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Cross Talk between Glucocorticoid and Estrogen Receptors Occurs at a Subset of Proinflammatory Genes

Aleksandra Cvoro,*† Chaoshen Yuan,‡ Sreenivasan Paruthiyil,*† Oliver H. Miller,*† Keith R. Yamamoto,§ and Dale C. Leitman*†‡§

Glucocorticoids exert potent anti-inflammatory effects by repressing proinflammatory genes. We previously demonstrated that estrogens repress numerous proinflammatory genes in U2OS cells. The objective of this study was to determine if cross talk occurs between the glucocorticoid receptor (GR) and estrogen receptor (ER)α. The effects of dexamethasone (Dex) and estradiol on 23 proinflammatory genes were examined in human U2OS cells stably transfected with ERα or GR. Three classes of genes were regulated by ERα and/or GR. Thirteen genes were repressed by both estradiol and Dex (ER/GR-repressed genes). Five genes were repressed by ER (ER-only repressed genes), and another five genes were repressed by GR (GR-only repressed genes). To examine if cross talk occurs between ER and GR at ER/GR-repressed genes, U2OS-GR cells were infected with an adenovirus that expresses ERα. The ER antagonist, ICI 182780 (ICI), blocked Dex repression of ER/GR-repressed genes. ICI did not have any effect on the GR-only repressed genes or genes activated by Dex. These results demonstrate that ICI acts on subset of proinflammatory genes in the presence of ERα but not on GR-activated genes. ICI recruited ERα to the IL-8 promoter but did not prevent Dex recruitment of GR. ICI antagonized Dex repression of the TNF response element by blocking the recruitment of nuclear coactivator 2. These findings indicate that the ICI–ERα complex blocks Dex-mediated repression by interfering with nuclear coactivator 2 recruitment to GR. Our results suggest that it might be possible to exploit ER and GR cross talk for glucocorticoid therapies using drugs that interact with ERs. The Journal of Immunology, 2011, 186: 4354–4360.

A major event in the activation of the immune response is the synthesis and release of several cytokines that trigger inflammatory pathways. If overexpressed proinflammatory cytokines can cause excessive inflammation and tissue damage that can lead to the development of variety of human diseases. Glucocorticoids are the most widely used immunosuppressive and anti-inflammatory agents for treating inflammatory disorders. The anti-inflammatory effects of glucocorticoids are mediated through the glucocorticoid receptor (GR). A major mechanism whereby glucocorticoids exert anti-inflammatory actions is by causing the repression of proinflammatory genes (1). Glucocorticoids initiate gene repression by binding to GR. This allows GR to interact directly with transcription factors, such as NF-κB (2), which is a key transcription factor that binds to and activates many proinflammatory genes (3). GR binds to the C-terminal activation domain in the p65 subunit of NF-κB (4). However, GR does not interfere with the binding of NF-κB to DNA to cause repression. Instead, GR inhibits the transcriptional activation function of NF-κB after it binds to the promoter of target genes by different mechanisms depending on the gene (5, 6). For instance, when GR binds to NF-κB at the IL-8 promoter, it inhibits the phosphorylation of serine 2 (Ser2) in the C-terminal domain (CTD) of the largest subunit of RNA polymerase II (RNAPII) (7). GR does this by competing with Ser2 CTD kinase positive transcription elongation factor b (P-TEFb) (8), which consists of a heterodimer of the C-type cyclin and cyclin-dependent kinase 9. Cyclin-dependent kinase 9 phosphorylates the CTD of RNAPII at Ser2 and Ser5 (9). Because P-TEFb is required for productive elongation of the RNAPII initiation complex (9), the inhibition of P-TEFb binding by GR prevents elongation of transcripts.

