The Arginase II Gene Is an Anti-inflammatory Target of Liver X Receptor in Macrophages*

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The liver X receptors (LXRs) are ligand-dependent transcription factors that have been implicated in lipid metabolism and inflammation. LXRs also inhibit the expression of inflammatory genes in macrophages, including inducible nitric oxide synthase (iNOS). Some of these actions are mediated through LXR antagonism of NF-κB activity. The potential for LXRs to positively regulate the expression of anti-inflammatory molecules, however, has not been explored. Here we show that the arginase II (ArgII) gene is a direct target for LXR regulation. ArgII catalyzes the conversion of l-arginine into l-ornithine and urea, leading to the synthesis of polyamines. Expression of ArgII is induced by LXR agonists in macrophage cell lines and primary murine macrophages in a receptor-dependent manner. The ArgII promoter contains a functional LXR response elements that mediates promoter induction by LXR/RXR (retinoid X receptor) in transfection assays. Since ArgII and iNOS utilize a common substrate, induction of ArgII expression has the potential to exert anti-inflammatory effects by shifting arginine metabolism toward polyamine synthesis at the expense of NO production. In support of this hypothesis, we demonstrate that forced expression of ArgII mimics the inhibitory effect of LXR activation on macrophage NO production. Furthermore, inhibition of arginase activity partially reverses the inhibitory effect of LXR agonists on NO production. These studies suggest that regulation of ArgII may contribute to the immunomodulatory effects of LXRs.

The liver X receptors (LXRs)3 are members of the superfamily of nuclear receptors activated by oxysterol ligands (1, 2).

These receptors are highly expressed in macrophages and have been shown to mediate lipid and cholesterol homeostasis and to impact the progression of atherosclerosis (3, 4). LXRs are positive regulators of a series of genes involved in lipid metabolism and cholesterol efflux, including ABCAI, ABCGI, PLTP, and apolipoprotein E (5–8). The ability of LXR ligands to inhibit the development of atherosclerosis in mice is likely to result, at least in part, from the promotion of the macrophage cholesterol efflux pathway (9, 10).

In addition to their function in metabolism, LXRs have also been implicated in inflammation and immune regulation. Ligand activation of LXRs negatively regulates macrophage inflammatory gene expression. For example, genes such as inducible nitric oxide synthase (iNOS), interleukin-6 (IL-6), COX-2, and MMP-9 are induced in macrophages exposed to bacterial components or pro-inflammatory cytokines but can be repressed by activation of LXRs (11, 12). These actions are observed in macrophages lacking either LXRα or LXRβ but not in Lxraβ−/− cells, suggesting that both receptors can mediate the anti-inflammatory activity of LXR agonists. Currently, the mechanism by which LXRs repress inflammatory gene expression is poorly understood. Previous studies have shown that LXRs inhibit the action of NF-κB on promoters of inflammatory genes encoding factors such as COX-2 and MMP-9. However, the potential for LXR to up-regulate the expression of anti-inflammatory molecules has not been explored.

Previous studies have also revealed the existence of cross-talk between Toll-like receptor (TLR) signaling and LXR (13). The TLR family of proteins recognizes pathogen-associated molecular patterns and triggers a potent antimicrobial response through NF-κB and IRF families of transcription factors (14). Viral or bacterial components such as double-stranded RNA or lipopolysaccharide (LPS) acting through TLR3 or TLR4 antagonize the expression of LXR target genes in macrophages. TLR-LXR cross-talk is mediated by the viral response transcription factor IRF3, which inhibits LXR activity through an undefined mechanism (13).

Arginase is the final enzyme of the urea cycle that converts arginine to urea. This process metabolizes excess nitrogen in the liver and prevents accumulation of the more toxic product ammonia. Two forms of arginase, known as arginase I and II (ArgI and ArgII), are encoded by different genes and are found receptor; IRF, interferon regulatory factor; LPS, lipopolysaccharide; BEC, S-2-boronooethyl-L-cysteine; WT, wild type; PGE2, prostaglandin E2.
in the cytoplasm and mitochondria, respectively (15). ArgI is mainly expressed in the liver, whereas ArgII is primarily expressed in kidney and prostate, and to a lesser extent, in immune cells such as macrophages (16). Both ArgI and ArgII convert L-arginine into L-ornithine. In extrahepatic tissues that lack the urea cycle, this process provides the substrate for synthesis of polyamines such as spermine and spermidine (17, 18).

