Characterization of an Interferon-stimulated Response Element (ISRE) in the \textit{Il23a} Promoter\textsuperscript{**}

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We have demonstrated previously that IFN-\(\gamma\) plays a protective role in the initiation of chronic intestinal inflammation through attenuation of Toll-like receptor-mediated IL-23 induction in macrophages. Here, an interferon-stimulated response element (ISRE) is identified in a region of conserved nucleotide sequences in the \textit{Il23a} promoter. This ISRE mediated, in part, \textit{Il23a} promoter induction by LPS and inhibition of LPS-induced activity by IFN-\(\gamma\). LPS and IFN-\(\gamma\) recruit interferon regulatory factors (IRFs) to the \textit{Il23a} ISRE in murine bone marrow-derived macrophages (BMMs). Functionally, IRF-1 is a negative regulator of \textit{Il23a} in LPS-stimulated BMMs. IRF-1\(^{-/-}\) BMMs demonstrated enhanced LPS-induced \textit{Il23a} expression compared with WT BMMs. Moreover, IRF-1 deficiency resulted in prolonged occupancy of RelA on the \textit{Il23a} promoter. Consequently, IRF-1\(^{-/-}\) mice were more susceptible to colonic injury by trinitrobenzenesulfonic acid, and IL-10/IRF-1 double-deficient (IL-10/IRF-1\(^{-/-}\)) mice demonstrated more severe colonic inflammation compared with IL-10\(^{-/-}\) mice. The severity of colitis in both models correlated with increased colonic IL-23. CD11b\(^+\) lamina propria mononuclear cells, comprising predominantly macrophages, were identified as the major source of IL-23 in colitis-prone mice. Basal and heat-killed \textit{Escherichia coli}-stimulated levels of \textit{Il23a} were increased in IL-10/IRF-1\(^{-/-}\) compared with WT and IL-10\(^{-/-}\) colonic CD11b\(^+\) lamina propria mononuclear cells. In conclusion, these experiments characterize IRF-ISRE interactions on the \textit{Il23a} promoter, which have \textit{in vivo} relevance as a homeostatic checkpoint in chronic intestinal inflammation.

\textit{Il23a} is a heterodimeric cytokine composed of a p19 subunit (\textit{Il23a}) and a p40 subunit (\textit{Il12b}) also shared with IL-12. \textit{Il23a} is expressed in macrophages and dendritic cells and is induced by microbial products such as LPS (1). The importance of IL-23 in the pathogenesis of chronic inflammatory disorders is supported by the recent identification of IL-23 receptor susceptibility alleles associated with the inflammatory bowel diseases, psoriasis, and spondyloarthropathies (2). IL-23-driven inflammation has been linked to its role in the development and persistence of a pathogenic subset of T-helper type 17 (\(T_\text{H}17\)) cells (3).

Recently, we demonstrated a protective role for IFN-\(\gamma\), the signature T\(_{\text{H}1}\) cytokine, in chronic intestinal inflammation through attenuation of \textit{Il23a} gene expression (4). IFN-\(\gamma\)-receptor deficiency exacerbated IL-23-mediated colitis in IL-10-deficient (IL-10\(^{-/-}\)) mice. Although the molecular regulation of IL-12 p35 (\textit{Il12a}) and IL12b expression has been well studied, many important questions remain about \textit{Il23a} regulation in macrophages (1).

Toll-like receptors (TLRs)\(^{2}\) induce \textit{Il23a} expression in macrophages through recruitment of NF-\(\kappa\)B family members to binding sites in the proximal promoter (5). We demonstrated that IFN-\(\gamma\) inhibited LPS-induced NF-\(\kappa\)B RelA while enhancing p50 subunit recruitment to the \textit{Il23a} promoter (4). IFN-\(\gamma\)-mediated IL-23 inhibition was in striking contrast to its augmentation of LPS-induced IL-12 p40 and IL-12 p70 expression (4). Notably, TLR and IFN-\(\gamma\)-transcriptional response in the \textit{Il12a} and \textit{Il12b} promoters is mediated by interferon regulatory factor (IRF) family members (1). Specifically, mice deficient in IRF-1, IRF-2, and IRF-8 demonstrate defects in \textit{Il12a} and \textit{Il12b} production (6). The reciprocal regulation of IL-12 family members by IFN-\(\gamma\) highlights its complex biological role in the maintenance of immune homeostasis.

