Crosstalk among Jak-STAT, Toll-like receptor, and ITAM-dependent pathways in macrophage activation

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Abstract: Macrophage phenotype and activation are regulated by cytokines that use the Jak-STAT signaling pathway, microbial recognition receptors that include TLRs, and immunoreceptors that signal via ITAM motifs. The amplitude and qualitative nature of macrophage activation are determined by crosstalk among these signaling pathways. Basal ITAM signaling restrains macrophage responses to TLRs and other activating ligands, whereas strong ITAM signals synergize with the same ligands to activate cells strongly. Similarly, basal ITAM signaling augments IFN signaling and function of receptor activator of NF-κB, but extensive ITAM activation inhibits Jak-STAT signaling. Thus, intensity and duration of ITAM signaling determine whether ITAM-coupled receptors augment or attenuate TLR and Jak-STAT responses. IFN-γ synergizes with TLRs in part by suppressing TLR-induced feedback inhibition, mediated by IL-10 and Stat3, by a mechanism that depends on glycogen synthase kinase (GSK)3 regulation of AP-1 and CREB. IFN-γ suppresses TLR2 and TLR4 induction/activation of AP-1 by overlapping mechanisms that include regulation of MAPKs, GSK3-dependent suppression of DNA binding, and decreased Fos and Jun protein expression and stability. IFN-γ suppression of TLR-induced activation of AP-1 and downstream target genes challenges current concepts about the inflammatory role of AP-1 proteins in macrophage activation and is consistent with a role for AP-1 in the generation of noninflammatory osteoclasts. Jak-STAT, TLR, and ITAM pathways are basally active in macrophages and strongly induced during innate responses. Thus, signal transduction crosstalk is regulated in a dynamic manner, which differs under homeostatic and pathologic conditions, and dysregulation of signal transduction crosstalk may contribute to pathogenesis of chronic inflammatory diseases. *J. Leukoc. Biol.* 82: 237–243; 2007.

Key Words: *cytokine · AP-1/CREB · RANK*

REGULATION OF MACROPHAGE ACTIVATION

Macrophages play important roles in innate immunity, acquired immunity, and tissue homeostasis. During an innate immune response, macrophages serve recognition and effector functions. Macrophages recognize microbial pathogens via multiple cell surface, endosomal, and cytoplasmic receptors, which interact with microbial structures such as cell wall components or nucleic acids (reviewed in ref. [1]). This recognition event leads to activation of macrophage effector functions important in host defense, such as enhanced microbial killing and production of chemokines, cytokines, and mediators, which coordinate the inflammatory response. TLRs mediate cellular responses to microbial lipopeptides (TLR1, -2, and -6), LPS (TLR4), flagellae (TLR5), and nucleic acids (TLR7, -8, and -9) and are among the most potent activators of macrophage inflammatory responses.

It is important to regulate the amplitude and the qualitative nature of TLR-induced inflammatory responses. The amplitude of the response needs to be fine-tuned to achieve effective clearance of pathogens while limiting the amount of inflammation to avoid toxicity and collateral tissue damage. The qualitative nature of the TLR/activating response needs to be modulated to coordinate the most effective innate and acquired immune responses to clear the invading pathogen. For example, gram-negative bacteria elicit classical macrophage activation (also called M1, characterized by production of NO, reactive oxygen intermediates, and inflammatory cytokines such as TNF-α) and downstream Th1 responses, whereas helminths elicit alternative macrophage activation (M2, characterized by production of arginase and a fibrogenic environment) and downstream Th2 responses (reviewed in ref. [2]). M2 macrophages are also important for wound healing and tissue remodeling, which follow infection or tissue injury. The nature of the inflammatory response needs to be modulated to be appropriate for the tissue environment. For example, it is important to avoid ocular inflammatory infiltrates, which would compromise vision, while mounting an appropriate antibody-mediated (IgA) response [3]. The amplitude and qualitative nature of macrophage responses, including those elicited by TLRs, are regulated by endogenous factors produced by the host and its specific tissues in response to the pathogen and related inflam-

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mation [4]. Among the most important of these regulatory factors are cytokines that signal using the Jak-STAT pathway and immunoreceptors that use ITAM-dependent signaling pathways. Cytokines and ITAM-coupled receptors regulate TLR-induced macrophage activation at the level of signal transduction crosstalk (**Fig. 1**).