Previous studies have suggested that both arginase isoforms are involved in inflammatory responses to LPS and pathogens (19, 20); however, their precise role in inflammatory signaling is poorly understood. One potential mechanism for arginase involvement in inflammation is through competition with iNOS for the common substrate arginine. Studies have suggested that arginase and iNOS have reciprocal activities that may shift nitrogen metabolism to either polyamine homeostasis or cytotoxic NO production, respectively (16, 21). Therefore, altering the expression of arginase and iNOS has the potential to alter inflammatory signaling within the macrophage. Here we demonstrate that the ArgII gene is a direct target for regulation by LXR in macrophages. We also provide evidence that regulation of ArgII expression contributes to the anti-inflammatory effects of LXR in this cell type.

EXPERIMENTAL PROCEDURES

Reagents and Plasmids—The specific LXR agonists GW3965 and T0901317 were provided by Tim Willson and Jon Collins (GlaxoSmithKline). RXR agonist, LG268, was provided by Rich Heyman (Ligand Pharmaceuticals). Ligands were dissolved in dimethyl sulfoxide before use in cell culture. LXR ligands were used at 1 μM, whereas RXR ligand was used at 50 nM. LPS from Salmonella typhimurium and lipoteichoic acid from Staphylococcus aureus were purchased from Sigma. Poly(I:C) was from Amersham Biosciences, and phosphorothioate-modified CpG oligonucleotide (tccatgacgttcctgacgtt) was synthesized by IDT. The arginase inhibitor S-2-boronoethyl-l-cysteine (BEC) was from Calbiochem (22, 23). The cDNA encoding full-length
murine AII was provided by Wayne Grody (UCLA). Plasmids expressing full-length murine LXRα, an AF-2 domain deletion mutant of LXRα (amino acids 1–435, LXRαAF2), full-length LXRα fused to the VP16 activation domain (LXRα-VP16), full-length LXRβ, and ArgII were subcloned into the pBabe retroviral system and packaged into retrovirus by transfection into Phoenix A cells. RAW cells were infected with retrovirus to produce stably transfected cell lines. Cells were selected using 6 μg/ml puromycin for 1 week.

Cell Culture—Macrophage cell lines RAW264.7, RAW-vec- tor, RAW-LXRα, LXRα-ΔAF2, LXRα-VP16, RAW-LXRβ, and RAW-ArgII cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. Primary peritoneal macrophages were obtained from thioglycollate-injected mice as described (24) and cultured in RPMI containing 10% fetal bovine serum (Omega Scientific). Macrophages were derived from LXRαβ+/+ and LXRαβ−/− mice (Sv129/C57bl/6 background), provided by David Mangelsdorf (University of Texas Southwestern) and were maintained on standard chow under pathogen-free conditions. Wild type mice C57/B6 control mice and IRF3−/− mice on a C57/B6 background were provided by Tadatsugu Taniguchi (University of Tokyo) and Genhong Cheng (UCLA). Macrophages were cultured in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum for 24 h. Cells were washed with phosphate-buffered saline and cultured in Dulbecco’s modified Eagle’s medium with 1% bovine serum albumin (fatty acid-free, Sigma) before stimulation with LXR ligands and inflammatory products.

Enzymatic Activities—iNOS activity was determined as accumulation of nitrite and nitrate in the culture medium by a colorimetric assay with Griess reagent as described (25). Supernatant of cultured cells was collected after 18–24 h of inflammation. Equal amounts of supernatant and Griess reagent were combined and read on a spectrophotometer at 540 nm. Values were referred to a standard curve. COX-2 activity was measured as PGE2 accumulation in the culture media with a commercial enzyme-linked immunosorbent assay kit (Amer- sham Biosciences). Arginase activity was determined spectrophotometrically as described previously by Corraliza et al. (26).