In these experiments, we functionally characterize an interferon-stimulated response element (ISRE) in the \textit{Il23a} promoter. This ISRE mediated \textit{Il23a} promoter induction by LPS and inhibition of LPS-induced activity by IFN-\(\gamma\). LPS- and IFN-\(\gamma\)-induced IRF-1 is identified as a negative regulator of IL-23 in macrophages and experimental colitis.

**EXPERIMENTAL PROCEDURES**

\textit{Mice—}WT, IL-10\(^{-/-}\), NF-\(\kappa\)B p50\(^{-/-}\), and IRF-1\(^{-/-}\) mice on a C57BL/6 background were matched for age in all experiments. IRF-1/IL-10\(^{-/-}\) mice were obtained by crossing IL-10\(^{-/-}\) and IRF-1\(^{-/-}\) mice. Heterozygous offspring were then bred to obtain homozygous IL-10/IRF-1\(^{-/-}\) mice. Age-
matched littermates were used as controls. 8–10-week-old WT and IRF-1<sup>−/−</sup> mice were administered 2.5% (w/v) trinitrobenzenesulfonic acid (TNBS) in 40% ethanol intrarectally, and intestines were harvested for analyses after 5 days of treatment. All mice were housed in accordance with guidelines from the American Association for Laboratory Animal Care, and research protocols were approved by the Institutional Animal Care and Use Committee of the University of North Carolina.

**Reagents and Plasmids**—Highly purified LPS and IFN-γ were obtained from InvivoGen (San Diego, CA). Heat-killed E. coli K12 cells were prepared as described previously (4). Murine IL-12 p40, IL-12 p70, and immunoassay kits (R&D Systems, Minneapolis, MN) and IL-23 (eBioscience, San Diego, CA) were used according to the manufacturers’ instructions. The 1.8-kb Il23a-luciferase construct was provided Dr. Y. H. Chen (University of Pennsylvania School of Medicine). The QuikChange XL system (Stratagene, Santa Clara, CA) was used to make mutations in the ISRE in the Il23a murine promoter. Mutations were confirmed by sequencing.

**Bone Marrow-derived Macrophages**—Bone marrow-derived macrophages (BMMs) were prepared as described (7).

**Western Immunoblot**—Western blotting was performed on whole cell extracts as described (7). Anti-IRF-1 antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and anti-β-actin antibodies were from Abcam (Cambridge, MA).

**Quantitative Real-time PCR**—Real-time PCR was performed as described previously (8). Primer sequences are included in supplemental Table 1.

**Transient Transfections**—BMMs were transiently transfected using Amaxa Nucleofector technology (4). Cells were cotransfected with a constitutively active heat shock protein promoter that expresses β-galactosidase to monitor transfection efficiency. IRF-1 siRNA and control scrambled siRNA were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and the QuikChange XL system (Stratagene, Santa Clara, CA) was used to make mutations in the ISRE in the Il23a murine promoter. Mutations were confirmed by sequencing.

**Colonic Tissue Explant Cultures**—Sections of the transverse colon were processed for cytokine analysis as described previously (8).

**Colonic Macrophage Isolation**—Lamina propria mononuclear cells (LPMCs) were isolated from mouse colon by a modified enzymatic method (9). LPMCs were separated into CD11b<sup>+</sup> cells using anti-CD11b microbeads (Miltenyi Biotec, Auburn, CA). Purity was >90% by flow cytometric analysis (data not shown).

**Histology**—Histological colitis scores were determined on colonic tissue sections by a staff pathologist (Tara Rubinas), who was blinded to the experimental protocols, as described (8).

**ChIP**—ChIP was performed with the ChIP-IT Express kit (Active Motif, Carlsbad, CA) as described (4). DNA-protein complexes were immunoprecipitated with anti-IRF-1, anti-RelA, and rabbit polyclonal IgG antibodies (Santa Cruz Biotechnology, Inc.). Real-time PCR primers (supplemental Table 1) amplified a 198-bp product in immunoprecipitated and input DNA (diluted 10-fold).

