Inhibition of Aldose Reductase Prevents Lipopolysaccharide-Induced Inflammatory Response in Human Lens Epithelial Cells

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PURPOSE. Bacterial infections are one of the major causes of human eye disease. Because the bacterial endotoxin lipopolysaccharide (LPS) is known to cause cytotoxicity through oxidative stress and an earlier study has shown that aldose reductase (AR) mediates oxidative stress signals, the purpose of this study was to investigate the anti-inflammatory effects of AR inhibition on LPS-induced activation of NF-κB–dependent signals in human lens epithelial cells (HLECs).

METHODS. Growth-arrested HLECs were cultured without or with AR inhibitors or transfected with an AR small interfering (si)RNA. Subsequently, the cells were stimulated with LPS (1-10 μg/mL) for 24 hours. The cell viability was assessed by cell counts and MTT assay, and apoptosis was measured by nucleosomal degradation. Electrophoretic mobility gel shift assays were performed to determine the activation of NF-κB and AP1. The levels of nitric oxide, MMP-2, MMP-9, Cox-2, and TNF-α were measured by using specific ELISA kits. Western blot analysis was performed to determine the cleavage of poly(ADP-ribose) polymerase (PARP) and the activation of PKC and mitogen-activated protein kinase (MAPK).

RESULTS. Bacterial LPS caused apoptosis of HLECs. Inhibition of AR by two structurally unrelated inhibitors, sorbinil and tolrestat, or ablation by AR siRNA prevented the LPS-induced apoptosis, activation of caspase-3 and cleavage of PARP protein. Inhibition of AR in HLECs also prevented the LPS-induced activation of redox-sensitive transcription factors such as NF-κB and AP1 and their downstream signals that lead to expression of Cox-2, MMP-2, MMP-9, and TNF-α proteins. In addition, inhibition of AR prevented LPS-induced activation of protein kinases upstream to NF-κB activation such as PKC and MAPK in HLECs.

CONCLUSIONS. The results indicate that AR mediates the bacterial endotoxin signaling that could damage HLECs by regulating the signals that activate the redox-sensitive transcription factor NF-κB and cause inflammation. Thus, inhibition of AR could be a therapeutic target for Gram-negative bacterial infection-induced visual complications. (Invest Ophthalmol Vis Sci. 2006; 47:5395–5403) DOI:10.1167/iovs.06-0469