RNA and Protein Analysis—Total RNA was harvested using TRIzol reagent (Invitrogen). Real-time quantitative PCR (SYBRgreen) assays were performed using an Applied Biosys- tems 7900 sequencer detector as described previously (27). Primers are as follows: AII forward primer 5’-AGCGCCTCCCGTCTCCT-3’ and AII reverse primer 5’-GCTACAGAGTGGACGGATCTCG-3’. Values were normalized to 36B4. Protein levels were determined by Western blot using whole cell soluble extracts from macrophages. Specific polyclonal antibodies against AII, iNOS, COX-2, and β-actin were obtained from Santa Cruz Biotechnology, and the anti-IRF3 antibody was from Zymed Laboratories Inc. ABCA1 protein levels were detected using an ABCA1 specific anti-serum, kindly provided by Michael L. Fitzgerald and Mason W. Freeman (Massa- chusetts General Hospital, Boston MA) (28). Antisera to LXRα was a gift of Jae Bum Kim (Seoul National University).
Electrophoretic Mobility Shift Assay and Transient Transfections—The following oligonucleotides corresponding to an LXR binding site were used (one strand shown): wild type 5'-GATATAGTTGGC-CTCTAGTAACCAGTGCTCTT-3' and mutant 5'-GATATAGTTGG-AATCTAGTAATTAGTGCTCTT-3'. Oligonucleotides were annealed and labeled using Klenow enzyme (Promega). In vitro translation of pCMX-RXRα was generated using a TNT quick coupled transcription/translation system from Promega. Purified bacterially expressed LXRα protein and in vitro translated RXRα were combined with labeled DNA and incubated with 100 μM NaCl, 1 mM EDTA, 20 μM Hepes,

FIGURE 4. LXR/RXR heterodimers activate the ArgII promoter. 293T cells were transiently transfected with wild type and mutant LXR DR4 reporter plasmids along with LXR and RXR expression plasmids. Cells were then treated with either vehicle or GW3965 (1 μM) for 24 h. Luciferase activity was normalized to β-galactosidase activity. Experiments were performed in triplicate, and results were averaged for each point. DMSO, Me2SO.

FIGURE 5. ArgII expression in RAW cells inhibits NO production. A, RAW264.7 cells were transduced with retroviral vectors to create stable cell lines overexpressing either LXRα or ArgII. Overexpression of ArgII was confirmed by Western blot. B, cells were first treated with GW3965 (1 μM) for 18–24 h and then treated with LPS for 24 h. LPS treatments were either 10 ng/ml or 100 ng/ml. Nitrite production was detected by colorimetric assay using Griess reagent to determine iNOS activity. DMSO, Me2SO.
Regulation of ArgII Expression by LXR

Previous work has shown that LXRs down-regulate the expression of inflammatory genes in macrophages (11, 12). In an effort to identify direct LXR target genes that may contribute to these effects, we performed transcriptional profiling on RAW264.7 cells that overexpressed LXRα or LXRβ (RAW-LXRα or RAW-LXRβ) cells. These studies suggested that ArgII expression was induced in cells expressing LXRs, whereas expression of the related ArgI gene was not changed (data not shown). Real-time quantitative PCR analysis of gene expression confirmed the induction of ArgII and Abca1 expression by synthetic LXR ligand (T1317, 1 μM) and/or RXR ligand (LG268, 50 nM) in RAW264.7 cells (Fig. 1A). ArgII was up-regulated in cells treated with LXR ligand, and an additive effect was observed when both LXR and RXR ligands were present. Interestingly, induction of ArgII was more prominent in cells expressing LXRα as compared with those expressing LXRβ. LXR agonist was unable to induce expression of ArgII in RAW264.7 cells expressing a truncated form of LXRα lacking the AF2 activation domain (LXRα-ΔAF2). On the other hand, expression of a constitutively active VP16-LXRα receptor induced expression of ArgII even in the absence of synthetic LXR agonist (GW3965, Fig. 1B). Induction of ArgII expression was observed as early as 6 h after ligand addition, suggestive of a primary effect of this nuclear receptor (Fig. 1C). To confirm that regulation of ArgII by LXR also occurred in primary macrophages, we treated thioglycollate-elicited peritoneal macrophages with LXR and RXR ligands. As shown in Fig. 1D, LXR ligands also increased ArgII expression in primary macrophages, although the magnitude of this response was smaller than in RAW264.7 cells. We have not observed significant changes in ArgII expression in response to LXR ligands in limited studies of human monocyte-derived macrophages (data not shown).