INFLAMMATORY SIGNALING BY TLRs: WHAT IS THE ROLE OF AP-1 PROTEINS?

TLRs activate complex signaling cascades (reviewed in ref. [5]). TLR signaling results in the downstream activation of three major families of proteins important in activating inflammatory gene expression (Fig. 1): NF-κB/Rel proteins; IRFs; and MAPKs—the ERKs and the stress kinases JNK and p38. NF-κB proteins are transcription factors whose nuclear translocation is triggered by TLR ligands, and NF-κB plays a key role in the transcriptional activation of multiple inflammatory genes. IRFs are induced or activated by TLR ligation and are particularly important in driving Type I IFN production and downstream activation of IFN-inducible genes. MAPKs induce the expression of AP-1 family transcription factors such as Fos and Jun and activate the transcriptional activity of AP-1 proteins by phosphorylation of their transcription activation domains. AP-1 proteins have been implicated in invasive cell growth and matrix metalloprotease (MMP) production and in cell line models, have been suggested to mediate induction of inflammatory genes such as TNF-α. The current paradigm is that AP-1 proteins are key mediators of the inflammatory effects of MAPKs. However, evidence for an inflammatory role of AP-1 proteins in the responses of primary cells to physiological or microbial factors is sparse. Indeed, Fos actually suppresses the expression of IL-12 [6, 7], and gene knockout studies have revealed that a primary role for AP-1 in myeloid lineage cells is the generation of osteoclasts [8], bone-resorbing cells, which have lost the capacity to produce inflammatory cytokines [9]. Thus, the role of AP-1 proteins in inflammatory macrophage activation has not been clarified. Although MAPKs are clearly proinflammatory, they can contribute to inflammation by many mechanisms other than activation of AP-1. For example, MAPKs can activate Ets and CREB/activating transcription factor, stabilize mRNAs, which encode inflammatory cytokines, induce efficient translation of TNF-α mRNA, and lead to phosphorylation of histones at inflammatory gene loci [10].

ITAM-COUPLED RECEPTORS AND CROSSTALK WITH TLRs

Macrophages express a panoply of cell surface receptors which are coupled with the ITAM-containing adaptor molecules FcRγ chain and DNAX-activating protein 12 (DAP12). FcγR-coupled receptors include receptors for the Fc region of Igs (FcRs), osteoclast-associated receptor (OSCAR), paired Ig-like receptor A (PIR-A), and leukocyte Ig-like receptor (ILT/LIR; reviewed in ref. [11]). DAP12-coupled receptors include triggering receptor expressed on myeloid cell 1 (TREM1), TREM2, and signal regulatory protein-β1 (SIRPβ1). Investigation of the biology of these receptors has been limited by the lack of known ligands, except in the case of FcRs and the recent description of a TREM2-Plexin A1 receptor complex, which is activated by Semaphorin 6D [12]. However, a macrophage-activating function for many of these receptors was inferred initially based on the activating nature of signaling pathways triggered downstream of ITAMs (see below), studies using receptor crosslinking antibodies, and the phenotypes of FcRγ- and DAP12-deficient mice, which showed diminished immune and inflammatory responses [13–16]. ITAM-mediated signaling is initiated by Src family kinase phosphorylation of the two ITAM motif tyrosines, leading to the recruitment and activation of the PTK Syk and downstream signaling pathways, which include activation of PTKs, NF-κB, MAPKs, and protein kinase C (PKC) and calcium-mediated signaling pathways (via activation of phospholipase Cγ).

