture containing pGRE-Luc in the presence or absence of the GR expression plasmid was mixed with TransIT-LT1 reagent (Panvera Corp., Madison, WI) and added to the culture. The total amount of plasmid was kept constant by adding an irrelevant plasmid (pGEM3Z) unless otherwise specified. After 6 h of incubation, the medium was replaced with fresh Dulbecco’s modified Eagle’s medium supplemented with 2% dextran-coated charcoal-treated FCS, and the cells were further cultured in the presence or absence of various ligands for 24 h. Luciferase enzyme activity was determined using a luminometer (Berthold GmbH & Co. KG, Bad Wildbad, Germany) essentially as described before (38).

**Visualization of Intracellular Trafficking of GFP Fusion Proteins in Living Cells—** For analysis of nuclear translocation of the GFP-GR, we transiently expressed GFP-tagged human GR or its mutants in COS7 cells as previously described (39). The cells were cultured on the silane-coated coverslips in 8-cm diameter plastic dishes, and the medium was changed by OPTI-MEM medium before transfection. The plasmid mixture containing 6 μg of the expression plasmids was mixed with 12 μl of TransIT-LT1 reagent and added to the culture. After 6 h of incubation, the medium was replaced with Dulbecco’s modified Eagle’s medium supplemented with 2% dextran-coated charcoal-treated FCS, and the cells were cultured at 37 °C. GFP was expressed at detectable levels between 24 and 72 h after transfection.
Effect of UDCA on the Subcellular Localization of the GR—As described in the Introduction, we previously suggested that, although UDCA does not bind to the GR, the receptor translocates into the nucleus in the presence of UDCA in GR-overexpressing CHO cells (37). In the present study, we first addressed the specificity of such UDCA action and examined the effect of treatment with UDCA on subcellular localization of various steroid receptors, all of which are believed to be predominantly docked in the cytoplasm in the absence of cognate ligands. For this purpose, we transfected the expression plasmids for GFP-tagged MR, PR, and AR as well as GFP-GR and microscopically observed their subcellular localization after 6-h treatment with their cognate ligands (100 nM) or 200 μM UDCA. As shown in Fig. 1, some of those receptors showed weak nuclear fluorescence in the absence of ligand; however, treatment with their ligands promoted complete nuclear condensation of green fluorescence, indicating that the chimeric proteins between GFP and these nuclear receptors are capable of ligand-dependent nuclear localization. Treatment with UDCA did not significantly influence the subcellular localization of either the MR, PR, or AR; however, it preferentially induced nuclear localization of the GR (Fig. 1).

To further confirm UDCA's effect on subcellular trafficking of the GR, we transfected COS7 cells with the GR expression plasmid pCMX-GR and cultured cells in the presence of UDCA for the indicated periods of time. Immunocytochemical analysis revealed that expressed GR localized in the cytoplasm in the absence of ligand (data not shown) and rapidly translocated into the nucleus after the addition of dexamethasone (Fig. 2). In the presence of UDCA, the GR revealed nuclear localization in a concentration- and time-dependent manner at a slower rate when compared with that of dexamethasone-induced translocation (Fig. 2).

The Ligand-binding Domain Is a Target of UDCA—We previously predicted that the LBD is involved in UDCA action on the GR (37). To confirm this, we tested the effect of treatment with UDCA on the subcellular localization of the chimeric protein of Gal4 DBD and the COOH-terminal half of the GR, Gal4-GR LBD, which encompasses the NH2-terminal nuclear localization signal, NL1, and the entire LBD of the GR. After transfection of this Gal4-GR LBD expression plasmid into COS7 cells, we immunocytochemically examined the effect of treatment with UDCA on the subcellular localization of the expressed protein using anti-Gal4 antibody. In the absence of ligand, Gal4-GR LBD exclusively showed cytoplasmic localization (data not shown). After treatment with dexamethasone, Gal4-GR LBD moved into the nucleus in a time-dependent manner (Fig. 3A). Indeed, treatment with UDCA promoted nuclear translocation of the Gal4-GR LBD even in the absence of dexamethasone (Fig. 3A). When the transactivational potential of this chimeric protein was assessed in a transient transfection assay, dexamethasone, not UDCA, induced expression of the reporter gene (Fig. 3B), as in the case of the wild-type GR (37).
with hsp90 in the absence of ligand, with slightly less efficiency in the case of GFP-GR-(1–730). When the cells expressing those mutant proteins were heat-shocked at 43 °C, all mutants moved into the nucleus at similar rates (data not shown), suggesting that those mutant proteins associate with hsp90, and dissociation of hsp90, by heat shock in this case, results in movement toward the nucleus. Given this, we tested the effect of UDCA on the subcellular localization of those mutants. When transfected cells were treated with dexamethasone, nuclear translocation was observed only in the GFP-fused wild-type GR and not in GFP-GR-(1–730), GFP-GR-(1–750), or GFP-GR-(1–770) (Fig. 4C). In contrast, treatment with UDCA caused nuclear translocation of every GFP-GR mutant except for GFP-GR-(1–730) (Fig. 4C). GFP-GR-(1–730) partially docked in the nucleus before treatment with ligand, and the effect of dexamethasone and UDCA was not apparent (Fig. 4C). This leakiness and unresponsiveness to the ligand of GFP-GR-(1–730) might be related to weak association of this mutant with hsp90 (Fig. 4B). In any case, we may conclude that the COOH-terminal end of the LBD is essential not only for eliciting the effect of UDCA but also for dissociation of UDCA and dexamethasone in terms of GR activation.

