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*J Immunol* 2001; 166:2674-2680; doi: 10.4049/jimmunol.166.4.2674
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IL-10 Is a Central Regulator of Cyclooxygenase-2 Expression and Prostaglandin Production

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IL-10 is a potent anti-inflammatory and immune regulatory cytokine. IL-10−/− mice produce exaggerated amounts of inflammatory cytokines when stimulated with LPS, indicating that endogenous IL-10 is a central regulator of inflammatory cytokine production in vivo. PGs are lipid mediators that are also produced in large amounts during the inflammatory response. To study the role of IL-10 in the regulation of PG production during the acute inflammatory response, we evaluated LPS-induced cyclooxygenase (COX) expression and PG production in wild-type (wt) and IL-10−/− mice. LPS-induced PGE2 production from IL-10−/− spleen cells was 5.6-fold greater than that from wt spleen cells. LPS stimulation resulted in the induction of COX-2 mRNA and protein in both wt and IL-10−/− spleen cells; however, the magnitude of increase in COX-2 mRNA was 5.5-fold greater in IL-10−/− mice as compared with wt mice. COX-1 protein levels were not affected by LPS stimulation in either wt or IL-10−/− mice. Neutralization of IFN-γ, TNF-α, or IL-12 markedly decreased the induction of COX-2 in IL-10−/− spleen cells, suggesting that increased inflammatory cytokine production mediates much of the COX-2 induction in IL-10−/− mice. Treatment of IL-10−/− mice with low doses of LPS resulted in a marked induction of COX-2 mRNA in the spleen, whereas wt mice had minimal expression of COX-2 mRNA. These findings indicate that, in addition to IL-10’s central role in the regulation of inflammatory cytokines, endogenous IL-10 is an important regulator of PG production in the response to LPS. The Journal of Immunology, 2001, 166: 2674–2680.

Prostaglandins are lipid mediators that have an important role in multiple physiologic processes including blood clotting, ovulation, initiation of labor, wound healing, and kidney function (1). PGs have also been implicated as key mediators of acute inflammatory responses (2, 3) and chronic inflammatory states such as arthritis (4, 5) and inflammatory bowel disease (6).

PG biosynthesis is tightly controlled. The key regulatory enzyme of the PG biosynthesis pathway, PG synthe (EC 1.14.99.1), also known as cyclooxygenase (COX), is the first enzyme in the biosynthetic pathway leading to PG, thromboxanes, and prostacyclins. The COX enzyme exists in two isozymes: COX-1, a constitutive form that is expressed in multiple cell types and is thought to produce PGs central to physiologic homeostasis (7); and COX-2, an inducible form that is rapidly up-regulated in response to LPS, cytokines, and mitogens (7–9). LPS is a potent inducer of PG synthesis. It has been demonstrated that LPS-stimulated monocytes rapidly induce COX-2 at both the mRNA and protein level (10–12). Multiple cytokines have been demonstrated to alter COX-2 expression in vitro. Proinflammatory cytokines including TNF-α (13, 14), IL-1α (14, 15), and IFN-γ (16, 17) have been demonstrated to induce COX-2 expression, whereas anti-inflammatory cytokines such as IL-4 (18), IL-13 (19, 20), and IL-10 (21) can inhibit COX-2 induction. The pleiotropic cytokine TGF-β can enhance (22) or inhibit (23) COX-2 expression, depending upon the cell type tested. The functional significance of these in vitro observations has not been established in vivo.

IL-10 is a cytokine with potent anti-inflammatory activity. IL-10 has been shown to be a potent macrophage deactivator, blocking the induced synthesis of TNF-α, IL-1, IL-6, IL-8, and GM-CSF by human monocytes (24) and mouse peritoneal macrophages (25).

IL-10 also indirectly suppresses the synthesis of IFN-γ by Th cells (26) and NK cells (27). Interestingly, IL-10 was found to be produced by monocytes after exposure to LPS (24), which suggested that IL-10 may regulate the inflammatory response to LPS. Indeed, we found that LPS-induced cytokine production was markedly increased in IL-10−/− mice and that the lethal dose of LPS for IL-10−/− mice was 20-fold lower than that for wild-type (wt) mice. These data demonstrate that endogenous production of IL-10 is a central regulator of cytokine production in response to LPS (28). It has recently been reported that IL-10 in vitro can inhibit COX-2 induction in human monocytes and neutrophils (18, 21). These studies suggested that IL-10 may regulate the production of lipid mediators.