Gram-negative bacteria can initiate fulminating and highly destructive infections in human eyes that may result in decreased visual acuity or blindness.1–5 The increase in the widespread use of contact lenses also has increased the incidence of bacterial infections of ocular tissues.4,5 Although bacteria are capable of producing a large number of potential virulent factors, lipopolysaccharide (LPS), a major component of the Gram-negative bacterial outer membrane, is a principal mediator of host innate immune responses.6–7 LPS causes many of the pathologic effects by stimulating host cells to synthesize large quantities of bioactive inflammatory mediators such as nitric oxide, prostaglandins, and cytokines, such as TNF-α, IL-1, IL-6, and IFN-γ.8–10 At low concentrations, the cytokines trigger a variety of host responses that eliminate invading bacteria; however, at overwhelming concentrations, they can induce significant morbidity due to the increase in oxidative stress and the reactive oxygen species (ROS)–mediated increase in NF-κB that could cause an inflammatory response leading to septic shock marked by high fever and severe inflammatory reactions.9,11,12 The eye can also be exposed to various cytokines and chemokines generated locally as well as peripherally that are released as a result of infection.13,14 Particularly, the lens can also be exposed to various inflammatory mediators that on bacterial infections are present in the aqueous humor.15–17 In fact, we and others have shown that incubation of HLECs with cytokines such as TNF-α increases the activation of NF-κB and cause cytoxicity.18,19 NF-κB is a redox sensitive transcription factor comprising p65 (RelA) and p50 subunits.20 Under normal conditions, it is present in the cytoplasm and is sequestered by the inhibitory protein IκB, which renders it inactive. Various stress conditions activate NF-κB by initiating phosphorylation and the dissociation of IκB-α, permitting the translocation of the active NF-κB dimer to the nucleus where it can bind to its cognitive elements in the target genes involved in cytoxicity.20,21 Recently, we have shown that aldose reductase (AR) mediates cytokine-induced activation of NF-κB in various cells.19,22–25 AR, a member of the aldo-keto reductase superfamily, is a rate-limiting enzyme of the polyol pathway of glucose metabolism that converts glucose to sorbitol in the presence of NADPH (reduced nicotinamide adenine dinucleotide phosphate). AR is expressed in all the ocular tissues, and its activity in all the human tissues studied has been shown to increase during hyperglycemia and other oxidative stress response diseases.26,27 Therefore, AR has been implicated in the pathophysiology of diabetic complications. We have recently shown that inhibition of AR prevents oxidative stress–induced and cytokine or hyperglycemia-initiated cell signals leading to proliferation of vascular smooth muscle cells and apoptosis of vascular endothelial cells and HLECs.19,22–24 We have further shown that AR inhibition or ablation prevents the activation of PKC/NF-κB signals that cause cytoxicity.25,26 Although there...
has been significant progress in understanding LPS-induced oxidative stress signals in monocytes, macrophages, and leukocytes, little is currently known about LPS responses in non-immune cells such as lens epithelial cells. Therefore, in the present study, we have investigated the effect of AR inhibition/ablation on LPS-induced cytotoxic signals in HLECs, leading to activation of NF-κB and inflammation. Our results show that inhibition or ablation of AR prevents LPS-induced activation of NF-κB and production of inflammatory markers such as nitric oxide, PGE2, Cox-2, TNF-α, MMP-2, and MMP-9.

**Materials and Methods**

Eagle’s minimum essential medium (MEM), phosphate-buffered saline (PBS), gentamicin solution, trypsin, and fetal bovine serum (FBS) were purchased from Invitrogen-Gibco (Grand Island, NY); the nuclear dye Hoechst 33342 from Invitrogen-Molecular Probes (Eugene, OR); antibodies against rabbit anti-human PARP from Santa Cruz Biotechnology (Santa Cruz, CA); and antibodies against phospho-p38 and JNK from Cell Signaling Inc. (Beverly, MA). Sorbinil and tolrestat were the gifts of Pfizer (New York, NY) and American Home Products, (Wyeth, Philadelphia, PA) respectively. Mouse anti-rabbit glycerinaldehyde phosphate dehydrogenase (GAPDH) antibodies was from Research Diagnostics, Inc., (Flanders, NJ); transfection reagent (LipofectAMINE Plus) and reduced-serum cell-culturing medium (OptiMEM) were obtained from Invitrogen-Life Technologies (Gaithersburg, MD); consensus oligonucleotides for NF-κB (5’-AGTTGAGGGACCTTCCAGG-3’) and AP1 (5’-CGCTTGAAGTGCAGCCGGAA-3’) transcription factors from Promega Corp. (Madison, WI); nitrite/nitrate, Cox-2 and PGE2 assay kits from Cayman Chemical Inc. (Ann Arbor, MI); and a human TNF-α ELISA kit from BD Biosciences (San Diego, CA). 3-(4,5-dimethylthiazol-2-y)-2,5-diphenyl tetrazolium bromide (MTT) and other reagents used in the electrophoretic mobility gel shift assay (EMSA) and Western blot analysis were obtained from Sigma-Aldrich (St. Louis, MO). All other reagents used were of analytical grade.