Recent studies have demonstrated that expression of ArgI and ArgII is regulated by Th2 cytokines (IL-4 and IL-10) and endotoxin (LPS) in macrophages (29–31). Up-regulation of arginase activity under these circumstances is associated with down-regulation of the inflammatory response. We tested the effect of a combination of LPS and LXR agonist on ArgII expression in macrophages obtained from WT and Lxraβ−/− mice. As shown in Fig. 2, the addition of LXR and RXR agonist induced ArgII protein expression in WT macrophages, whereas this induction was not observed in Lxraβ−/− macrophages. Expression of ABCA1 is shown as a control for LXR activation. LPS stimulation promoted ArgII expression in both WT and double knockout macrophages. Interestingly, stimulation with LPS in the presence of LXR/RXR agonists further induced ArgII expression in WT macrophages but not in Lxraβ−/− macrophages. Together, these results indicate that the ArgII gene is responsive to the LXR pathway in macrophages.

Next, we analyzed the DNA sequence of the ArgII promoter for binding sites for LXR/RXR heterodimers. A potential DR4 sequence (direct repeat with a four-nucleotide spacer) was identified 942 bp upstream of the transcription start site in the ArgII promoter (Fig. 3A). We tested the ability of LXRα and RXRα protein to bind to an oligonucleotide containing this element using electromobility shift assays. We observed LXR/RXR heterodimer binding to the ArgII LXR but not to a mutated

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5% glycerol, 0.01% Nonidet P-40, and 2 μg/μl poly(dI:dC). Protein-DNA complexes were electrophoresed on a polyacrylamide gel and visualized by autoradiography. Competition assays using unlabeled wild type and mutant oligonucleotides were performed and used in 20-, 100-, or 200-fold excess. Transient transfections of 293T cells were performed in triplicate using calcium phosphate. 5% glycerol, 0.01% Nonidet P-40, and 2

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FIGURE 6. ArgII inhibits nitrite production but does not affect PGE2 production. A, stable RAW-vector and RAW-ArgII cell lines were stimulated with LPS at concentrations of 1, 10, or 100 ng/ml. Nitrite production was detected by colorimetric assay using Griess reagent. PGE2 accumulation was measured by enzyme-linked immunosorbent assay to determine COX-2 activity. B, iNOS, COX-2, ArgII, and β-actin protein levels were measured by Western blot in RAW-vector or RAW-ArgII stimulated by LPS at concentrations of 1, 10, or 100 ng/ml. ctrl, control.
Regulation of ArgII Expression by LXR

Expression of ArgII in RAW cells was sufficient to reduce LPS-stimulated nitrite accumulation to a level similar to that observed in LXR agonist-treated cells. These data suggest that expression of ArgII shifts nitrogen use away from the iNOS pathway and demonstrate that ArgII expression mimics the effects of LXR activation on macrophage NO production.

To rule out the possibility that expression of ArgII in RAW cells led to a global or nonspecific inhibition of inflammatory responses, we compared the effect of ArgII expression on iNOS and COX-2 activity. Similar to the previous results, RAW-ArgII cells produced greater amounts of nitrite in the presence of LPS, indicating that iNOS activity was induced (Fig. 5B). Treating these cells with LXR ligand (GW3965) significantly inhibited iNOS activity. As expected, the effect of ligand was more pronounced in RAW-LXRα cells.