The generation of mice with a targeted disruption of the IL-10 gene (IL-10−/−) (29) has allowed further definition of the actions of IL-10 in complex physiologic systems. We have compared LPS-induced COX expression and PG production in IL-10−/− and wt mice to determine the importance of endogenous IL-10 in the regulation of PG production.

Materials and Methods

Animals

Healthy 5- to 6-wk-old IL-10−/− mice on a 129/SvEv background were used for this study (30). Wild-type 129/SvEv mice were obtained from Taconic Farms (Germantown, NY). Mice were maintained in microisolator cages under specific pathogen-free conditions at the animal care facility at the University of Iowa.
Reagents

LPS from Escherichia coli (serotype 0111:B4) was obtained from Difco (Detroit, MI) and resuspended in pyrogen-free saline. Aminoguanidine (hemisulfate salt) and A23187 were obtained from Sigma (St. Louis, MO). NS-398 was obtained from Cayman Chemicals (Ann Arbor, MI). Neutralizing Ab to IL-10 (JESS-2A5) and recombinant IL-10 (expressed in E. coli) were kind gifts of D. Remnick (DNAX, Palo Alto, CA). Rabbit polyclonal anti-murine COX-2 was obtained from Cayman Chemicals; and rabbit polyclonal anti-COX-1 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-cytokine Abs (anti-TNF-α, XT22; anti-IFN-γ, XMG-1.2; anti-IL-12, C17-8; and isotype control Ab, MOPC-21) were kind gifts of J. Weinstock (University of Iowa).

HPLC

For the HPLC assay of eicosanoids, spleen cell cultures were incubated for 30 min at 37°C with up to 7.5 μM [5,6,8,9,11,12,14,15]-5-hydroxyeicosatetraenoic acid (155 Ci/mmol; New England Nuclear, Boston, MA) in serum-free medium. After incubation, the medium was collected and centrifuged to remove cellular debris. In some experiments, after incubation with [3H]AA, the medium was removed and replaced with serum-free medium containing the calcium ionophore A23187 (2 μM). Lipids were then extracted with a 2:1 (v/v) mixture of chloroform/methanol containing 1% acetic acid, dried under N2 and resuspended in acetonitrile. Lipids were separated on a 332 HPLC system (Beckman Coulter, Fullerton, CA) equipped with a C18 reverse-phase column containing 5-μm spherical packing with the use of a gradient of water (adjusted to pH 3.5 with phosphoric acid) and acetonitrile. The column effluent was mixed with scintillation solution at a 1:3 ratio before passing through an on-line radioactivity detector (Flo-1, Radiomatic Instruments and Chemicals, Tampa, FL). The system was standardized with the following eicosanoids: 6-keto-PGF1α, PGE2, PGE3, leukotriene B4 (LTB4), and AA (Amersham Pharmacia Biotech, Piscataway, NJ). Control studies performed with empty culture dishes incubations or extractions.

Cell culture protocols

Spleen cells from wt or IL-10−/− mice were cultured at 5 × 106 cells/ml in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 0.05 mM 2-ME, penicillin (100 U/ml), and streptomycin (100 U/ml) in 12-well tissue culture plates (Costar, Corning, NY). Cells were incubated in medium alone or supplemented with LPS at 10 μg/ml; this concentration of LPS gave maximal stimulation of PG production from both wt and IL-10−/− spleen cell cultures. Supernatants from replicate cultures were harvested after 24 h and stored at −70°C before analysis for PG concentration. Spleen cells were subsequently harvested for either RNA or protein isolation. In some cultures, after 24 h of LPS stimulation the supernatant was removed, cells were incubated in PBS for 30 min in the presence or absence of NS-398 (10 μM), and the supernatant was subsequently assayed for PGE2 production.