**EMSA**—BMM nuclear extracts were prepared by the modified Dignam protocol as described previously (10). BMMs were untreated or treated with LPS (100 ng/ml) with or without IFN-γ (10 ng/ml). Synthetic double-stranded oligonucleotides were designed to span the Il23a promoter region from −368 to −388 bp. The EMSA probe 368/388m contains a mutated sequence from −372 to −378 bp. EMSAs were performed as described previously (10). For competition experiments, a 100-fold molar excess of unlabeled oligonucleotides was added to the reaction mixture before adding labeled probes. For supershift assays, 1.5 μg of rabbit polyclonal antibodies to IRF-1, -2, and -8 (Santa Cruz Biotechnology, Inc.) was added on nuclear extracts 30 min prior to the probe.

**RESULTS**

**Characterization of an ISRE in the Il23a Promoter**—To localize potentially important regulatory regions, conserved nucleotide sequences were identified in multiple species in the Il23a gene locus (supplemental Fig. 1). Within a conserved nucleotide sequence in the murine Il23a promoter, a putative ISRE was identified at −378 to −384 bp with respect to the transcription start site (Fig. 1A).

IRFs Interact with the Il23a ISRE—To determine DNA-protein interactions at this ISRE, EMSA and ChIP experiments were performed. An EMSA probe that spans Il23a promoter sequence −368 to −388 (368/388) containing the ISRE demonstrated enhanced DNA-protein interactions (Fig. 1B, complexes I and II, lanes 3–11) when incubated with nuclear extracts from LPS- and IFN-γ-activated BMMs compared with extracts from unstimulated cells (Fig. 1B, lane 2). An EMSA probe (368/388m) with a mutated sequence from −378 to −384 bp within the ISRE demonstrated decreased DNA binding of complexes I and II (Fig. 1B, lane 12). Likewise, competition experiments with unlabeled double-stranded oligonucleotides corresponding to probe 368/388 (368/388u) (Fig. 1B, lane 6) revealed competition of complex I and II DNA binding. When nuclear extracts from LPS- and IFN-γ-stimulated BMMs were preincubated with specific antibodies to IRF-1 but not IRF-2, IRF-8, and c-Rel, inhibition and supershift of complexes I and II were observed (Fig. 1B, lanes 7–10).

Next, BMMs were cultured with LPS/IFN-γ, and occupancy of IRF-1 on the ISRE site was analyzed by ChIP using primers that span the Il23a promoter from −343 to −521 bp. LPS induced IRF-1 recruitment to the ISRE on the Il23a promoter. IRF-1 promoter occupancy was enhanced by the addition of IFN-γ (Fig. 1C).