ITAM signaling, similar to TLR signaling, activates NF-κB and MAPKs and thus can augment TLR-induced activation of these molecules (Fig. 1). In addition, ITAM-mediated induction of PTK cascades and calcium signaling offers the potential for synergistic activation of cells by TLRs and ITAM-coupled receptors. One clear example of synergy between TLRs and ITAM-coupled receptors is potentiation of LPS-induced endotoxemia by TREM-1, a DAP12-coupled receptor [17]. A potential molecular mechanism underlying such synergy is provided by reports describing collaboration between TLR2 and dectin-1, a β-glucan receptor that contains a cytoplasmic, ITAM-like motif, which signals via Srcs and Syk. Dectin-1

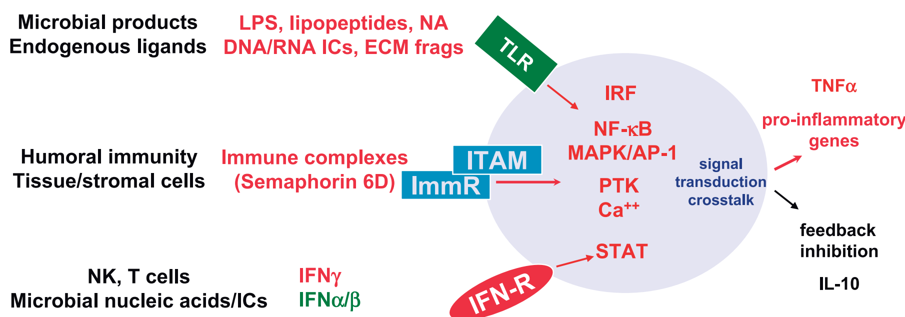

Fig. 1. Crosstalk among major macrophage classical activation pathways. TLR signaling results in the downstream activation of three major families of proteins important in activating inflammatory gene expression: NF-κB/Rel proteins, IFN regulatory factors (IRFs), and MAPKs. ITAM signaling, similar to TLR signaling, activates NF-κB and MAPKs and thus can modulate TLR-induced activation of these molecules and also, more strongly activates tyrosine kinase cascades and calcium signaling. IFN-γ is one of the most potent endogenous macrophage-activating factors and uses the Jak-STAT signaling pathway. Semaphorin 6D–ITAM-containing receptor interactions are indirect and mediated by plexins. NA, nucleic acids; ICs, immune complexes; ECM frags, extracellular matrix fragments; ImmR, immune receptors; PTK, protein tyrosine kinase; IFN-R, IFN receptor.

activates a respiratory burst and collaborates with TLR2 in promoting inflammatory cytokine production [18].

It is becoming increasingly clear that ITAM-coupled receptors can also negatively regulate TLR function (reviewed in ref. [19]). Cells deficient in DAP12 are hyper-responsive to TLR stimulation, and the FcRγ chain mediates negative regulation of FcεR and FcγR function by FcαR. TREM2 mediates, in large part, the suppressive effects of DAP12 on TLR responses [20, 21], and additional ITAM-coupled receptors, including NKp44, PIR-A, and ILT-7, exert negative effects on cell activation [22]. The inhibitory functions of these receptors are dependent on the ITAM motif, which mediates activating functions. The mechanisms underlying inhibitory actions of ITAMs have not been fully clarified but include a role for ERK and recruitment of phosphatases [Src homology-2-containing tyrosine phosphatase 1 (SHP-1), SHP-2, SHIP] to the ITAM. A model has been proposed, whereby low-level ITAM signaling (such as occurs in physiological settings) negatively regulates cell activation by other receptors such as TLRs, whereas high-level ITAM signaling (such as may occur during infection) activates macrophages in synergy with parallel signaling pathways.

IFN-γ AND Jak-STAT SIGNALING

IFN-γ is one of the most potent endogenous macrophage-activating factors. In contrast to TLRs, IFN-γ is at best a weak activator of NF-κB and MAPKs. Instead, IFN-γ uses the Jak-STAT signaling pathway (Fig. 1). Binding of IFN-γ to its cell surface receptor leads to the activation of receptor-associated Jak protein tyrosine kinases, followed by tyrosine phosphorylation and activation of latent cytoplasmic proteins—STATs—which in turn, dimerize and translocate to the nucleus, where they bind to promoter sequences and activate transcription. STAT transcriptional activity is potentiated by serine phosphorylation of transcription activation domains, which can be mediated by multiple kinases, including MAPKs, PKC, and calmodulin-dependent protein kinase II. IFN-γ activates Stat1 predominantly, which mediates the activating functions of IFN-γ, including enhanced microbial killing, increased antigen presentation, and enhanced inflammatory cytokine production.