To assess the role of inflammatory cytokines in the induction of COX-2, LPS-stimulated spleen cells from IL-10−/− mice were incubated in the presence of anti-cytokine Abs (anti-TNF-α, XT22, 2.5 μg/ml; anti-IFN-γ, XMG-1.2, 5.0 μg/ml; or anti-IL-12, C17-8, 3.0 μg/ml) or isotype control Ab (MOPC-21, 5.0 μg/ml).

Quantification of PGE2

PGE2 levels in tissue culture supernatants were determined using the PGE2 enzyme immunoassay kit (Cayman Chemicals) according to the manufacturer’s instructions.

Northern blotting

RNA from spleens or spleen cell cultures was prepared using RNA-STAT-60 (Tel-Test, Friendswood, TX) exactly as per the manufacturer’s instructions. Total RNA (15 μg/lane) was electrophoresed through a 1% agarose gel and transferred onto a nylon membrane. cDNA fragments specific for murine COX-1 and COX-2 (Oxford Biomedical, Oxford, MI) were 32P-labeled by the random prime method and used for sequential hybridizations. Membrane hybridization was conducted in 50% formamide at 42°C overnight with 2 × 106 cpm/ml cDNA probe. After a series of washings in 2× SSC at room temperature, in 0.2× SSC, 0.1% SDS at 60°C, and in 0.1× SSC at room temperature, the membranes were exposed to Fuji RX film (Tokyo, Japan) at −70°C. Ethidium bromide staining of 18S and 28S ribosomal RNA served as an internal loading control.

Western blotting

Protein was isolated from cultured spleen cells by resuspending in lysis buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 100 μg/ml PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM diethyldithiocarbamic acid, 1% Nonidet P-40, and 1% sodium deoxycholate). Cells were lysed by sonication (20, 4°C). Debris was eliminated by centrifugation (15 min, 1000 × g). Protein concentration was measured using a commercial reagent based on bichinonic acid staining (Pierce, Rockford, IL) using BSA as an internal standard. Equal amounts of cellular protein were loaded onto a 10% polyacrylamide gel and separated by electrophoresis (200 V for 45 min). Proteins were then transferred to nitrocellulose (100 V for 1 h), and the membrane was blocked with 5% nonfat dry milk. The nitrocellulose was then incubated with a rabbit polyclonal primary Ab (anti-COX-2, 1:1000; Cayman Chemicals; anti-COX-1, 1:1000; Baxter Healthcare Corporation, Detroit, MI) and resuspended in pyrogen-free saline. Aminoguanidine (hemisulfate salt) and A23187 were obtained from Sigma (St. Louis, MO), and the supernatant was subsequently assayed for PGE2 production using an electrochemical sandwich immunoassay (ECL, Amersham) as per the manufacturer’s instructions. Specificity of the anti-COX Ab was confirmed with the use of rat seminal vesicle COX-1 (Oxford Biomedical) and sheep placenta COX-2 (Cayman Chemicals).

Films and photographs of Northern or Western blots were scanned in at 600 dpi using an Epson Expression 1600 scanner. Densitometric analysis was performed using Vtrace (developed at the University of Iowa Image Analysis Facility) operating on a Silicon Graphics O2 workstation (Mountain View, CA). Average and integrated OD measurements were made on user-selected regions. A Kodak photographic step tablet (Rochester, NY) was used to calibrate OD.