**Cell Culture**

The HLEC line B3 was obtained from American Type Culture Collection (catalog no., CRL-11421). This cell line (transfected with Ad12-SV-40 virus) was derived from human lens obtained within 24 hours from 5- to 1-month-old patient of retinopathy and characterized by their ability to synthesize β- and γ-crystallins as monitored by immunoblot analysis. The cells were grown in MEM with 20% fetal bovine serum, with or without indicated ARIs (10 µM) for another 24 hours. (A) The cells were growth arrested in 0.5% serum, little is currently known about LPS responses in non-immune cells such as lens epithelial cells. Therefore, in the present study, we have investigated the effect of AR inhibition/ablation on LPS-induced cytotoxic signals in HLECs, leading to activation of NF-κB and inflammation. Our results show that inhibition or ablation of AR prevents LPS-induced activation of NF-κB and production of inflammatory markers such as nitric oxide, PGE2, Cox-2, TNF-α, MMP-2, and MMP-9.

**RNA Interference Ablation of AR in HLECs**

The ablation of AR mRNA was performed essentially as described earlier. Briefly, HLECs were incubated with serum-free medium containing AR-small interfering (si)RNA (AAGCGATTGGAGGACTTCA) or scrambled siRNA (AACAGCGCTGGAATGACTATA; control) to a final concentration of 100 nM with transfection reagent (RNAiFect; Qiagen, Chatsworth, CA). After 15 minutes of incubation at 25°C, the medium was aspirated and replaced with fresh MEM containing 10% serum. The cells were cultured for 48 hours at 37°C, and AR expression was determined by measuring AR protein by Western blot analysis using anti-AR antibodies and by measuring AR activity in the total cell lysates.25

**Cell Viability Assays**

The cells were grown to confluence in MEM, harvested by trypsinization, and plated at 5000 cells/well in a 96-well plate. The cells were growth arrested for 24 hours by replacing fresh medium containing 0.5% FBS and 50 µg/mL of gentamicin. The low serum levels were maintained during growth arrest to prevent the slow apoptosis that accompanies complete serum deprivation in the cell lines. After 24 hours, LPS (1-10 µg/mL) was added to the medium, and the cells were incubated for an additional 24 hours. Cell viability was detected by the MTT assay, in which the MTT is converted into formazan granules in the presence of molecular oxygen. After the incubation, 10 µL of 5 mg/mL MTT was added to each well of the 96-well plates and incubated at 37°C for 2 hours. The formazan granules obtained were dissolved in 100% dimethyl sulfoxide (DMSO), and absorbance at 562 nm was detected with a 96-well ELISA reader. The cell viability was also calculated by cell counting with a hemocytometer. Briefly, the cells were harvested by trypsinization, washed with PBS, and mixed with an equal amount of trypan blue dye. The percentage of the cells excluding trypan blue was

![Image](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933233/ on 06/24/2017)
Determination of Apoptosis

Apoptosis was evaluated by using a cell death-detection ELISA kit (Roche Inc., Indianapolis, IN) that measures cytoplasmic DNA-histone complexes generated during apoptotic DNA fragmentation. Cell death detection was performed according to the manufacturer’s instructions and monitored spectrophotometrically at 405 nm. LPS (5 μg/mL)-induced apoptosis was also analyzed by using nuclear staining with Hoechst 33342, a DNA-binding fluorescent dye. Briefly, after various treatments, the HLECs were incubated with 5 μg/mL of Hoechst 33342 for 30 minutes at 4°C. The morphologic characteristics of apoptotic cells were identified with the aid of a fluorescence microscope (Eclipse E800; Nikon, Tokyo, Japan), with excitation at 405 nm. The cells with fragmented and/or condensed nuclei were classified as apoptotic cells.

Caspase-3 Activity

The activity of caspase-3 was measured by using the specific caspase-3 substrate Z-DEVD-AFC (CBZ-Asp-Glu-Val-Asp-AFC) which was incubated with cell lysate and the fluorescence (excitation, 480 nm; emission 505 nm) released by the cleavage of substrate was measured by using a 96-well fluorescence plate reader. The in situ activation of caspase-3 was also measured by using cleavage of poly-(ADP-ribose)-polymerase (PARP) by activated caspase-3 in LPS-induced cells in the absence and presence of AR inhibitors (ARIs), by performing Western blot analysis.