Although glucocorticoids are the most extensive anti-inflammatory steroids studied, other compounds that interact with nuclear receptors also have anti-inflammatory properties. One important action of estrogens that is relatively unappreciated is its anti-inflammatory effects (10). Estrogens exert their effects through binding to two distinct estrogen receptors (ERs), ERα or ERβ (11). Similarly to GR, it has been demonstrated that ERs exert their anti-inflammatory activity by repressing the expression of multiple NF-κB–driven cytokine genes (12–15). Early in vitro studies suggested that ERs repress genes by blocking the binding of NF-κB to the promoter (12, 13), but other studies revealed a much more complex mechanism for ER-mediated repression. Our studies with U2OS cells stably transfected with ERs (U2OS-ERα and U2OS-ERβ) showed that TNF-α treatment leads to the recruitment of unliganded ERs to the composite c-Jun/NF-κB binding site in the TNF-α promoter (16). Once unliganded ERα associates with NF-κB and c-Jun, it functions as a coactivator that activates the TNF-α gene. Estradiol (E2) treatment causes repression of the TNF-α gene by recruiting nuclear coactivator 2 (NCOA2), which

*Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, San Francisco, CA 94143; †Center for Reproductive Sciences, University of California, San Francisco, San Francisco, CA 94145; ‡Department of Nutritional Science and Toxicology, University of California, Berkeley, CA 94720; and §Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, CA 94143

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Address correspondence and reprint requests to Dr. Dale C. Leitman, Department of Nutritional Science and Toxicology, University of California, Berkeley, Berkeley, CA 94720. E-mail address: dale@leitmanlab.com

The online version of this article contains supplemental material.

Abbreviations used in this article: Ad-ERα, adenovirus expressing human estrogen receptor α; CTD, C-terminal domain; Dex, dexamethasone; Dox, doxycycline; E2, estradiol; ER, estrogen receptor; GR, glucocorticoid receptor; ICI, ICI 182780; MOI, multiplicity of infection; NCOA2, nuclear coactivator A2; P-TEFb, positive transcription elongation factor b; qPCR, quantitative PCR; RNAPII, RNA polymerase II; Ser, serine; TNF-RE, TNF-α response element.

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functions as a corepressor in this context (16). A corepressor function for NCOA2 was previously reported for GR-mediated repression of the collagenase-3 gene (17). The complexity of ER-mediated transcriptional repression is also revealed on different NF-κB response elements in MCF-7 cells (18). In the IL-6 promoter, ERα displaced p65 and associated coregulators from NF-κB binding site, whereas in the case of the MCP-1 and IL-8 promoters, ERα displaces CREB binding protein from NF-κB sites (18). The displacement of these transcription factors and coregulators prevents them from activating these proinflammatory genes.

The studies with GR and ER show that the two receptors share some common mechanisms, but they also exploit different mechanisms to repress proinflammatory genes depending on the target gene, cell type, and probably interactions with other transcription factors. Despite the findings that both GR and ER repress proinflammatory cytokine genes, little is known about the potential cross talk between GR and ER. However, in an experimental lung inflammation rat model, the ERα antagonistICI 182780 (ICI) blocked the anti-inflammatory effects of glucocorticoids (19). This finding suggests that when ER is bound to an antagonist, it can displace CREB binding protein from NF-κB sites (18). The studies with GR and ER show that the two receptors share some common mechanisms, but they also exploit different mechanisms to repress proinflammatory genes depending on the target gene (21) upstream of the minimal thymidine kinase promoter in pGL4.22 (Promega) into U2OS-ERα cells. U2OS-ERα–TNF-RE cells were selected and maintained in 50 μg/ml hygromycin B, 500 μg/ml zeocin, and 1 μg/ml puromycin. U2OS-GR cells were a generous gift from Dr. Michael Garabedian. U2OS-GR cells were supplemented with 500 μg/ml geneticin (G418; Invitrogen). ECC-1 cells were obtained from American Type Culture Collection. All cell lines were cultured continuously in phenol red-free DMEM/F-12 medium (Invitrogen) containing 5% charcoal/dextran-stripped FBS (Gibco-BioProducts), 2 mM glutamine, 100 U/ml penicillin and streptomycin, and 50 μg/ml fungizone. Adenoviruses expressing human ERα (Ad-ERα) and the control virus (Ad-LacZ) were purchased from Viraquest.