RNase protection assay

A murine COX-2 cDNA fragment (nt-205–505, GenBank accession no. M87324) was synthesized by RT-PCR using mouse brain RNA as a template and cloned into pGEM-4. Fragments of the RPL32-4A gene (31) and the inducible NO synthase (iNOS) gene (32) were also cloned into pGEM-4. L32 served as an internal loading control. RNase protection assay was done for the detection of COX-2 and iNOS as performed previously described (33). Briefly, for the synthesis of a 32P-labeled antisense RNA probe, equimolar mixtures of the linearized COX-2, iNOS, and L32 templates were used. Hybridization reactions were performed overnight at 56°C. Following RNase digestion, the RNA duplexes were isolated by electrophoresis in a standard 7.5% acrylamide/12 M urea/0.5% Tris/borate/89 M phosphoric acid) and acetonitrile. The column effluent was mixed with scintillation solution at a 1:3 ratio before passing through an on-line radioactivity detector (Flo-1, Radiomatic Instruments and Chemicals, Tampa, FL). The system was standardized with the following eicosanoids: 6-keto-PGF1α, PGE2, albeit in smaller amounts than the IL-10−/− spleen cells was PGE2. Under these culture conditions, spleen cells from wt mice did not produce significant amounts of PGE2 or leukotrienes even when stimulated with LPS (Fig. 1, A and B). In contrast, incubation of IL-10−/− spleen cells for 48 h in medium alone resulted in the production of small amounts of PGE2 as well as LTB4 (Fig. 1C). When stimulated with LPS, IL-10−/− spleen cells produced more PGE2, while not appreciably changing their release of LTB4 (Fig. 1D). Qualitatively similar results were obtained when spleen cells were incubated with LPS for 24 h. Stimulation of the 3H-labeled cells with the calcium ionophore A23187 (2 μM) produced a qualitatively similar eicosanoid profile (data not shown).

Next, we quantified PGE2 accumulation in LPS-stimulated spleen cell cultures. Marked accumulation of PGE2 was found in the culture supernatant of LPS-stimulated IL-10−/− spleen cells (Fig. 2). Spleen cells from LPS-stimulated wt mice also produced PGE2, albeit in smaller amounts than the IL-10−/− spleen cells.
We next assessed whether endogenous IL-10 would alter the level of protein expression of COX-1 and COX-2. Stimulation of IL-10−/− mice cultured for 48 h in medium supplemented with LPS (10 μg/ml) resulted in significant decreases in PGE2 production from wt spleen cells as compared with wt (Fig. 3). Incubation of the cells with NS-398, a COX-2 selective inhibitor, blocked PGE2 production, demonstrating that the PGE2 production was derived from the COX-2 enzyme.

**Effect of endogenous IL-10 on COX mRNA levels**

Because we had found that LPS stimulation markedly increased PG production from IL-10−/− spleen cells, we next determined whether endogenous IL-10 would regulate the expression of COX-2 mRNA. Spleen cells from wt or IL-10−/− mice were incubated in medium or LPS (10 μg/ml) for varying time points, and COX-2 mRNA levels were subsequently determined. As demonstrated in Fig. 4, no COX-2 mRNA was seen in unstimulated wt and IL-10−/− spleen cells. Within 2 h of LPS stimulation, COX-2 mRNA levels increased in both wt and IL-10−/− spleen cells; however, at 24 h the magnitude of increase was 6.3-fold greater in the IL-10−/− cells than in the wt cells (Fig. 4).

**Effect of endogenous IL-10 on protein expression of COX-1 and COX-2**

We next assessed whether endogenous IL-10 would alter the level of protein expression of COX-1 and COX-2. Stimulation of IL-10−/− spleen cells with LPS resulted in a marked induction of COX-2 protein as compared with wt spleen cells (Fig. 5A). In contrast, the absence of IL-10 had no reproducible effect on the level of protein expression of COX-1 (Fig. 5B). Wild-type spleen cells were incubated with LPS in the presence of neutralizing Abs to IL-10 to further assess the role of endogenous IL-10 in COX-2 expression. Neutralization of IL-10 resulted in a 1.8-fold increase in COX-2 protein expression (Fig. 5C).

**Effect of inflammatory cytokines on the induction of COX-2 protein**

Inflammatory cytokines such as TNF-α are known to be potent inducers of COX-2 expression (34, 35). Because IL-10−/− mice markedly overproduce inflammatory cytokines in response to LPS (29), we tested whether the marked induction of COX-2 mRNA and protein in IL-10−/− spleen cells was secondary to increased production of inflammatory cytokines. Spleen cells from IL-10−/− mice were incubated with LPS in the presence or absence of neutralizing Ab to TNF-α, IFN-γ, or IL-12, and the induction of COX-2 protein was assessed by Western blotting (Fig. 6). Neutralization of IFN-γ and TNF-α resulted in significant decreases in LPS-induced COX-2 expression (IFN-γ, 64.0 ± 16.5%; TNF-α, 75 ± 5.57%; mean percent decrease ± SD). Neutralization of IL-12 also resulted in a significant decrease in protein expression of COX-2 (49 ± 15.8%). As reported previously (21), incubation of spleen cells with IL-10 (10 U/ml) inhibited LPS induction of COX-2 protein (data not shown).