Next, a 1.8-kb Il23a promoter-luciferase reporter plasmid containing the ISRE was transiently transfected into BMMs. As reported previously, LPS strongly induced Il23a promoter activity and IFN-γ inhibited luciferase activity (4). BMMs transfected with a reporter plasmid containing a site-directed mutation within the ISRE also demonstrated LPS-induced luciferase activity (Fig. 1D). Importantly, IFN-γ strongly inhibited the LPS-induced luciferase activity of the WT pro-
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**FIGURE 1.** Characterization of an ISRE in the Il23a promoter. A, schematic representation of the murine Il23a promoter region. The NF-κB sites designated k1, k2, and k3 are 82, 618, and 960 bp, respectively, and ISRE is −378 upstream of the transcription initiation site (TIS). The luciferase reporter construct (Il23a luc) contains all three putative NF-κB sites and the ISRE upstream of the firefly luciferase (luc) open reading frame. B, EMSA probe 368/388 spans Il23a promoter sequence −368 to −388. The EMSA probe 368/388m contains a mutant ISRE from −378 to −384 bp. 32P-Labeled probes 368/388 (lanes 2–4) and 368/388m (lane 12) were incubated with nuclear extracts from untreated (lane 2), LPS-activated (lane 3), IFN-γ-activated (lane 4), and IFN-γ and LPS-activated (lanes 5–12) BMMs. Lane 1 represents free probe (FP). Two DNA-protein complexes (I and II) were detected with probe 368/388. A 100-fold molar excess of unlabeled probe (368/388u) (lane 6) was added to compete for binding. Probe 368/388 was incubated with nuclear extracts from IFN-γ/LPS-treated BMMs cells for 30 min, and then polyclonal antibodies against IRF-1, IRF-2, and IRF-8 (lanes 1–12) and 368/388m (lanes 2–4) were added. The figure represents similar results from one of three independently conducted experiments. C, chromatin samples from BMMs treated with LPS (100 ng/ml) and IFN-γ (10 ng/ml) were immuno precipitated with anti-IRF-1 and rabbit polyclonal IgG antibodies. Quantitative real-time PCR analysis was performed on precipitated and input DNA. Results are reported as enrichment (%) input of IRF-1 DNA binding. Data represent the mean ± S.E. of three independent experiments. **, p < 0.01 versus unstimulated (Un) Il23a promoter. D, WT BMMs were transfected with an Il23a promoter-luciferase reporter plasmid (Il23a luc) or a plasmid containing a mutant ISRE (Il23a mut luc), and IRF-1−/− BMMs were transfected with the Il23a-luciferase reporter and then cultured with LPS (100 ng/ml; black bars) + IFN-γ (10 ng/ml; gray bars) for 18 h. Reporter activity is presented as luciferase units normalized to heat shock protein promoter β-galactosidase activity. Data represent the mean ± S.E. of three independent experiments. *, p < 0.05 versus the LPS-stimulated Il23a promoter-luciferase reporter in WT BMMs.

**FIGURE 2.** Characterization of an ISRE in the Il23a Promoter—Two NF-κB sites have been implicated previously in LPS-induced Il23a promoter (4), but when the ISRE was mutated, the inhibitory effect of IFN-γ was abrogated. Given that IRF-1 interacts with the ISRE, the WT Il23a promoter-luciferase plasmid was expressed in IRF-1−/− BMMs. Interestingly, significantly higher basal and LPS-induced promoter activities were demonstrated, and the inhibitory effect of IFN-γ on LPS-induced activity was diminished (Fig. 1D). These results show that interactions on or around this ISRE are important for the induction and inhibition of Il23a by LPS and IFN-γ and suggest a prominent role for IRF-1 in Il23a regulation.

**IRF-1 Negatively Regulates Il23a Gene Expression in BMMs**—The functional role of IRF-1 in endogenous Il23a regulation was determined next. LPS-activated IRF-1−/− BMMs demonstrated increased expression of Il23a (Fig. 2A) and IL-23 protein (Fig. 2B) compared with WT BMMs. In the presence of IFN-γ, IL-23 levels were higher in IRF-1−/− BMMs than in LPS-activated WT BMMs, although inhibition of LPS-induced IL-23 was still apparent (Fig. 2, A and B). LPS- and IFN-γ-stimulated IRF-1−/− BMMs showed reduced levels of IL-12 p70 (Fig. 2C) and minimal differences in IL-12 p40 (Fig. 2D) compared with WT BMMs, as reported previously (11). To validate these findings, IRF-1 expression in WT BMMs was inhibited using siRNA. Irf1 siRNA effectively decreased IRF-1 expression (Fig. 2E). LPS-induced Il23a expression was increased in WT BMMs transfected with Irf1 siRNA compared with cells transfected with scrambled siRNA (Fig. 2F). An inhibitory effect of IFN-γ on LPS-induced Il23a expression was still observed in Irf1 siRNA transfected BMMs. These results implicate IRF-1 as a negative regulator of LPS-induced Il23a. However, IRF-1 is not solely responsible for IFN-γ-mediated inhibition of IL-23.