Real-time PCR
Total RNA was prepared using the Aurum Total RNA Kit (Bio-Rad) according to the manufacturer’s protocol. A total of 1 μg total RNA was reverse transcribed using the iScript Kit (Bio-Rad). Real-time quantitative PCR was performed with the Bio-Rad iCycler Thermal Cycler System using SYBR Green Supermix (Bio-Rad). The sequences of the primers are listed in Supplemental Table I. The data were collected and analyzed using the comparative threshold cycle method using β-actin as the reference gene. Experiments were performed at least three times, and the mean ± SEM was calculated using the Prism curve-fitting program (GraphPad Software, version 3.03; GraphPad).

Western blotting
U2OS-GR cells were infected with Ad-LacZ or Ad-ERα for 24 h. Total proteins were extracted in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors. Immobiloblotting of proteins was performed following standard procedures using an Ab to ERα (1D5; DakoCytomation). Western blots for GR were done in U2OS–ERα–TNF-RE cells using an Ab to GR (PA1-512; Thermo Scientific). An ECL detection system (GE Healthcare) was used to visualize the proteins.

Luciferase assays
U2OS–ERα–TNF-RE cells were treated with doxycycline (Dox) for 18 h to induce ERα, and then drugs were added to the medium as indicated in

### Table 1. Dex and E2 repress common and unique proinflammatory genes

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<th>Gene</th>
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<th>Fold TNF-α Activation</th>
<th>Percent of Repression</th>
<th>p Value</th>
<th>Fold TNF-α Activation</th>
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\*U2OS-GR and U2OS-ERα stable cells were treated with 2.5 ng/ml TNF-α for 2 h in the absence or presence of 10 nM Dex (U2OS-GR) or 10 nM E2 (U2OS-ERα) for 2 h, total RNA was isolated, and real-time RT-PCR was performed. Real-time RT-PCR results are shown. Each data point is the average obtained from three individual U2OS cell samples ± SEM. Analyses were performed using the Prism curve-fitting program.

\*TNFAIP3 is activated by DEX but repressed by E2.
the figure legends for 18 h. The cells were solubilized with lysis buffer, and then the plates were frozen. The samples were thawed and assayed for luciferase activity using the Luciferase Assay System (Promega). Triplicate samples for each treatment were measured for luciferase activity using a luminometer. The experiments were repeated at least three times.

Chromatin immunoprecipitation

U2OS-GR cells were infected with Ad-LacZ or Ad-ERα for 24 h. After the treatments as indicated in the figure legends, the cells were cross-linked and washed, and collected chromatin was sheared with sonication. Immunoprecipitations were performed overnight at 4°C with anti-ERα (1D5, or HC-20) or anti-GR Abs (N499 or PA1-510A) as previously described (16, 22). U2OS–ERα–TNF-RE cells were treated with drugs, and then chromatin immunoprecipitation (ChIP) was done using anti-GR or KAT13C/NCOA2 (ab9261-50; Abcam) Abs. Chromatin samples were purified using the QIAquick Purification Kit (Qiagen), and amplification of samples at the IL8 gene was done using quantitative PCR (qPCR). ChIP primers that spanned the TNF-RE in pGL4.22 or NF-κB element in the IL8 promoter (8) were used. Experiments were done in triplicate, the mean ± SE was calculated, and statistical analysis was performed as previously described (23).