**Effect of endogenous IL-10 on COX-2 mRNA expression in vivo**

To ascertain whether endogenous IL-10 would regulate the expression of COX-2 in vivo, wt and IL-10−/− mice were treated with a low dose of LPS (25 μg, i.p.), and splenic mRNA expression of COX-2 was assessed by Northern blotting (Fig. 7). Neutralization of IL-10 resulted in a 1.8-fold increase in COX-2 mRNA (Fig. 7). Neutralization of IL-12 also resulted in a significant decrease in COX-2 expression (75 ± 5.57%; mean percent decrease ± SD). Neutralization of IFN-γ and TNF-α resulted in significant decreases in LPS-induced COX-2 expression (IFN-γ, 64.0 ± 16.5%; TNF-α, 75 ± 5.57%; mean percent decrease ± SD).

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**FIGURE 1.** Eicosanoid profiles produced by LPS-stimulated spleen cells. These reverse-phase HPLC radiochromatograms were generated from spleen cells from wt or IL-10−/− mice cultured for 48 h in medium or medium supplemented with LPS (10 μg/ml). At the end of the culture period, the spleen cells were washed and subsequently incubated for 30 min in medium supplemented with 7.5 μM [3H]AA. A, Eicosanoid production from wt spleen cells incubated in medium for 48 h. B, Eicosanoid production from wt spleen cells stimulated with LPS (10 μg/ml) for 48 h. C, Eicosanoid production from IL-10−/− spleen cells incubated in medium for 48 h. D, Eicosanoid production from IL-10−/− spleen cells stimulated with LPS (10 μg/ml) for 48 h. The PGE2 and LTB4 peaks are identified on the basis of the retention time. Data are representative of four independent experiments.

**FIGURE 2.** Effect of endogenous IL-10 on PGE2 accumulation from LPS-stimulated spleen cells. Spleen cell cultures (5 × 106 cells/ml) from wt or IL-10−/− mice were incubated for 12 or 24 h in medium alone or medium supplemented with LPS (10 μg/ml). At the end of the culture period, the culture medium was removed and assayed for PGE2 as described in Materials and Methods. Data are expressed as means ± SD of three observations and are representative of three independent experiments.
COX-2 was assessed by RNase protection (Fig. 7). The induction of COX-2 mRNA in IL-10−/− mice was markedly greater than that of wt mice. COX-2 mRNA was not detected in control wt or IL-10−/− mice. Interestingly, iNOS mRNA was also markedly up-regulated in the IL-10−/− mice, potentially increasing the production of NO, which may up-regulate COX-2 enzyme activity (36).

Discussion

We have used IL-10−/− and wt mice to further define the regulatory role of endogenous IL-10 in the immune/inflammatory responses elicited by LPS. Our studies show that LPS-stimulated production of PGs is significantly increased in spleen cells from COX-2 determination. Data are expressed as means ± SD and are representative of three independent experiments.

FIGURE 3. Effect of endogenous IL-10 on PGE2 production from LPS-stimulated spleen cells. Spleen cell cultures (5 × 10⁶ cells/ml) from wt or IL-10−/− mice were incubated for 18 h in culture medium alone or medium supplemented with LPS (10 μg/ml). At the end of the culture period, the medium was removed, and cells were cultured in PBS with or without NS-398 (10 μM). After 30 min, the culture medium was harvested for PGE2 determination. Data are expressed as means ± SD and are representative of three independent experiments.