Cell Cycle Analysis

The cells were grown to confluence in MEM, harvested by trypsinization, and plated at 100,000 cells/well in six-well plates. The cells were grown arrest for 24 hours by replacing fresh medium containing 0.5% FBS and 50 μg/mL of gentamicin. After 24 hours, LPS (5 μg/mL) without or with ARIs (10 μM) was added to the medium, and the cells were incubated for an additional 24 hours. Cells were harvested, washed, and fixed in 70% chilled ethanol for 2 hours followed by staining with propidium iodide (50 μg/mL) for 20 minutes. Cell cycle analysis was performed by flow cytometry (FACSCanto; BD Biosciences).

EMSAs for NF-κB and AP1

The cells were pretreated with ARIs for 24 hours and then with LPS (1 μg/mL) for 2 hours at 37°C. The cytosolic as well as nuclear extracts were prepared and electrophoretic mobility gel shift assays (EMSAs) were performed as described earlier.22 Briefly, nuclear extracts prepared from various control and treated cells were incubated with oligonucleotides for NF-κB or AP1 for 15 minutes at 37°C, and the DNA-protein complex formed was resolved on 6.5% native polyacrylamide gels. The specificity of the binding was also examined by competition with excess of unlabeled oligonucleotide. Supershift assays were also performed to determine the specificity of NF-κB binding to its specific consensus sequence by using specific antibodies to p65.

Prostaglandin E2 Assay

HLECs were plated in 6-well plates at a density of 1 × 10⁵ cells/well. After 24 hours, the medium was replaced with serum-free medium with or without ARIs (10 μM). The growth-arrested cells were treated with 1 μg/mL of LPS for another 24 hours. The medium was collected from each well and analyzed for PGE2 by using an ELISA kit according to the manufacturer’s instructions (Cayman Chemical Co., Inc.). Briefly, 50 μL of diluted standard/sample was pipetted into a 96-well plate precoated with goat polyclonal anti-mouse IgG. Aliquots (50 μL) of a PGE2 monoclonal antibody and PGE2 acetylcholine esterase (AChE) conjugate. (PGE2 tracer) were added to each well and allowed to incubate at 4°C for 24 hours. After incubation, the wells were washed five times with wash buffer containing 0.05% Tween 20, followed by the addition of 200 μL of Ellman’s reagent containing acetylthiocholine and 5,5-dithio-bis(2-nitrobenzoic acid). Wells were read after 60 minutes at 412 nm with an ELISA reader.

Cox Activity Assay

After various treatments, the HLECs were harvested and homogenized in cold buffer containing 0.1 M Tris-HCl (pH 7.8) and 1 mM EDTA, and the activity was measured in a 96-well plate, according to the manufacturer’s instructions (Cayman Chemical Co., Inc.). Briefly, 10 μL of standard and sample was incubated in the presence of arachidonic acid and a colorimetric substrate, N, N, N, N-tetramethyl-phenylenediamine (TMPD), in a total reaction volume of 210 L. The Cox-2 peroxidase activity was measured colorimetrically by monitoring the appearance of oxidized TMPD at 590 nm with an ELISA reader.

Matrix Metalloproteinase Assays

Levels of MMP-9 and -2 were measured in the culture medium by using an MMP-2 and -9 activity assay system (Biotrak; GE Healthcare, Piscataway, NJ). Parallel measurements of standards and samples were performed by applying them to an antibody precoated microplate, and the procedure was followed as indicated by the manufacturer’s protocol. The absorbance of the samples was determined at 450 nm using a
microplate reader. MMP-9 and -2 concentrations were determined from the best line curve drawn with the absorbance of standards versus their concentrations.

**Western Blot Analysis**

Western blot analyses were performed with antibodies against PARP and phospho-P38, P38, phospho-JNK, JNK, and AR. Transfected and untransfected HLECs were either untreated or pretreated with tolrestat or sorbinil for 24 hours and then stimulated with 1 μg/mL of LPS for different durations of exposure. Equal amounts of protein were subjected to Western blot analysis using different antibodies, and the antigen-antibody complexes were detected by enhanced chemiluminescence (Pierce, Rockford, IL).