It was recognized early on that a key function of IFN-γ is to synergize with activating stimuli such as TLR ligands [23]. In addition, pretreatment of macrophages with IFN-γ sensitizes these cells to exhibit enhanced responses to TLRs, ITAM-coupled FcγRs, and IFNs themselves; this phenomenon has been termed priming. Mechanisms underlying synergy/priming have been studied extensively (reviewed in ref. [24]) and include IFN-γ-induced increases in TLR and FcγRI expression; synergistic activation of promoters by NF-κB and Stat1, which bind to their distinct cognate promoter elements; enhanced activation of NF-κB; and additive functions of NF-κB/MAPK-induced and Stat1-induced target genes. We have proposed that IFN-γ can also potentiate TLR activity by interrupting TLR-induced feedback-inhibition loops, and underlying mechanisms will be discussed below.

CYTOKINE BALANCE AND HOMEOSTASIS

Production of anti-inflammatory cytokines is an important mechanism for limiting inflammation and establishing homeostasis (**Fig. 2**). Among the most potent suppressive factors for myeloid cells are IL-10, TGF-β, IL-1RA, and glucocorticoids. It is interesting that these factors are induced or activated post-translationally by TLRs, thereby establishing feedback inhibition loops, which limit cell activation. The balance between activating factors and homeostatic factors will determine the extent of inflammation (Fig. 2). Relative expression of opposing cytokines is an important determinant of cytokine balance. In addition, it is important to consider that crosstalk among these various cytokines will impact on their biological activity and thus, on cytokine balance. For example, attenuation of IL-10 signaling and target gene activation contributes to the dysregulation of cytokine balance in chronic inflammation in rheumatoid arthritis (RA) [25, 26].

IL-10 is a potent suppressor of TLR-induced gene expression and inflammatory cytokine production and can also suppress IFN-γ responses [27]. All of the known suppressive effects of IL-10 are dependent on Stat3. Thus, Stat1, which is activated by IFN-γ, and Stat3, activated by IL-10, oppose each other's activity in the regulation of inflammation (Fig. 2). Pleiotropic cytokines such as Type I IFNs and IL-6 activate Stat1 and Stat3, and the balance between Stat1 and Stat3 activation by these cytokines can determine the balance of their pro- and anti-inflammatory functions [28]. The balance between opposing Stat1 and Stat3 activity represents one axis, which receives inputs from many cytokines and regulates the inflammatory status of macrophages (Fig. 2). As discussed below, Stat1 and Stat3 activity is regulated by many factors in addition to cytokines, and thus, regulation of these STATs represents a mechanism for integrating multiple environmental signals.

REGULATION OF Jak-STAT SIGNALING BY ITAM-DEPENDENT PATHWAYS

Our laboratory reported recently that IFN-α-induced activation of Stat1 is amplified by the ITAM-containing adapters FcRγ

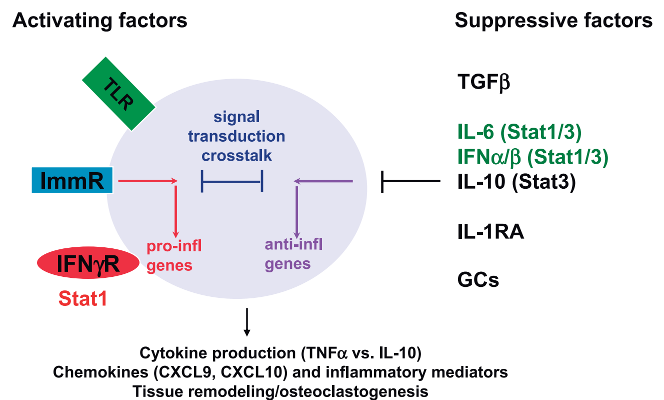

Fig. 2. Balance of cytokine action determines severity of inflammation. Many suppressive factors are induced as part of homeostatic feedback inhibition. Crosstalk at the signal transduction level contributes to cytokine balance. pro-infl, Proinflammatory; anti-infl, anti-inflammatory; IL-1RA, IL-1 receptor antagonist; GCs, glucocorticoids.