**IRF-1 Abrogates RelA Binding to the Il23a Promoter**—Two NF-κB sites have been implicated previously in LPS-induced Il23a regulation (4). The NF-κB sites designated k1, k2, and k3 are 82, 618, and 960 bp, respectively, and ISRE is −378 upstream of the transcription initiation site (TIS). The luciferase reporter construct (Il23a luc) contains all three putative NF-κB sites and the ISRE upstream of the firefly luciferase (luc) open reading frame. EMSA probe 368/388 spans Il23a promoter sequence −368 to −388. The EMSA probe 368/388m contains a mutant ISRE from −378 to −384 bp. 32P-Labeled probes 368/388 (lanes 2–4) and 368/388m (lane 12) were incubated with nuclear extracts from untreated (lane 2), LPS-activated (lane 3), IFN-γ-activated (lane 4), and IFN-γ and LPS-activated (lanes 5–12) BMMs. Lane 1 represents free probe (FP). Two DNA-protein complexes (I and II) were detected with probe 368/388. A 100-fold molar excess of unlabeled probe (368/388u) (lane 6) was added to compete for binding. Probe 368/388 was incubated with nuclear extracts from IFN-γ/LPS-treated BMMs cells for 30 min, and then polyclonal antibodies against IRF-1, IRF-2, and IRF-8 (lanes 1–12) and 368/388m (lanes 2–4) were added. The figure represents similar results from one of three independently conducted experiments. C, chromatin samples from BMMs treated with LPS (100 ng/ml) and IFN-γ (10 ng/ml) were immuno precipitated with anti-IRF-1 and rabbit polyclonal IgG antibodies. Quantitative real-time PCR analysis was performed on precipitated and input DNA. Results are reported as enrichment (%) input of IRF-1 DNA binding. Data represent the mean ± S.E. of three independent experiments. **, p < 0.01 versus unstimulated (Un) Il23a promoter. D, WT BMMs were transfected with an Il23a promoter-luciferase reporter plasmid (Il23a luc) or a plasmid containing a mutant ISRE (Il23a mut luc), and IRF-1−/− BMMs were transfected with the Il23a-luciferase reporter and then cultured with LPS (100 ng/ml; black bars) + IFN-γ (10 ng/ml; gray bars) for 18 h. Reporter activity is presented as luciferase units normalized to heat shock protein promoter β-galactosidase activity. Data represent the mean ± S.E. of three independent experiments. *, p < 0.05 versus the LPS-stimulated Il23a promoter-luciferase reporter in WT BMMs.
activation of the Il23a promoter (5, 12). The ISRE is critically positioned between two NF-κB sites implicated in LPS-induced activation of the Il23a promoter (5, 12). We recently demonstrated that IFN-γ abrogates LPS-induced RelA occupancy on the distal Il23a NF-κB-binding site (4). Therefore, the role of IRF-1 in RelA recruitment was assessed in ChIP assays using PCR primers (from −549 to −680 bp) spanning the distal NF-κB site on the Il23a promoter. As described previously (4), in LPS-activated WT BMMs, RelA promoter occupancy was increased by 30 min but rapidly returned to near base line by 1 h. In LPS-induced IRF-1−/− BMMs, RelA occupancy was prolonged through 1 h compared with WT BMMs (Fig. 3A).

Recruitment of the NF-κB p50 subunit to gene promoters is associated with transcriptional repression (12). The role of IRF-1 in p50 recruitment to the distal NF-κB site on the Il23a promoter was next determined in ChIP experiments. In contrast to RelA, in WT BMMs, no basal occupancy of NF-κB p50 on the Il23a promoter was detected, but promoter occupancy was increased 60 min following LPS stimulation (Fig. 3B), correlating with attenuation of Il23a mRNA expression (4). Unlike WT BMMs, IRF-1−/− BMMs demonstrated elevated base-line association of NF-κB p50 with the Il23a promoter that was markedly abrogated 60 min following LPS activation (Fig. 3B). Validating the functional significance of IRF-1-mediated differential recruitment of NF-κB subunits to the Il23a promoter, Il23a mRNA (Fig. 3C) and IL-23 protein (Fig. 3D) induction was significantly greater in LPS-activated NF-κB p50−/− BMMs compared with WT BMMs (Fig. 3).

Increased Mucosal Expression of IL-23 Correlates with Severe Colonic Inflammation in TNBS-treated IRF-1−/− Mice and IRF-1−/− IL-10−/− Mice—To discern the biological effects of IRF-1 deficiency on the initiation of colonic inflammation, colitis was induced in WT and IRF-1−/− mice by rectal administration of the hapten TNBS. Compared with WT mice on the C57BL/6 background relatively resistant to acute TNBS-induced colonic injury (13), IRF-1−/− mice demonstrated severe colonic inflammation with significantly worsened histological scores (Fig. 4A). The severity of colonic inflammation correlated with increased colonic Il23a mRNA and IL-23 protein expression (Fig. 4, B and C).