**Determination of PKC Activity**

The membrane-bound PKC activity was determined as described earlier using a total PKC assay system (SignaTect; Promega). Briefly, aliquots of the reaction mixture (25 mM Tris·HCl [pH 7.5] 1.6 mg/mL phosphatidylserine, 0.16 mg/mL diacylglycerol, and 50 mM MgCl2) were mixed with [γ-32P]-ATP (3000 Ci/mmol, 10 μCi/μL) and incubated at 30°C for 10 minutes. The extent of phosphorylation was detected by measuring the radioactivity retained on the paper.

**Statistical Analysis**

Data are presented as the mean ± SEM, and the probabilities were determined with the unpaired Student’s t-test (P < 0.05 considered as statistically significant).

**RESULTS**

**Effect of AR Inhibition on LPS-Induced HLEC Growth**

To investigate the role of AR in LPS-induced cytotoxic signals leading to HLEC death, we determined the effect of two structurally unrelated ARIs, sorbinil and tolrestat, on cell growth. Treatment of HLECs with various concentrations of LPS ranging from 1 to 10 μg/mL for 24 hours significantly diminished HLEC growth, determined by MTT assay (Fig. 1A). The decrease in growth was reversed by incubation of the HLECs with 10 μM of the ARIs sorbinil or tolrestat. However, sorbinil or tolrestat in the absence of LPS had no effect on the growth, indicating that AR inhibition by itself does not affect HLEC growth. Similar results were obtained when the cell growth was estimated by counting cell number (data not shown). To rule out nonspecific effects of pharmacological inhibitors, we ablated the AR message by transient transfection of HLECs with AR siRNA or control (scrambled) siRNA and investigated the effect of AR ablation of LPS-induced cell growth. The transfection of HLECs with AR siRNA but not control siRNA caused >95% ablation of AR protein (Fig. 1B, inset) and no detectable amount of AR activity (measured using gliceraldehyde as substrate) was observed in siRNA-transfected cells (data not shown). Consistent with the pharmacological data, transfection with AR siRNA but not control siRNA oligonucleotides restored LPS-induced loss of cell growth (Fig. 1B). Together, these observations suggest that the inhibition of AR and the reaction product(s) of AR catalysis may be involved in the LPS-induced signaling.

**Effect of AR Inhibition on LPS-Induced HLEC Apoptosis**

To examine the nature of the LPS-induced decrease in HLEC growth, we measured free histones released on nucleosomal degradation, which is a hallmark of apoptotic cell death. LPS (5 μg/mL) caused degradation of nucleosomal histones, suggesting that LPS causes apoptosis of HLECs. Preincubation of the cells with either sorbinil or tolrestat and ablation of AR by siRNA prevented these changes (Figs. 1C, 1D). Under similar conditions, neither AR inhibition nor ablation alone caused apoptosis of HLECs. For additional confirmation of apoptosis, we stained HLECs with Hoechst 33342 and propidium iodide, which can detect apoptotic cells with morphologic changes leading to nuclear fragmentation and condensation, whereas, preincubation with sorbinil or tolrestat prevented the cells from undergoing apoptosis induced by LPS. Under identical conditions, no significant necrotic cells were found either with LPS or LPS+ARIs (Fig. 2B).

**Effect of AR Inhibition on LPS-Induced Caspase-3 Activity**

Apoptosis was further confirmed by demonstrating that incubation of HLECs with 5 μg/mL LPS caused increased caspase-3 activity (Fig. 3).