and DAP12 and the downstream kinase Syk [29]. Syk-mediated amplification of Stat1 activation is most apparent in IFN-γ-primed macrophages, which express high Stat1 levels. The shift in IFN-α-induced STAT activation toward increased Stat1 activation relative to Stat2 and Stat3 activation results in enhanced expression of Stat1-dependent inflammatory genes such as the chemokines CXCL9 and CXCL10 and a shift to a more inflammatory phenotype. We proposed a model whereby IFN-γ induced expression of a FcRγ- or DAP12-coupled receptor, which mediates interactions with the IFN-α receptor (IFNAR; **Fig. 3**). Syk can phosphorylate Stat1 directly [29] or could phosphorylate IFNAR Stat1-docking sites (Fig. 3). These results establish positive crosstalk between ITAM-dependent and Jak-STAT pathways. The ITAM-coupled receptor, which mediates crosstalk with IFNAR, is not yet known. As basal Syk activity is detected in unstimulated macrophages, this receptor is likely stimulated at a low level by endogenous ligands acting in an autocrine or paracrine manner, similar to basal activity of other ITAM-coupled receptors, such as TREM2 [20]. In addition to increasing tyrosine phosphorylation, ITAM-coupled receptors can increase Stat1 serine phosphorylation, thereby enhancing transcriptional activity [30].

Osteoclastogenesis represents another scenario, where low-level basal ITAM signaling enhances signaling by a different receptor system—in this case signaling by RANK, a member of the TNFR family [31]. Ligation of the DAP12-associated, ITAM-coupled receptors TREM2 and SIRPβ1 by endogenous ligands, expressed on myeloid lineage osteoclast precursors, "costimulates" RANK signaling by regulating calcium oscillations, leading to expression and activation of NFATc1 and osteoclastogenesis (Fig. 3). A recent, exciting discovery identified Semaphorin 6D as an endogenous ligand for a receptor complex containing plexin A1-TREM2-DAP12 [12]. The current model is that plexin A1 is involved in direct ligand interaction, and TREM2 and DAP12 contribute to signaling induced by Semaphorin 6D. Costimulation of RANK signaling is also achieved by the FcRγ-coupled receptors OSCAR and PIR-A, whose ligands appear to be expressed by osteoblasts, mesenchymal cells that regulate osteoclastogenesis. Thus, ITAM-mediated costimulation of RANK signaling can be accomplished in trans, making possible the regulation of this macrophage signaling system by other cell types. Collectively, these studies establish that low-level/basal ITAM signals can augment signaling by different macrophage-activating pathways, including Jak-STAT- and RANK-mediated pathways.

In sharp contrast, high-intensity activation of ITAM signaling pathways can inhibit Jak-STAT signaling. Extensive crosslinking of TCRs or of FcγRs by opsonized erythocytes or plate-bound (insoluble), immune complexes suppresses STAT activation by IL-2, IL-4, and IL-10 in, respectively, T cells and macrophages [26, 32, 33]. In addition, activation of the atypical ITAM motif in the Dectin-1 β-glucan receptor likely contributes to zymosan-mediated inhibition of IL-10 signaling [34]. The mechanisms of inhibition of IL-2, IL-4, and IL-10 are rapid and dependent on MAPKs and PKC, which possibly work by targeting cytokine receptors. Similar to ITAM-mediated inhibition in other systems [19], ITAM-mediated inhibition of Jak-STAT signaling can also involve recruitment of SHP-2 [35]. ITAM-mediated signaling can also suppress Jak-STAT signaling indirectly, via induction of IL-10 production [36]. Finally, prolonged stimulation of FcRγ-associated FcγRIII by immune complexes leads to attenuation of IFN-γ signaling by a mechanism, which is not ITAM-dependent but involves suppression of IFN-γ receptor subunit 2 expression [37]. Thus, ITAM-dependent signals can augment or attenuate Jak-STAT signaling. Similar to ITAM regulation of TLRs, whether the effect is positive or negative appears to depend on the intensity and duration of ITAM signaling.