FIGURE 4. A. Effect of endogenous IL-10 on LPS-stimulated COX-2 mRNA expression. Spleen cell cultures (5 × 10⁶ cells/ml) from wt or IL-10−/− mice were incubated for varying time periods in culture medium supplemented with LPS (10 μg/ml). Total RNA was isolated and COX-2 mRNA expression was analyzed by Northern blot using a COX-2-specific probe. Ethidium bromide staining of the RNA gel demonstrates equivalent RNA loading. Quantitation of COX-2 transcripts was assessed by image analysis. COX-2 mRNA levels were normalized to 28S rRNA levels. Data are representative of three independent experiments.

FIGURE 5. Effect of endogenous IL-10 on COX-1 and COX-2 protein expression. Spleen cell cultures (5 × 10⁶ cells/ml) from wt or IL-10−/− mice were incubated in the presence or absence of LPS (10 μg/ml). After 24 h, cell lysates were prepared, equivalent amounts of protein (25 μg/ lane) were separated on 10% SDS-PAGE, and COX expression was assessed by Western blot analysis. A. COX-2 expression in control and LPS-stimulated wt or IL-10−/− spleen cells. The COX-2 band is indicated by the arrow. B. COX-1 expression in control and LPS-stimulated wt or IL-10−/− spleen cells. C. Effect of neutralization of endogenous IL-10 on COX-2 expression. Spleen cells from wt mice were stimulated in the presence or absence of neutralizing Ab to IL-10, and COX expression was assessed by Western blot analysis. Data are representative of three independent experiments.

IL-10−/− mice as compared with wt mice. Consistent with this finding, LPS-stimulated spleen cells from IL-10−/− mice had markedly increased levels of COX-2 mRNA and protein as compared with wt mice. Treatment of IL-10−/− mice with a low dose of LPS resulted in marked expression of COX-2 mRNA in the spleen, whereas minimal expression was noted in wt mice following the same dose. Taken together, these results suggest that endogenous IL-10 is a central regulator of LPS-induced PG production.

PGs are potent bioactive lipid mediators, and thus PG production is tightly controlled. The increased PG production observed in IL-10−/− spleen cells in response to LPS occurs together with markedly increased levels of mRNA and protein for the inducible form of COX, COX-2. This finding is consistent with previous studies that demonstrated that PG production in response to LPS, inflammatory cytokines, and/or mitogens is due almost entirely to the rapid induction of the COX-2 isoform (10, 11). Our finding that NS-398, a selective COX-2 inhibitor, blocks the increased PG production in our system was secondary to increased COX-2 expression and/or activity. In contrast, IL-10 appeared to exert no regulatory effect on COX-1 protein expression, similar to previous reports indicating that COX-1 expression is generally constitutive rather than inducible (37).

IL-10 may regulate COX-2 mRNA expression through either indirect or direct means. Direct regulation of COX-2 by IL-10 may occur at the transcriptional level via regulation of NF-κB. The COX-2 promoter contains two NF-κB motifs (38) that are clearly involved in the regulation of COX-2 expression in both mouse (39) and human (40) macrophage cell lines. IL-10 has been shown to inhibit inflammatory cytokine production via inhibition of NF-κB function (41). Therefore, the absence of IL-10 in the IL-10−/−
mouse may have led to enhanced NF-κB activity, resulting in increased levels of COX-2 mRNA.

IL-10 may regulate COX-2 gene expression at the posttranscriptional level as well. One of the mechanisms by which IL-10 suppresses inflammation is via induction of instability in the mRNAs of inflammatory cytokines, resulting in their degradation. The mRNAs for multiple inflammatory cytokines (TNF-α, IL-1α, IL-1β, GM-CSF) and chemokines (KC, macrophage inflammatory protein 1α, macrophage inflammatory protein 1β) all contain AU-rich elements in their 3′ untranslated regions, a sequence that has been found to be important for mRNA stability and translation (42, 43). IL-10 has been reported to destabilize the mRNA for all of these cytokines (44). The 3′ untranslated region of COX-2 mRNA has 20 copies of the pentamer AUUUA (38), suggesting that COX-2 mRNA expression may be regulated posttranscriptionally. Indeed, LPS and IL-1β enhance COX-2 mRNA stability (45), whereas exogenous IL-10 in vitro accelerates the degradation of COX-2 mRNA in human monocytes (21). Therefore, the absence of IL-10 may have resulted in increased COX-2 mRNA through an increase in the mRNA stability.