**FIGURE 3.** Effect of AR inhibition and ablation on LPS-induced activation of caspase-3 in HLECs. Cells were growth-arrested in 0.5% serum with (A) or without (C) ARIs (10 μM). (B) Cells were transfected with control or AR siRNA oligonucleotides, growth arrested in 0.1% serum followed by incubation with 5 μg/mL of LPS for 24 hours. The caspase-3 activity was determined (A, C) in vitro ELISA kit and (B) in situ PARP cleavage by Western blot using antibodies against human PARP. Data are expressed as the mean ± SEM (n = 4). *P < 0.001 compared with LPS-treated cells; #P < 0.001 control cells.

activation of caspase-3. As shown in Figures 3A and 3B, LPS-caused a near twofold induction of caspase-3 activity, measured in vitro by using caspase-3–specific synthetic peptide and inhibition or ablation of AR significantly (>85%) prevented LPS-induced caspase-3 activity. For additional documentation we also investigated in situ activation of caspase-3, which cleaves 116-kDa PARP substrate to an 85-kDa form. As shown in Figure 4C, LPS caused PARP cleavage and inhibition of AR prevented it, suggesting that AR inhibition prevents LPS-induced caspase-3 activation and thus prevents apoptosis.

**Effect of AR Inhibition on LPS-Induced Cell Cycle Arrest in HLECs**

Because lipid aldehyde detoxification is known to prevent oxidative stress-induced toxicity in corneal epithelial cells by prolonging the cell cycle and LPS toxicity is mediated by oxidative stress, we next examined the effect of AR inhibition on the LPS-induced cell cycle. Treatment of HLECs with LPS caused a significant decrease in the cells entering the synthesis (S)-phase of the cell cycle (Fig. 4), and cells accumulated in the G2/M phase, suggesting that the cell cycle arrests at the S-
phase. Inhibition of AR in the presence of LPS causes accumulation of cells in the S- and G2/M phases, suggesting that AR inhibition prevents the LPS-induced arrest of cell cycle.

**Effect of AR Inhibition on LPS-Induced Production of Inflammatory Markers in HLECs**

Because the autocrine and paracrine effects of inflammatory cytokines and chemokines produced by LPS are responsible for the propagation of LPS-toxicity, we next examined the effect of AR inhibition on the LPS-induced inflammatory markers. As shown in Figures 5A and B, treatment of HLECs with 1 μg/mL LPS for 24 hours caused 4.5- and 5-fold increases in the synthesis of PGE2 and activation of Cox, respectively, and inhibition of AR significantly (>85%) prevented it. However, sorbinil or torestat by itself did not alter the total PKC activity in these cells. The phosphorylated forms of p38, MAPK, and JNK were markedly enhanced in HLECs stimulated with LPS, but there was no change in the expression of total JNK and p38 MAPK. Inhibition of AR prevented LPS-induced phosphorylation of p38 and JNK but not their protein synthesis in HLECs (Figs. 8B-E). Collectively, these results suggest that inhibition of AR in HLECs prevents PKC activation by interrupting upstream signaling of the NF-κB by LPS.

**DISCUSSION**

LPS is a common initiator of inflammation, triggering tyrosine phosphorylation and activation of protein kinases such as PKC and MAPK, which in turn regulate a variety of transcription factors, leading to the expression of various inflammatory genes. It is also well known that, in several cell types, LPS is the major inducer of the redox-sensitive transcription factor NF-κB, which regulates the expression of a variety of genes essential for cellular immune response, inflammation, growth,
development, and apoptotic processes. During Gram-negative bacterial infections, excessive cytokines and chemokines are generated that, in an autocrine and paracrine manner, cause tissue damage that leads to multiorgan failure and septic shock. In general, the ocular tissues are exposed to various cytokines and other proinflammatory markers that are released as a result of injury, infection, and/or disease processes. Even though the lens is located within the very center of the eye, it can also be exposed to various inflammatory markers present in the aqueous humor subsequent to bacterial infection, and the lens epithelium has also been shown to produce cytokines.

Herein, we have presented several lines of experimental evidence to suggest that LPS predominantly induces apoptosis in HLECs that can be inhibited by either pharmacological inhibition of AR or by using RNA interference ablation of the AR message. Our studies suggest that the salutary effects of AR inhibition may be related to inhibition of inflammatory signaling mediated by transcription factors (NF-kB and AP-1) and stress-activated MAPKs (JNK and p38).