Previous work has shown that LXR activation inhibits iNOS expression and NO production in macrophages. Both iNOS and ArgII utilize arginine to synthesize either nitric oxide or polyamines, respectively. Given that ArgII and iNOS utilize a common substrate for which they can compete, we investigated whether induction of ArgII by LXR in macrophages might contribute to suppression of NO production. First, we performed gain of function assays in which RAW264.7 cells were engineered to overexpress either LXRα or ArgII (RAW-LXRα or RAW-ArgII, Fig. 5A). These two cell lines and a control RAW-vector cell line were treated with LPS (10 ng/ml or 100 ng/ml) in the presence or absence of GW3965 (1 μM). The control and RAW-LXRα cells produced greater amounts of nitrite in the presence of LPS, indicating that iNOS activity was induced (Fig. 5B). Treating these cells with LXR ligand (GW3965) significantly inhibited iNOS activity. As expected, the effect of ligand was more pronounced in RAW-LXRα cells.

FIGURE 7. ArgII inhibits nitrite production in response to multiple inflammatory stimuli. A, RAW cells expressing a control vector or ArgII were treated with ligands for different TLRs for 24 h. Nitrite production was detected by colorimetric assay using Griess reagent. B, PGE2 production was measured by enzyme-linked immunosorbent assay to determine COX-2 activity. LTA, lipoteichoic acid; PGN, prostaglandin.

version of this element (Fig. 3B, left). Furthermore, the addition of LXRα antisera inhibited binding and resulted in formation of a supershifted complex. Finally, complex formation was competed by an excess of unlabeled ArgII LXRE but not the mutated version (Fig. 3B, right). These data indicate that LXR/RXR heterodimers bind directly to the ArgII promoter.

We performed transient transfection assays to test the functional importance of this element for ArgII promoter regulation. Luciferase reporter plasmids were constructed containing ArgII promoter sequences from −942 bp to −12 bp (that contain the LXRE) or from −916 bp to −12 bp (in which the LXRE has been deleted at the 5’ end). These constructs were transiently transfected into 293T cells along with expression vectors for LXRα and RXRα. Following transfection, cells were treated with vehicle or LXR agonist GW3965 as indicated. As expected, the empty luciferase vector was not affected by LXR expression or ligand (not shown), whereas a reporter driven by multiple consensus LXREs was highly induced (Fig. 4). The −942-bp ArgII promoter construct containing the LXRE was transactivated by LXRα/RXRα in a ligand-dependent manner (Fig. 4). By contrast, the −916-bp construct lacking the LXRE showed minimal responsiveness to transfection of LXR. These data indicate that the ArgII promoter is a direct target for regulation by LXR/RXR heterodimers.
To evaluate the contribution of arginase activity to the anti-inflammatory action of LXR ligands, we tested the effect of a specific arginase inhibitor in macrophages. Primary macrophages isolated from WT and LXR<sup>−/−</sup> mice were cultured for 48 h in the presence of LXR/RXR ligands (1 μM GW3965 and 100 nM LG268 (GW+LG)) or vehicle control. Ligands were washed with phosphate-buffered saline, and cells were restimulated with LPS (100 ng/ml) with or without the arginase inhibitor BEC (10 μM) in the absence of LXR/RXR ligands. Nitrite accumulation in the culture media was measured after 36 h using the Griess reagent.

Next we addressed whether the ability of ArgII to inhibit NO production in macrophages was dependent on the nature of the inflammatory stimulus. In addition to LPS, ligands for other TLRs are potent inducers of NO production in macrophages (32). We examined NO production in control and RAW-ArgII cells in response to treatment with ligands for TLR2 (lipopolysaccharide and PGN), TLR3 (poly(I:C)), TLR9 (CpG), or TLR4 (LPS). Control RAW cells produced greater amounts of nitrite as compared with RAW-ArgII cells in response to every stimulus (Fig. 7A). By contrast, production of PGE<sub>2</sub> in response to TLR ligands was equivalent in RAW-vector and RAW-ArgII cells (Fig. 7B). Collectively, the data presented above strongly suggest that the decrease in NO production in RAW-ArgII cells is not due to decreased iNOS protein expression or to inhibition of a specific TLR signaling pathway but rather to competition for limiting substrate and a shift in nitrogen metabolism. Furthermore, these studies suggest that LXR-dependent arginase activity constitutes an anti-inflammatory mechanism in macrophages that functions to limit TLR-stimulated inflammatory signaling.