**FIG. 4.** Mutational analysis of the ligand binding domain of the GR. **A,** schematic drawing of the GR and its mutants used in the present study. **B,** bar graph showing the positions of α-helices (H1 to H12). **C,** Western blot analysis of the association of the GR and hsp90. COS7 cells were transfected with pCMX vector alone or the expression plasmids for the full-length and COOH-terminal deleted GR with GFP tag. Whole cell lysates were, either directly (IP−) or after immunoprecipitation with anti-hsp90 antibodies (IP+) analyzed in Western blots using anti-GFP or anti-hsp90 antibodies. C, cellular distribution of the GFP-GR after treatment with 100 nM dexamethasone (D) or 200 μM UDCA (U).
When Gal4-GR LBD was introduced, treatment with dexamethasone increased reporter gene expression as a function of dexamethasone concentration, most possibly due to ligand-dependent transactivation potential of Gal4-GR LBD (Fig. 5B). Moreover, coexpression of VP16AD-TIF2/NID further enhanced reporter gene expression, indicating the interaction between Gal4-GR LBD and TIF2/NID (Fig. 5B). Since the nuclear translocation of Gal4-GR LBD was shown to be less efficient in the presence of UDCA than dexamethasone (Fig. 3), we took another immunocytochemical approach to confirm the interaction between the GR and TIF2 in situ. It has already been shown that ligand-activated steroid receptors tend to be condensed in the particular regions in the nucleus with forming discrete foci (41), in which coactivators are often found with the receptors (42). We overexpressed the expression plasmids for the GR and TIF2, and intracellular localization of these molecules was studied using anti-GR and anti-TIF2 polyclonal antibodies with a confocal laser microscopy. As shown in Fig. 5C, the GR showed diffuse cytoplasmic distribution in the absence of ligand, whereas TIF2 revealed distinct dot-like localization in the nucleus. Upon exposure to dexamethasone, GR-specific fluorescence tended to form distinct foci, most of which overlapped with TIF2 foci (Fig. 5C). On the contrary, treatment with UDCA resulted in diffuse nuclear fluorescence of the GR, which did not merge with TIF2-related foci (Fig. 5C). It may thus be concluded that UDCA-activated GR does not communicate with TIF2 in the nucleus.

Effect of UDCA on GR-NF-κB Interaction—We next examined the effect of dexamethasone and UDCA on another function of the GR, repression of NF-κB. Treatment with PMA induced expression of NF-κB-responsive reporter gene by ~10-fold (Fig. 6, columns 1 and 2). Dexamethasone alone did not significantly suppress the PMA-induced response of reporter gene expression (columns 3 and 4). When the wild-type GR expression plasmid pCMX-GR was cotransfected, treatment with dexamethasone resulted in marked reduction of NF-κB activity (columns 5–10). The inhibitory effects on NF-κB activity were abolished when the COOH-terminal truncated receptor GR1–750 was expressed (columns 13–16), indicating that dexamethasone-mediated suppression of NF-κB may again require the COOH-terminal end of the LBD. When the effect of UDCA was assessed, NF-κB activity was slightly decreased in the absence of the GR expression plasmid (columns 5 and 6). Moreover, transfection of the wild-type GR expression plasmid, as expected, resulted in further suppression of NF-κB activity in the presence of UDCA (column 12). Notably, the COOH-terminal truncated mutant GR(1–750) still maintained this suppressive effect of UDCA (column 18). The UDCA-mediated suppression of NF-κB activity was increased along with the increment of GR expression plasmid or UDCA concentrations (data not shown).