IFN-γ INHIBITS TLR2-INDUCED FEEDBACK INHIBITION BY SUPPRESSING IL-10 PRODUCTION

We reported recently that IFN-γ suppresses TLR-mediated induction of IL-10 expression and downstream Stat3 activation, thereby interrupting a TLR2-induced feedback inhibition loop (ref. [38] and **Fig. 4**). Suppression of IL-10 expression results in increased production of the inflammatory cytokines TNF-α and IL-6, demonstrating that abrogation of feedback inhibition contributes to synergistic activation of macrophages by IFN-γ and TLR ligands. The mechanism of inhibition of TLR2-induced IL-10 production involves attenuation of TLR2-induced MAPK activation, which results in diminished AP-1

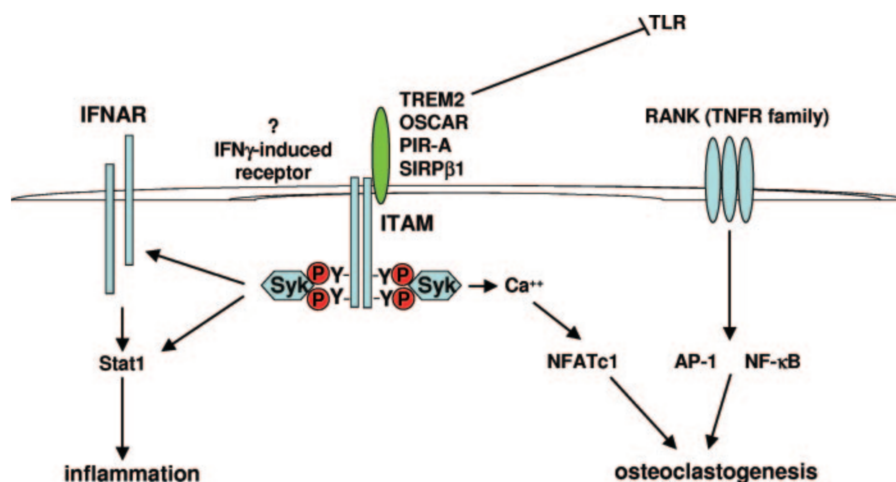

Fig. 3. Costimulation of macrophage responses and osteoclastogenesis by ITAM-dependent signaling pathways. IFN-γ may induce expression of a FcRγ- or DAP12-coupled receptor, which mediates interactions with the IFNAR. IFN-α-induced activation of Stat1 is amplified by the ITAM-containing adapters FcRγ and DAP12 and the downstream kinase Syk, which can phosphorylate Stat1 directly or could phosphorylate IFNAR Stat1-docking sites. On myeloid lineage osteoclast precursors, ligation of the DAP12-associated, ITAM-coupled receptors by endogenous ligands costimulates receptor activator of NF-κB (RANK) signaling by regulating calcium oscillations, leading to expression and activation of NFATc1 and osteoclastogenesis. Basal TREM2 and DAP12 signaling negatively regulates TLR responses.

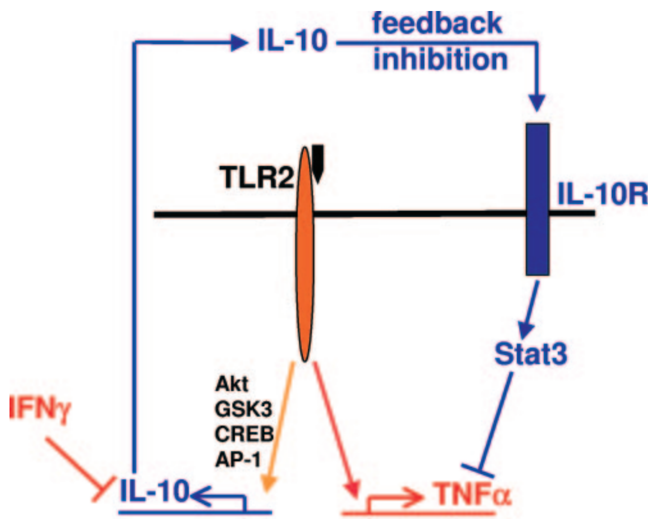

Fig. 4. IFN-γ suppresses TLR2-induced feedback inhibition by decreasing IL-10 production and downstream Stat3 activation, thereby interrupting a TLR2-induced feedback-inhibition loop. Suppression of IL-10 expression results in increased production of the inflammatory cytokines such as TNF-α. GSK3, Glycogen synthase kinase 3.