Recent investigations have also shown the involvement of various caspases and cytokines associated with the decline in lens clarity. Alexander et al. have shown the activation of NF-kB in the mouse lens, whereas Dudek et al. have shown the activation of NF-kB by TNF-α in HLECs. Moreover, our earlier studies have suggested that TNF-α causes activation of NF-kB and apoptosis in HLECs. However, the apoptosis of HLECs as a causative factor of cataractogenesis is controversial. Recently, it has been shown that lens epithelial cell apoptosis is an initiating factor in noncongenital cataract formation, whereas Harocopoulos et al. have shown that apoptosis of HLECs is not the major cause of age-related cataract formation. In contrast, Li et al. have shown that exposure of rat lens to hydrogen peroxide causes lens epithelial cell death followed by lens opacification. Further, oxidative stress caused by various stimulants such as infections, UVB radiation, and environmental contaminants could cause HLEC apoptosis that leads to the opacification of the rat lens. Andley et al. have shown that increased biosynthesis of PGE2 may be important in formation of posterior subcapsular cataracts in humans and in animals exposed to UVB radiation.

A general role of AR in mediating inflammation and cytokine generation is consistent with our observations showing that inhibition of AR prevents PKC and NF-kB activation by a variety of stimuli, such as TNF-α, FGF, PDGF, angiotensin-II, and high glucose and hyperglycemia-induced MAPK and JAK2. These findings suggest that AR could be an obligatory mediator of stress response including the activation of NF-kB and other PKC-sensitive transcription factors. Activation of NF-kB requires association of IKK (Inhibitor of kappaB kinase)-α, IKKβ, and IKKγ. In LPS signaling, the IKKα/β complex is assembled through a TAK1-dependent pathway that also activates JNK and p38. The observation that phosphorylation of both the JNK and p38 kinases in the HLECs was severely attenuated by AR inhibition (Figs. 3C, 8B) further suggests that the signals preceding TAK1 activation are prevented by ARIs and that the inhibition of AR does not directly interfere with the NF-kB and its downstream effectors. LPS-triggered signaling events further upstream to IKK activation are mediated by the activation of PKC, because macrophage PKC activity is increased by LPS stimulation, and PKC inhibitors prevent LPS-induced NF-kB activation and the release of cytokines. In agreement with a central role of PKC, we found a marked PKC activation with LPS (Fig. 8A). AR inhibition or ablation prevented LPS-induced activation of PKC, supporting our previous observation that inhibition of AR prevents PKC activation and thus modulates the activity of NF-kB. Although the mechanisms by which AR facilitates PKC activation...
remains unclear, we propose that inhibition of AR prevents the events that could lead to the activation of PLC isoforms, which are activated by LPS, as observed in high-glucose–treated vascular smooth muscle cells (VSMCs). Recently, we have reported that ARIs prevent phosphatidylinositol-specific phospholipase C (PI-PLC)–dependent synthesis of diacylglycerol in high-glucose–stimulated VSMCs. A similar mechanism could account for the AR mediation of LPS-induced PKC and NF-κB activation in HLECs. Alternatively, AR inhibition could affect signaling due to products of lipid peroxidation or their glutathione conjugates. Recent studies have shown that the oxidized phospholipids such as 1-palmitoyl, 2-oxovaleryl phosphocholine (POVPC), which is also a substrate of AR, inhibit NF-κB activation and increase mortality in mice injected with lethal doses of LPS. This indicates that the inhibition of AR could prevent activation of NF-κB by allowing oxidized phospholipids to accumulate in the cells.

In summary, our current results provide evidence of an unanticipated role of an aldehyde-degrading enzyme, AR, in mediating acute inflammatory responses and provide a novel concept that inhibition of AR could be therapeutically useful in preventing ocular tissue inflammation induced by Gram-negative bacterial infections.

References