Our study indicates that IL-10 may regulate COX-2 expression and PG production indirectly via regulation of proinflammatory cytokines. COX-2 mRNA is known to be induced by multiple cytokines including TNF-α and IL-1α (46, 47). In contrast, IL-10 is an important down-regulator of inflammatory cytokines (for example, TNF-α and IL-1α) from LPS-stimulated macrophages (25). We have previously demonstrated that LPS stimulation of IL-10−/− mice in vivo results in markedly increased and sustained production of TNF-α, IFN-γ, and IL-12 (28). Our findings clearly demonstrate that neutralization of TNF-α and IFN-γ resulted in a marked decrease in COX-2 induction in LPS-stimulated IL-10−/− cells. Interestingly, this study demonstrated that neutralization of IL-12 resulted in a significant decrease in COX-2 expression as well. IL-12 is produced by macrophages, neutrophils, and dendritic cells in response to infection or bacterial products such as LPS (48, 49). Ligation of the IL-12 receptor that is expressed on T cells and NK cells promotes the development of IFN-γ-producing Th1 CD4+ T cells and induces high-level production of IFN-γ (49). In mouse macrophages, IFN-γ enhances the effect of LPS on COX-2 expression (50). In contrast, IL-10 is a potent inhibitor of IL-12 production (51), and LPS-treated IL-10−/− mice markedly overproduce IL-12 (28). Therefore, neutralization of IL-12 may have indirectly decreased COX-2 production due to decreased IFN-γ production. Taken together, these results indicate that much of the LPS-induced increase in COX-2 expression in IL-10−/− mice is secondary to dysregulated inflammatory cytokine production in these mice.

The relationship between NO production and PG biosynthesis is quite complex. NO donors have been reported to stimulate or inhibit PG production in a variety of systems (36, 52–55). It has recently been reported that iNOS-deficient mice have markedly decreased PGE2 production in response to LPS and IFN-γ, clearly supporting the view that NO and/or NO-derived species modulate COX activity and PG production (56). In our in vitro studies, we did not observe increased NO production from LPS-stimulated IL-10−/− spleen cell cultures at 24 h (data not shown), whereas PGE2 production at this time point was clearly elevated, suggesting that NO production was not central to the increased PGE2 production from IL-10−/− spleen cells. Interestingly, in vivo we found marked induction of iNOS mRNA in response to LPS in the IL-10−/− mice, consistent with our previous study in which we demonstrated that IL-10−/− mice produce excessive amounts of NO in vivo in response to LPS (28). The kinetics of iNOS induction and NO production in vivo may differ significantly from that in vitro and it is quite possible that NO or NO-derived species such as peroxynitrite have important effects on in vivo production of PGs in IL-10−/− mice. Further studies will be required to resolve this issue.

This study clearly demonstrates that endogenously produced IL-10 is a key regulator of COX-2 expression and subsequent PG production. The regulatory effect of IL-10 on PG production may be both direct (via inhibition of mRNA expression and decreasing the half-life of the COX-2 mRNA) and indirect (via regulation of inflammatory cytokines that can induce COX-2 expression). PGs are potent lipid mediators of the immune and inflammatory response; however, their role is quite complex. For example, PGE2 appears to be a key mediator of the inflammatory response: in an animal model of arthritis, neutralization of PGE2 resulted in decreased edema, hyperalgesia, and IL-6 production (3). However,
PGE₂ may have anti-inflammatory effects: PGE₂ in vitro can inhibit cytokine production, including IL-12 production and function. We previously reported that IL-10⁻/⁻ mice have exaggerated inflammatory responses (28, 63) as well as dysregulated mucosal immune responses (30, 64) characterized by increased expression of peptide mediators. The results of the studies described herein suggest that increased PG production is also a major component of the altered immune and inflammatory responses seen in IL-10⁻/⁻ mice. Further studies will be required to determine the precise role played by PGs in the altered immune/inflammatory response in IL-10⁻/⁻ mice.

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