To analyze the molecular mechanism of GR-mediated suppression of NF-κB activity, we examined the effect of UDCA on subcellular localization of the p65 subunit of NF-κB, since it has been shown that the major constituents of NF-κB are p65 and p50, and transactivational activity of NF-κB is considered to rely on p65 (23, 24). For this purpose, pCMX-GR was transfected into HeLa cells, and subcellular localization of the exog-
test the protein levels of NF-κB components. In the absence of UDCA, PMA induced rapid phosphorylation of the inhibitory protein IκB, resulting in a gradual decrease in IκB protein levels. Protein expression of p65 and p50 appeared to be constant. In the presence of UDCA, almost identical results were obtained, as in the case of treatment with dexamethasone (Fig. 7B, data not shown). Taken together, it is strongly indicated that UDCA does not affect the activation process of NF-κB in the cytoplasm (e.g. phosphorylation and degradation of IκB and the following nuclear translocation of p65/p50 heterodimer). Alternatively, UDCA might affect the transcription function of NF-κB in the nucleus. To test this hypothesis, we assessed the effect on transactivational function of p65 using Gal4-p65 fusion proteins (Fig. 7C). Transactivational activity of Gal4-VP16 was not significantly influenced by treatment with either dexamethasone or UDCA. When Gal4 DBD was fused with full-length p65 (Gal4-p65/1–549), the transcriptional activity was repressed by dexamethasone and UDCA in the presence of introduced GR (Fig. 7C, columns 2 and 3). This suppressive effect of dexamethasone and UDCA is not observed when Gal4-p65/1–285 was used (columns 4 and 5), but was observed when Gal4-p65/286–549 was used (columns 6 and 7). Although the suppressive effect of dexamethasone was greater against Gal4-p65/1–549 than against Gal4-p65/286–549, UDCA equally repressed transactivation of either Gal4-p65/1–549 or Gal4-p65/286–549 (compare columns 3 and 7).

**DISCUSSION**

Although it is known that UDCA modulates various cellular and immunological processes in vitro and influences the clinical course and pathology of inflammatory liver diseases, its molecular mechanism has remained unknown (43). We previously reported that UDCA activates the GR into DNA binding species in the absence of steroid ligand (37). We here describe that UDCA interacts with the GR through the LBD, with neither recruitment of p160 coactivator TIF-2 nor eliciting transactivational function of the GR. Moreover, we indicate that UDCA-activated GR represses NF-κB-dependent transcription.

Recent emerging studies have widened the spectrum of ligand for the nuclear receptors. Among others, it is revealed that a number of bile acids, including chenodeoxycholic, cholic, deoxycholic, and lithocholic acids, bind and activate the nuclear receptor FXR/BAR in cultured cells (44–46). The identification of the target genes for FXR and FXR gene disruption in mice provided the first clues to the physiological function of the interaction between bile acids and the receptor (47, 48). FXR heterodimerizes with the common heterodimeric partner RXR and constitutively resides in the nucleus (48). Therefore, their ligands necessarily pass through not only the plasma membrane but also the nuclear envelope. In this line, the fact that FXR ligand bile acids have considerable lipophilicity appears to be rational. Moreover, overexpression of their transporter potentiates the effect of those bile acids on FXR (45). In clear contrast, UDCA, which is a relatively hydrophilic bile acid compared with FXR ligands, does not activate the FXR (45) but rather with the GR as we have shown (37). Previous reports showed that various actions of UDCA could chiefly be ascribed to its effects on the cell membrane, since it is not conceivable that the hydrophilic bile acid UDCA readily penetrates the cell membrane and directly influences intracellular processes (49). It may thus be speculated that UDCA initially interacts with the cell membrane and then modulates cytoplasmic events, one of which may be connected to GR activation. For example, UDCA, via interaction with an as yet unknown target machinery or receptor on the membrane, may generate such secondary signals that dissociate hsp90 from the GR even in the absence of glucocorticoid ligands. This perspective is indirectly supp-
ported by the fact that overexpression of the ileal bile acid transporter did not influence UDCA-dependent nuclear translocation or transactivation of the GR (data not shown). In the case of heat shock experiments, it has been shown that hsp90 is a target of heat-shock-induced cellular signals (50, 51). Taking the relative GR specificity of UDCA action into consideration, however, not hsp90 but GR itself might be the final target of such signals, since all steroid receptors used in the present study have been shown to associate with hsp90. Although the precise mechanism still remains unknown, the interplay between UDCA and the GR could be mechanistically distinct from that between other hydrophobic bile acids and FXR.