Results

ER and GR repress similar and unique proinflammatory genes

We previously identified proinflammatory genes activated by TNF-α that are repressed by E2 in U2OS osteosarcoma cells stably transfected with human ERα or ERβ (16, 23). To determine if GR produces similar anti-inflammatory effects as ERs, we tested if glucocorticoids also repress these genes in U2OS cells stably transfected with GR. U2OS-GR or U2OS-ERα cells were treated with TNF-α for 2 h in the absence or presence of dexamethasone (Dex) or E2, respectively. We found that E2 and/or Dex regulated three classes of proinflammatory genes. Thirteen genes tested by real-time PCR were activated by TNF-α and repressed by E2 and Dex (ER/GR-repressed genes, Table I). Five TNF-α–activated genes were repressed only by ERα (ER-only repressed genes, Table I) including TNFAIP3, which was activated by GR. Another five genes were repressed by GR, but not ERα (GR-only repressed genes, Table I). Representative real-time PCR shows that Dex and E2 blocked the induction of the TNF-α and CD69 genes in U2OS-GR (Fig. 1A, left panels) and U2OS-ERα (Fig. 1A, right panels) cells, respectively. The TNFAIP3 gene (Fig. 1B, upper panels) was repressed only by E2, whereas only Dex repressed the LIF gene (Fig. 1B, lower panels). These data demonstrate that Dex and E2 commonly repress multiple proinflammatory genes, but some genes are differentially repressed by ER and GR.

FIGURE 1. Effect of Dex and E2 on repression of the inflammatory genes in U2OS cells. U2OS-GR and U2OS-ERα stable cells were treated with 2.5 ng/ml TNF-α for 2 h in the absence or presence of 10 nM Dex (U2OS-GR) and 10 nM E2 (U2OS-ERα) for 2 h, total RNA was isolated, and real-time RT-PCR was performed. A, TNF-α–activated genes that are repressed by Dex and E2. Real-time RT-PCR results for representative genes (TNF-α and CD69) repressed by both Dex and E2 are shown. Each data point is the average obtained from three individual U2OS cell samples ± SEM. B, Differential transcriptional repression of inflammatory genes by ER and GR in U2OS cells. Real-time RT-PCR results for representative genes repressed by Dex (LIF) or by E2 only (TNFAIP3) are shown. Each data point is the average obtained from three individual U2OS cell samples ± SEM.

FIGURE 2. Effect of Dex and E2 on gene regulation in U2OS cells expressing both GR and ER. U2OS-GR stable cells were infected with 50 MOI Ad-LacZ (A) or Ad-ERα (B) for 24 h. The cells were then treated with 2.5 ng/ml TNF-α for 2 h in the absence or presence of 10 nM Dex, 10 nM E2, or both drugs for 2 h. Cells were treated with ICI 30 min prior to Dex and E2, and real-time RT-PCR for IL8 was performed. C and D, U2OS-GR stable cells were infected as in B and treated with Dex or E2 for 4 h. ICI (1 μM) and RU486 (1 μM) were administered 30 min prior to Dex, and real-time RT-PCR for IGFBP and IRF8 genes was performed. Error bars represent the mean ± SEM.

FIGURE 2. Effect of Dex and E2 on repression of the inflammatory genes in U2OS cells. U2OS-GR and U2OS-ERα stable cells were treated with 2.5 ng/ml TNF-α for 2 h in the absence or presence of 10 nM Dex (U2OS-GR) and 10 nM E2 (U2OS-ERα) for 2 h, total RNA was isolated, and real-time RT-PCR was performed. A, TNF-α–activated genes that are repressed by Dex and E2. Real-time RT-PCR results for representative genes (TNF-α and CD69) repressed by both Dex and E2 are shown. Each data point is the average obtained from three individual U2OS cell samples ± SEM. B, Differential transcriptional repression of inflammatory genes by ER and GR in U2OS cells. Real-time RT-PCR results for representative genes repressed by Dex (LIF) or by E2 only (TNFAIP3) are shown. Each data point is the average obtained from three individual U2OS cell samples ± SEM.
ICI blocks Dex repression of ER/GR-repressed genes