To evaluate the contribution of arginase activity to the anti-inflammatory action of LXR ligands, we tested the effect of a specific arginase inhibitor in macrophages. Primary macrophages isolated from WT and Lxrαβ<sup>−/−</sup> mice were cultured in the presence of LXR/RXR agonists or vehicle control for 48 h. Cells were then challenged with LPS in the presence of the arginase inhibitor BEC (10 μM) or vehicle control, and NO production was measured after 36 h. As shown in Fig. 8, inhibition of iNOS activity was observed in WT cells stimulated with LXR ligands (black bars) but not in Lxrαβ<sup>−/−</sup> macrophages as expected (11, 13). Interestingly, inhibition of arginase activity with BEC compound partially restored NO production in WT cells but not in Lxrαβ<sup>−/−</sup> macrophages. Similar results were obtained with RAW-LXR<sup>+</sup>-expressing cells (data not shown). These results indicate that ArgII activity contributes to the ability of LXR agonists to inhibit iNOS activity.

Finally, we examined the impact of IRF3-LXR cross-talk on the regulation of macrophage AII expression. Previous studies have shown that activation of IRF3 by viral or bacterial components inhibits LXR-dependent gene expression and cholesterol efflux in macrophages (13). Thioglycollate-elicited peritoneal macrophages from wild type and IRF3 knock-out mice were treated with LXR ligands GW3965 and T1317 (1 μM). Expression of ArgII and Abca1 was analyzed by real-time PCR. As expected, LXR and RXR agonists induced the expression of both ArgII and ABCA1 in peritoneal macrophages (Fig. 9A). Consistent with the known inhibitory effect of IRF3 on LXR, expression of both genes was greater in cells lacking IRF3. Furthermore, levels of ArgII and ABCA1 protein expression correlated closely with levels of mRNA in these cells (Fig. 9B). This effect was particularly prominent for ArgII, the expression of which was prominently up-regulated in the absence of IRF3, indicating that LXR activity on the ArgII promoter is tightly controlled by basal IRF3 activity in activated (thioglycollate-elicited) macrophages as compared with other known LXR targets. These results indicate that LXR-IRF3 cross-talk exerts complex effects on inflammatory gene expression and is an important determinant of macrophage ArgII expression.

**DISCUSSION**

ArgII activity in extrahepatic tissues contributes to the depletion of L-arginine and accumulation of L-ornithine. Interestingly, O’Brien and colleagues (18) found that plasma levels of L-ornithine are moderately increased in ArgII<sup>−/−</sup> mice. Other studies have outlined the role of ArgII activity in the metabolism of nitrogen into polyamines in immune cells (15). This process is important for cellular proliferation and wound healing, as well as substrate competition with other arginine-dependent enzymes, such as iNOS (33, 34). In resting macrophages, there is little expression of ArgII, and arginine utilization is minimal. Under inflammatory conditions such as microbial infections, arginine is actively shuttled into the macrophage, whereas iNOS and ArgII expression are induced (35). iNOS activity is an important cytotoxic mechanism to control pathogen growth (36). Arginase activity, on the other hand, may function to prevent excessive immune activation and promote the resolution of inflammation and wound healing (37). Since both ArgII and iNOS utilize arginine as a common substrate, induction of ArgII would be expected to inhibit NO production. Defective regulation of this response during inflammation might cause deleterious effects. For example, uncontrolled NO production could lead to excessive vasodilation and hemodynamic instability. Deficient NO production, however, could compromise anti-microbial responses (38). A proper balance between macrophage inflammatory activation and resolution is crucial for effective immune responses.