We indicated here that one of the target domains of UDCA (or UDCA-generated signal) on the GR is the LBD, since expressed Gal4-GR LBD still moves into the nucleus after treatment with UDCA. This fusion protein has been shown to have a ligand-inducible transactivation potential (52). However, UDCA did not elicit induction of GRE-dependent reporter gene expression in the present study. Again, UDCA induces nuclear translocation and DNA binding activity of the wild-type GR, whereas UDCA-activated GR lacks transactivation potential (37). Our data may suggest that UDCA, despite interacting with the LBD, cannot activate activation function-2. Already a number of reports have described that the LBD has multiple functions distributed throughout the very domain, and not only steroid ligands but also various stimuli may differentially influence its functionality (53–55). For example, the interaction with coactivators is elicited by the majority of glucocorticoid ligands but not by certain steroids including RU486, although RU486 can bind with the LBD and RU486-bound GR could translocate into the nucleus as a DNA-binding species (56, 57). Since we showed in two independent experiments that UDCA-activated GR cannot communicate with a coactivator TIF2 in the nucleus, UDCA-activated GR appears to be extremely similar to RU486-activated GR. Interestingly, the domain requirement for UDCA-dependent nuclear translocation is distinct from that for dexamethasone. Our results indicate that UDCA or UDCA-provoked secondary signals influence a broader region of the LBD than dexamethasone; GR (1–765), in which the very COOH-terminal end of the LBD was chopped off, could not be activated by dexamethasone but was by UDCA, and the effect of UDCA was observed when COOH-terminal deletion progressed to amino acid position 740. Note that these deletion mutants of the GR lack helix 12, which forms an interaction surface with coactivators (Fig. 4A). We thus may speculate that UDCA, either directly or indirectly, modulates the LBD structure of the GR into such a unique conformation that the GR can translocate into the nucleus and bind DNA but no longer interacts with the coactivators to elicit transcriptional activation. Of course, for confirmation of this scenario, structural analysis of the GR is essential. At this moment, however, we are confronted with several difficulties in performing such experiments. For example, the three-dimensional structure of the LBD of the GR has not yet been made clear, and current structural discussion of the GR LBD, therefore, ought to be based on the knowledge of other nuclear receptors. More importantly, it is likely that the GR is indirectly modulated within the cells after treatment with UDCA via generation of as yet unidentified secondary signal, and at this moment there is no way to reconstitute such activation process in vitro. To overcome these issues, it is necessary to clarify the pathway for UDCA-mediated activation of the GR.

We also showed that UDCA suppresses NF-κB-dependent transcription, and this inhibitory effect, at least in part, is mediated by the GR. Various mechanisms have been presented for GR-dependent NF-κB suppression (See the Introduction). Given the data showing that the protein amounts of the NF-κB components (p65, p50, or IκBα), phosphorylation of IκBα, and nuclear translocation of p65 were not significantly influenced after treatment with UDCA, we, among others, favor the idea that UDCA represses transcriptional activity of NF-κB in the nucleus via activation of the GR. Notably, a helix 12-lacking GR (1–750) does not activate GRE-dependent transcription but suppresses NF-κB-dependent transcription in the presence of UDCA, suggesting that UDCA-dependent suppression of NF-κB does not involve either induction of IκB synthesis or competition of a limiting amount of coactivators. On the other hand, a one-hybrid assay indicates that COOH-terminal transactivation domain of p65 could be a target of UDCA-activated GR; UDCA-activated GR may interact with the p65 subunit in the nucleus and repress NF-κB activity. In the meantime, negative regulation by the GR was more marked in full-length p65 when treated with dexamethasone. However, UDCA-mediated repression was almost comparable between full-length and the COOH-terminal half of p65. It thus is tempting to speculate that UDCA-activated GR may suppress NF-κB in such a manner distinct from dexamethasone-activated GR, at least not involving the NH2-terminal half of p65.

From the pharmacological viewpoint, GR is still considered to be one of the therapeutic targets for anti-inflammatory and immunosuppression (14). Several steroid compounds have enabled partial dissociation of these pharmacological actions from metabolic side effects (33, 36, 58). However, it has not been elucidated whether these compounds reproduce such distinction in vivo (36). Moreover, their receptor specificity has not been critically evaluated. We here showed that UDCA is extremely specific to the GR and represses NF-κB without induction of transactivation function of the GR. Since UDCA is not defined as a classical glucocorticoid despite carrying a steroid structure but as a bile acid, it is plausible that UDCA is a prototype of a novel and selective GR modifier. However, we should state again that the UDCA concentrations required for nuclear translocation and transrepression in our experiments appear to be extraordinarily high as a therapeutic drug. Indeed, it is known that in patients treated with UDCA, serum levels of UDCA are lower than the concentrations used in the present study (59–61). We therefore cannot directly link UDCA action observed in the present study with the therapeutic mechanism of UDCA in inflammatory liver diseases. On the other hand, it has been shown that the concentrations of UDCA are extremely elevated in the liver and bile ducts in patients taking UDCA (62). Given the beneficial effect of UDCA in a number of hepatobiliary diseases, it is possible that UDCA, because of its differences in regional concentration, acts only in such regions where UDCA is concentrated (i.e. liver and bile ducts) as an organ-specific immunomodulator. Of course, further identification of the molecular mechanism for UDCA action (e.g. identification of its receptor and signal transducer) would develop a novel pharmacological approach that may act in a more systemic fashion.

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