To determine if cross talk exists between ERα and GR, we infected U2OS-GR stable cells with the control adenovirus Ad-LacZ (Fig. 2A) or Ad-ERα (Fig. 2B–D). TNF-α activated the IL8 gene in U2OS-GR cells infected with Ad-LacZ, which was repressed by Dex (Fig. 2A). In the absence of ER, E2 and the ERs antagonist ICI had no effect on the IL8 gene with or without Dex (Fig. 2A). When the U2OS-GR cells were infected with Ad-ERα, there was repression of the IL8 gene with both E2 and Dex (Fig. 2B). The combination of E2 and Dex did not produce a greater repression compared with the individual drugs (Fig. 2B). Similar findings were found with all 13 GR/ER-repressed genes (data not shown). The GR-antagonist RU486 did not block repression by E2 of the IL8 gene (Supplemental Fig. 1). RU486 also did not antagonize Dex repression of three TNF-α–activated genes in the presence or absence of ERα (Supplemental Fig. 2). In contrast, ICI blocked the repression of the IL8 gene by E2 (Fig. 2B). Surprisingly, ICI also blocked the Dex repression of the IL8 gene (Fig. 2B) and other ER/GR-repressed genes (data not shown). ICI did not antagonize Dex activation of the IGFBP (Fig. 2C) and IRF8 (Fig. 2D) genes in U2OS-GR cells, whereas RU486 did. These results demonstrate that RU486 blocks activation, but not repression by Dex. To determine if the antagonistic action of ICI was dependent on the levels of ERα, U2OS-GR cells were infected with increasing amounts of Ad-ERα. RT-PCR (Fig. 3A) and Western blots (Fig. 3B) show that there was a dose-dependent increase in amount of ERα in the cells with increasing multiplicity of infection (MOI) of Ad-ERα. The antagonistic action of ICI on Dex repression was dependent on the levels of ERα in the cells (Fig. 3C). Antagonism by ICI was first observed at 1 MOI Ad-ERα, which produced very low levels of ERα as shown by the Western blot (Fig. 3B). At 50 MOI Ad-ERα, ICI abolished Dex repression of the IL8 gene. These results demonstrate that the ER antagonist ICI but not the GR antagonist RU486 blocks GR-mediated repression of ER/GR-repressed genes and that the antagonistic effect of ICI is dependent on the presence of ERα in the cells.

ICI does not block GR-only repressed genes

To further explore the action of ICI, we also examined its effects on an ER-only (CCL20) or a GR-only (TNFAIP2) repressed gene. U2OS-GR cells were infected with Ad-ERα or Ad-LacZ and then treated with TNF-α in the absence or presence of E2 or Dex. E2 repressed TNF-α activation of the CCL20 gene in U2OS-GR cells infected with Ad-ERα (Fig. 4A), but not Ad-LacZ (Fig. 4B).
repression of the CCL20 gene by E2 was inhibited by ICI (Fig. 4A). E2 or ICI did not have any effect on the Dex-mediated repression of the TNFAIP2 gene in the presence (Fig. 4C) or absence (Fig. 4D) of ERα. The other GR-only genes were repressed by Dex, but not by E2 or ICI (data not shown). These data indicate that cross talk between ER and GR occurs only in the subset of genes that are repressed by both ER and GR.

ICI recruits ERα to the IL8 promoter

To examine the mechanism whereby ICI inhibits GR-mediated repression of proinflammatory genes, we performed ChIP to determine the effects of ICI on ERα and GR recruitment to the IL8 promoter. U2OS-GR cells were infected with Ad-ERα for 24 h. The cells were then treated with TNF-α for 2 h in the absence or presence of Dex with or without ICI. ChIP was performed using ERα and GR Abs. The data shown are derived from real-time qPCR analysis of the IL8 gene, as detailed in Materials and Methods. The fold change was determined using the raw values from the untreated control. Each data point is the average obtained from three independent experiments. Error bars represent the mean ± SEM.