Colonic CD11b+ LPMCs from IL-10−/− mice, predominantly composed of macrophages (4), were identified as the major source of Il23a, Il12a, and Il12b (Fig. 5A). CD11b+ LPMCs from IRF-1−/− IL-10−/− mice demonstrated increased basal and heat-killed E. coli-activated Il23a expression compared with IL-10−/− and WT CD11b+ LPMCs (Fig. 5B). To study the consequences of IRF-1 deficiency in the development of spontaneous colitis, colonic inflammation and IL-23 expression were determined in IL-10−/− and IRF-1−/− IL-10−/− mice. 8-week-old IL-10−/− mice demonstrated minimal or no
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Inflammatory changes. However, age-matched littermate IL-10/IRF-1−/− mice developed significant colonic inflammation (Fig. 5C). The severity of colonic inflammation correlated with increased colonic Il23a mRNA and IL-23 protein expression (Fig. 5, D and E). There were no significant differences in colonic Il12a and Il12b expression between Il-10−/− and IRF-1/IL-10−/− mice (supplemental Fig. 2, A and B). These experiments implicate IRF-1 as a negative regulator of IL-23 in colonic inflammation, and suggest that a primary defect in Il23a inhibition in colonic macrophages may underlie the phenotype of severe colitis in an inducible and a spontaneous experimental model.

DISCUSSION

We previously identified an anti-inflammatory role for IFN-γ signaling in macrophages and experimental colitis through attenuation of Il23a expression (4). In this study, an IFN-γ signaling pathway that plays a role in IL-23 regulation was further elucidated. An ISRE was identified within the Il23a promoter involved in LPS induction and IFN-γ inhibition of Il23a expression, respectively. Notably, IRF-1 is a negative regulator of Il23a expression. In mucosal immunity, IFN-γ and IRF-1 may maintain homeostasis through inhibition of IL-23. This hypothesis is supported in vivo through several experimental observations. In colitis-prone mice, colonic CD11b+ LPMCs are a primary source of IL-23 and a target for IFN-γ during the initiation of colonic inflammation. Moreover, IL23a was further elucidated. An ISRE was identified within the Il23a promoter involved in LPS induction and IFN-γ inhibition of Il23a expression (4). We identified an ISRE in the Il23a promoter as a site for IRF-1 recruitment. In the absence of IRF-1, LPS-induced Il23a mRNA and IL-23 protein expression in BMMs was significantly enhanced. Although IRF-1 deficiency did not completely abrogate IFN-γ-mediated inhibition of IL-23, LPS- and IFN-γ-induced IL-23 expression was significantly greater in IRF-1−/− BMMs than in LPS-activated WT BMMs (in the absence of IFN-γ). It is plausible that in...
the absence of IRF-1, other IRFs play a compensatory inhibitory role, consistent with the finding that in luciferase reporter assays, mutation of the ISRE abrogates the inhibitory effect of IFN-γ (15). Alternatively, a combination of IRF-dependent and IRF-independent mechanisms may exist through which IFN-γ regulates Il23a expression (4). It should also be noted that transient transfection of relatively small regulatory regions in episomal reporter plasmids, although historically useful to locate cis-acting elements, may not recapitulate the complex regulation of the endogenous locus (16). Therefore, complete reconciliation of the differences between endogenous gene expression and the Il23a promoter-reporter descriptors herein will require the development of in vivo transgenic reporters using bacterial artificial chromosome transgenes and/or knock-in strategies to track regulation of the entire Il23a locus (16). With these limitations, our work provides insight into paradoxical regulatory roles played by cytokines and transcription factors that have long been considered to drive pro-inflammatory responses. Whereas IFN-γ and IRF-1 are indispensable for optimal IL-12 expression by macrophages and dendritic cells (6), both act as negative regulators of IL-23.