(Fos and Jun) expression and diminished CREB phosphorylation on the transcription-activating site serine 133. In addition, IFN-γ suppresses TLR2-induced Akt activity, which is linked with decreased phosphorylation and increased activity of GSK3β, which in turn, contributes to inhibition of AP-1 DNA binding [38], likely by phosphorylation of the Jun DNA-binding domain [39]. Diminished AP-1 and CREB activity in IFN-γ-primed, TLR2-activated cells leads to decreased expression of AP-1 and CREB target genes, including MMP-1 (collagenase), plasminogen activator inhibitor-2 (PAI-2), and IL-10 [38]. More extensive analysis of gene expression using microarray analysis showed that IFN-γ inhibits the expression of ~15% of TLR2-inducible genes, including multiple putative AP-1 and CREB target genes (**Fig. 5**). Inhibition or ablation of GSK3β in vivo results in suppression of peritonitis and arthritis [38, 40]. These results along with work from other labs [41] suggest a central role for GSK3 in integrating inputs from macrophage-activating receptors and regulating the balance of pro- and anti-inflammatory cytokine production by regulating AP-1, CREB, and NF-κB (**Fig. 6**).

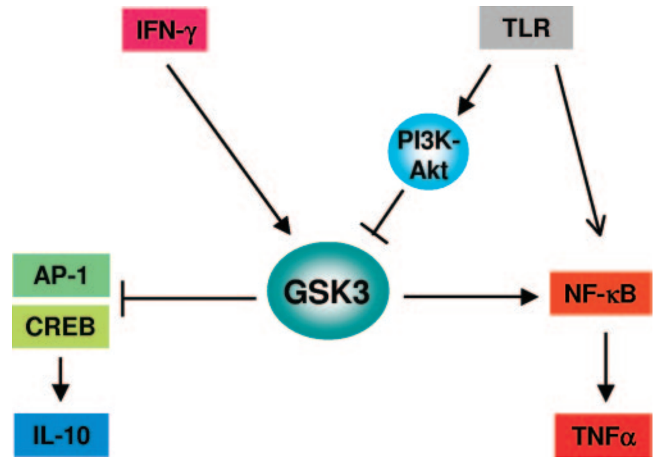

Fig. 6. GSK3 integrates inflammatory signals and regulates the balance of cytokine production. IFN-γ suppresses TLR2-induced Akt activity, linked with decreased phosphorylation and increased activity of GSK3β, which in turn, contributes to inhibition of AP-1 DNA binding. Diminished AP-1 and CREB activity in IFN-γ-primed, TLR2-activated cells leads to decreased expression of AP-1 and CREB target genes such as IL-10. In IFN-γ-primed macrophages, constitutively active GSK3 could also potentiate activation of NF-κB and promote the expression of proinflammatory mediators such as TNF-α.

REGULATION OF TLR4 RESPONSES BY IFN-γ: SUPPRESSION OF Fos AND Jun PROTEIN EXPRESSION

Similar to TLR2, IFN-γ suppresses TLR4-induced expression of IL-10 by a mechanism that involves GSK3 (ref. [38] and data not shown). However, in contrast to TLR2, IFN-γ does not suppress TLR4-induced MAPK activation [42]. This prompted us to investigate the effects of IFN-γ on TLR4 induction and activation of AP-1 proteins. Consistent with intact MAPK