ICI blocks Dex repression of the TNF-RE and recruitment of NCOA2 by GR

A, U2OS–ERα–TNF-RE cells were treated with Dox (100 ng/ml) for 18 h, then TNF-α (5 ng/ml) was added for an additional 18 h. Luciferase activity was measured in triplicate samples. B, E2 causes a dose-dependent repression of the TNF-RE in U2OS–ERα–TNF-RE cells. U2OS–ERα–TNF-RE cells grown on 12-well plates were treated with Dox (100 ng/ml) for 18 h to induce ERα expression. The cells were then treated with TNF-α (5 ng/ml) along with increasing doses of E2 for 18 h and then assayed for luciferase activity. C, U2OS–ERα–TNF-RE cells express endogenous GR by Western blot. A rabbit anti-GR IgG was used to detect a band ~97 kDa, which represents endogenous GR. D, Dex causes a dose-dependent repression of the TNF-RE. U2OS–ERα–TNF-RE cells were treated with Dox (100 ng/ml) for 18 h to induce ERα expression. The cells were then treated with TNF-α (5 ng/ml) along with increasing doses of Dex for 18 h and then assayed for luciferase activity. E, ICI blocks the Dex repression of the TNF-RE. U2OS–ERα–TNF-RE cells were treated with increasing amount of Dox for 18 h, and then TNF-α (5 ng/ml), Dex (10 nM), and ICI (10 nM) were added to the cells for an additional 18 h. F and G, ICI treatment prevents GR recruitment of NCOA2 to the TNF-RE. U2OS–ERα–TNF-RE were treated with Dox (100 ng/ml) for 18 h and then with TNF-α (5 ng/ml) for 2 h followed by Dex (10 nM) and ICI (10 nM) for an additional 3 h. ChIP assays were performed as described in Materials and Methods using anti-GR (F) or anti-NCOA2 (G) Ab. The data are derived from real-time qPCR. The fold changes were determined by comparison of threshold cycle values obtained from samples without Ab (control) and with specific Ab. All data are expressed as mean ± SEM obtained from triplicate samples and are representative of several experiments.
ICI blocks GR recruitment of NCOA2 to the TNF-RE

To further explore the mechanism whereby ICI antagonizes GR-mediated repression, we studied the effect of ICI on the TNF-RE in the TNF-α gene, which is one of the ER/GR-repressed genes (Table I). In our previous study, we demonstrated that the TNF-RE is responsible for TNF-α activation of the TNF-α gene (21), and it mediates the repression by E2 (14). The TNF-RE consists of a composite element that has AP-1 and NF-kB sites (21), which are known to be involved in GR-mediated repression of inflammatory genes (1, 7, 8, 24). We prepared a U2OS cell line (U2OS–ERα–TNF-RE) by stably transfecting the TNF-RE upstream of the minimal thymidine kinase promoter and luciferase cDNA into the Dox-inducible U2OS–ERα cells. Stable cell lines were screened for the activation of the TNF-RE by TNF-α, the expression of endogenous GR, and the repression by E2 and Dex. As expected, ICI blocked the repression of TNF-RE (Fig. 6A) that was repressed by E2 in a dose-dependent manner (Fig. 6B). The U2OS–ERα–TNF-RE cells expressed endogenous GR by Western blot analysis (Fig. 6C). GR was functional in these cells as shown by a dose-dependent repression of the TNF-RE by Dex (Fig. 6D). These results demonstrate that these cells can be used to study the mechanism whereby ICI antagonizes repression. The ICI antagonism of the Dexam repression of the TNF-RE was dependent on the expression level of ERα because there was a dose-dependent effect by Dox (Fig. 6E). ICI had no antagonistic effect in the absence of Dox, but completely blocked the Dexam repression of the TNF-RE when the cells were treated with 100 ng/ml Dox to induce the production of ERα.

We previously demonstrated that ER and GR recruit NCOA2 to cytokine genes and that NCOA2 mediates repression (16, 17). These observations suggest that ICI might antagonize GR effects at the TNF-RE by blocking the recruitment of NCOA2. The U2OS–ERα–TNF-RE cells were treated with Dexam and ICI after ERα was induced with Dox, and then ChIP was performed using Abs to GR or NCOA2. Dexam induced recruitment of GR to the TNF-RE by ∼6-fold (Fig. 6F). Consistent with data from the IL8 promoter (Fig. 5), ICI did not block recruitment of GR to the TNF-RE (Fig. 6F). In contrast, ICI blocked the Dexam-induced recruitment of NCOA2 to the TNF-RE (Fig. 6G). These results indicate that the ICI–ERα complex antagonizes GR-mediated repression by interfering with the recruitment of NCOA2 by GR.