Il23a expression is dramatically reduced in macrophages deficient in the NF-κB family members c-Rel and RelA (5). We recently demonstrated that IFN-γ inhibits LPS-induced Il23a expression through attenuation of RelA and enhancement of NF-κB p50 recruitment to the Il23a promoter (4). In contrast to c-Rel and RelA, NF-κB family members p50 and p52 have been shown to be important for transcriptional gene repression (12, 17). Il23a gene expression is enhanced in p52-deficient macrophages (17). IRFs also interact and cooperate with other transcription factors to regulate target genes. IRF-interacting transcription factors include NF-κB, NFKB, and STAT family members (10). Indeed, the ISRE in the Il23a promoter is situated between two NF-κB sites essential for TLR-mediated Il23a induction (5). In the absence of IRF-1, RelA recruitment to the Il23a promoter in macrophages is prolonged, whereas p50 binding is abrogated, suggesting that IRF-1 functionally interacts with NF-κB. As a functional correlate, we demonstrated that LPS-induced Il23a and IL-23 expression is in fact increased in NF-κB p50−/− BMMs.

The Il23a gene is activated through MyD88-dependent and MyD88-independent pathways (18). LPS-induced MAPK (ERK, JNK, and p38) activation has also been shown to regulate Il23a in macrophages (15, 18, 19). In the RAW 264.7 macrophage cell line, putative SMAD-2-, ATF-2-, IRF-3-, and IRF-7-binding sites were identified through sequence homologies in the Il23a promoter. Deletions in the IRF-3, SMAD-3, ATF-2, or NF-κB sites, but not the IRF-7 sites, resulted in significant loss of Il23a promoter activity in RAW 264.7 cells activated with TLR3 or TLR7 agonists (15). Our newly identified ISRE is distinct from these previously described sites. Moreover, mutations within our identified ISRE abrogate LPS-mediated Il23a induction, suggesting that multiple IRF-ISRE interactions may be essential for Il23a regulation.

Mice on a C57BL/6 background are relatively resistant to colitis development (20). Interestingly, IRF-1−/− mice developed more severe acute colonic injury following TNBS administration compared with WT mice, correlating with increased colonic IL-23 expression. Likewise, IRF-1 deficiency also severely exacerbated IL-23-mediated colitis in IL-10−/− mice on this resistant background. A limitation of this analysis is that IRF-1 deficiency affects multiple innate and adaptive immune pathways (21). For instance, IFN-γ directly inhibits T17 differentiation (22), and IRF-1−/− naïve T cells produce more IL-17 in response to IL-23 (21). However, as in vivo proof of concept that IRF-1 directly mediates regulation of Il23a, we demonstrated increased Il23a expression in the colon and in colonic CD11b+ cells from IRF-1/IL-10−/− mice compared with IL-10−/− mice. In vivo dissection of cell type-specific effects of IRF-1 deficiency on innate and adaptive immunity will require the development of cell type-specific IRF-1 deletions; nonetheless, these findings describe a novel and paradoxical role for IRF-1 in inflammation, implicating Il23a expression as an inciting event.

This study further elucidates signaling pathways that contribute to the protective effects of IFN-γ in chronic inflammation (4). Similarly, in other models such as experimental autoimmune encephalomyelitis and collagen-induced arthritis, IFN-γ gene deletion or administration of anti-IFN-γ antibodies leads to increased severity of disease (23, 24).

Differences in regulation of the p19 subunit of IL-23 and the common p40 subunit of IL-12 and IL-23 may represent an important in vivo checkpoint to shape the subsequent T cell response. T11 and T117 responses are counter-regulatory (3). IFN-γ and IRF-1 may act directly upon the macrophage to attenuate T117 responses through inhibition of IL-23. The IL23R gene contains single nucleotide polymorphisms that confer susceptibility to inflammatory bowel diseases (2). A recent genome-wide association study in ulcerative colitis patients revealed that the most significant chromosome 12q15 association signal was located in a region proximal to Ifng (25). Moreover, a 250-kb risk haplotype within the IBD5 locus on chromosome 5q31 associated with Crohn disease contains IRF1 (26). Therefore, converging genetic and functional evidence suggests that IL-23, IFN-γ, and IRF-1 define an important pathway in experimental and human inflammatory bowel diseases.

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