Previous studies have shown that LXR heterodimers play important roles in macrophage functions, including cholesterol metabolism, inflammation, and immune responses (10, 39). LXR agonists repress the expression of inflammatory factors such
as iNOS, COX-2, IL-6, and MMP-9 (11, 12). However, the mechanisms by which LXRs negatively regulate inflammatory pathways are poorly understood. For example, it is not known whether genes with anti-inflammatory activity may be direct targets for LXR regulation. Through transcriptional profiling, we have identified ArgII as a potential anti-inflammatory gene that is regulated by LXR. Gene expression and promoter analysis confirmed that ArgII is a direct target of LXR/RXR heterodimers. Activation of LXR up-regulates ArgII expression in both primary cells and macrophage cell lines. Furthermore, the ArgII promoter contains a functional LXRE as revealed by electromobility shift and transient transfection assays. Finally, we have presented data that support the hypothesis that LXRs may exert its anti-inflammatory and immunomodulatory actions, at least in part, through induction of ArgII expression.

We explored the potential consequence of LXR activation of ArgII by generating stable macrophage cell lines that constitutively express either ArgII or LXRα. Cells expressing either protein exhibited decreased NO production after TLR ligand treatment. Importantly, expression of ArgII did not alter expression of other inflammatory mediators such as the COX-2 product PGE₂, pointing to a selective effect on inflammatory pathways. These results are consistent with previous studies in macrophages showing that ligand activation of LXR inhibits NO production and that Lxra−/− macrophages produce higher levels of NO as compared with WT cells (11, 39). We also showed that inhibition of arginase activity with the specific inhibitor BEC significantly reduced the anti-inflammatory effect of LXR agonists in macrophages. Importantly, these effects were not observed in macrophages obtained from Lxra−/− mice. These results suggest that, in addition to inhibiting the action of pro-inflammatory genes through transcriptional repressive mechanisms, LXRs also participate in nitrogen metabolism in macrophages by controlling excess NO production during immune responses.

We also showed that the viral response transcription factor IRF3 plays an important role in the regulation of ArgII

FIGURE 9. LXR activation up-regulates ArgII expression in IRF3−/− macrophages. A, thioglycollate-elicited wild type and IRF3−/− mouse peritoneal macrophages were treated with vehicle or GW3965 or T1317 (1 μM) and LG268 (50 nM). GW+L, GW3965 and LG268; T+L, T1317 and LG268. Quantitative real-time PCR was performed to detect mRNA levels of ArgII and ABCA1. DMSO, Me2SO. B, protein levels of ArgII, ABCA1, and IRF3 were detected in total protein from thioglycollate-elicited wild type and IRF3−/− mouse peritoneal macrophages. Cells were treated with vehicle or GW3965 (GW) (1 μM) and LG268 (50 nM) for 24, 48, or 72 h. ctrl, control; GW/LG, GW3965 and LG268; ns, not significant.
mRNA and protein expression in macrophages. Previous studies have shown that IRF3 is located in the cytoplasm in resting macrophages and requires phosphorylation, dimerization, and translocation to the nucleus to activate antiviral gene expression (40, 41). Activated IRF3 participates with other transcription factors, including IRF7, ATF2-c-Jun, and NF-κB, to assemble a multiprotein complex called “enhancerosome” that is important for up-regulation of inflammatory gene expression (42). Recent studies by Glass and colleagues (43) have uncovered a molecular mechanism for glucocorticoid-selective immune suppression in which glucocorticoid receptor inhibits IRF3 assembly to its specific target sequences. In addition to its role as co-activator of antiviral genes, activation of IRF3 by viral or bacterial components inhibits LXR activity on the promoters of its target genes, suggesting an additional role for IRF3 in the control of metabolic pathways (13). Here we demonstrate that thiglycollate-elicited macrophages from IRf3−/− mice exhibit increased basal expression of ArgII and dramatically enhanced response to LXR agonists. A potential explanation for this result is that the absence of IRF3 derepresses LXR and allows its target genes to be transcribed at an increased level. Intriguingly, the effect of IRF3 on ArgII is particularly prominent among LXR target genes examined. Our data suggest that fully differentiated macrophages (thioglycollate-elicited, which mature from macrophage-colony-stimulating factor-differentiated), which are prepared to act in inflammatory situations, contain significant amounts of IRF3.

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