Discussion

Glucocorticoids are extensively used therapeutically for numerous inflammatory and autoimmune conditions because of their potent anti-inflammatory properties. Although the effects of estrogens on the reproductive system are well recognized, estrogens also can produce anti-inflammatory actions (10). We previously found that estrogens repress numerous proinflammatory genes in U2OS cells (16, 23). However, it is unclear if these proinflammatory genes are also repressed by glucocorticoids and if there is cross talk between ER and GR at these genes. To explore these possibilities, we examined the effects of Dexam and E2 in U2OS cells that express only ERα, only GR, or both ERα and GR. We found that Dexam and/or E2 repressed three classes of proinflammatory genes. Most of the proinflammatory genes were repressed by both ERα and GR. However, five genes were repressed by only ERα or GR. As expected, the ER antagonist ICI blocked E2-mediated repression. In contrast, the GR antagonist RU486 did not block Dexam-mediated repression. Instead, we unexpectedly found that ICI blocked Dexam repression of ER/GR-repressed genes. ICI did not have any effect on genes that were repressed with only GR. The effect of ICI was dependent on the expression of ERα and was limited to genes repressed by Dex, as genes activated by Dex were not affected by ICI.

There are several possible mechanisms whereby the ICI–ERα complex blocks the repression of genes in response to Dexam. It is known that GR causes repression of proinflammatory genes by binding to composite elements, such as AP-1 and NF-kB elements (7, 24). Once tethered to these transcriptional factors, GR recruits NCOA2, which mediates the repression of inflammatory genes (17). Interestingly, we found that estrogens repress proinflammatory genes through a similar mechanism involving NCOA2 (14, 16). Based on these observations, the simplest model is that the ICI–ERα complex might bind to the same element as the Dex–GR complex on the IL8 promoter and therefore compete for and prevent the binding of GR. A competition model is supported by the observation that ICI was more effective at blocking the effects of Dexam at higher levels of ERα in the cells. However, the observation that ICI did not inhibit the recruitment of GR to the IL8 gene or the TNF-RE does not support a competitive model. Another possibility is that the ICI–ERα complex directly interacts with GR to form a heterodimer on particular promoters and then blocks its repression function at these genes. However, there is no evidence that ER and GR can form a heterodimer at promoters. Finally, Dexam–GR and ICI–ERα complexes could bind to different sites on the promoter, allowing the ICI–ERα complex to interfere with the recruitment of transcriptional factors or cofactors to Dexam–GR that are essential for repression, such as NCOA2. Consistent with this model is our finding that ICI blocked the recruitment of NCOA2 by GR at the TNF-RE, which indicates that this is an important mechanism for the antagonistic effect of ICI on GR repression.

It has been demonstrated that ICI reverses the anti-inflammatory activity produced by Dexam in a rat model of carrageenan-induced pleurisy (19). The mechanism whereby ICI inhibited the anti-inflammatory effects of Dexam in this model is unclear. Our findings suggest that ICI might interfere with the Dex-induced repression of proinflammatory genes by blocking the recruitment of NCOA2. It is clear that the anti-inflammatory effects of glucocorticoids in certain tissues, particularly in the skin and lungs, have tremendous clinical value. However, prolonged reduction in cytokines by glucocorticoids could lead to adverse effects in other tissues because of their beneficial role in the growth, differentiation, and viability of cells. By understanding the mechanism of ER and GR cross talk, it might be possible to develop unique estrogens that could be used in conjunction with glucocorticoids to regulate proinflammatory genes. A combination of drugs that target both GR and ER simultaneously in some tissues might reduce the adverse effects of glucocorticoids alone.

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References