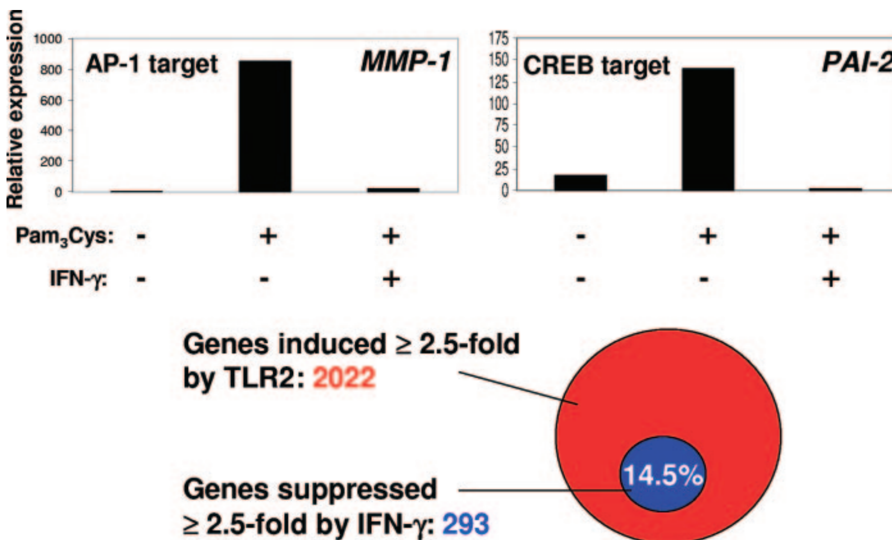

Fig. 5. Suppression of TLR2-induced gene expression by IFN-γ. Microarray analysis was performed using human primary macrophages, which were stimulated with IFN-γ (100 U/ml) for 1 day and then with the TLR2-activating lipopeptide Pam_3Cys for 6 h. IFN-γ inhibited the expression of ~15% of TLR2-inducible genes, including multiple putative AP-1 and CREB target genes.

activation, LPS-induced Fos and Jun mRNA expression was not inhibited in IFN-γ-preactivated macrophages (**Fig. 7A**). In contrast, pretreatment of macrophages with IFN-γ effectively suppressed basal and TLR4-induced expression of whole cell and nuclear Fos and Jun proteins, as measured by immunoblotting (Fig. 7, B and C). The proteasome inhibitor MG132 reversed IFN-γ suppression of Fos and Jun protein expression (Fig. 7C). These results indicate that IFN-γ negatively regulates Fos and Jun protein expression and suggest that the mechanism is increased proteasomal degradation of these already labile proteins. IFN-γ changes the composition of the proteasome and alters/increases proteasomal function [43], and mechanisms by which IFN-γ regulates Fos and Jun proteins will be investigated further in future work. These results clarify that IFN-γ suppresses AP-1 expression and function downstream of TLR2 and TLR4 and demonstrate that there are several mechanisms of suppression of AP-1, which can be differentially used downstream of different TLRs. In addition, this work highlights the suppression of AP-1 activity by IFN-γ during synergistic inflammatory activation of macrophages by IFN-γ and TLRs and raises questions about the role of AP-1 transcription factors in macrophage biology.

CONCLUSION

There is a complex crossregulation among major macrophage-activating pathways mediated by TLRs, ITAM-coupled immunoreceptors, and cytokines/STATs at the level of signal transduction. All three of these pathways are basally active at low levels under physiological, homeostatic conditions [4, 19, 20, 44, 45]. This basal activity will reflect the state of the host [4] and will help determine the magnitude and qualitative nature of cellular responses to other ligands. For example, basal ITAM activity can suppress TLR responses and enhance IFN and RANK responses. These three pathways are activated strongly during innate immune responses. It is interesting that strong activation of these receptors can have the opposite effects from basal activity. For example, ITAM-coupled receptors cooperate with TLRs in driving inflammation under conditions of infection, and strong activation of ITAM-mediated signals inhibits rather than amplifies Jak-STAT signaling. Thus, signal transduction crosstalk is regulated in a dynamic manner and differs under homeostatic and pathologic conditions. It is possible that dysregulation of signal transduction crosstalk contributes to the pathogenesis of chronic inflammatory diseases. For example, abrogation of IL-10 signaling and function, which may be

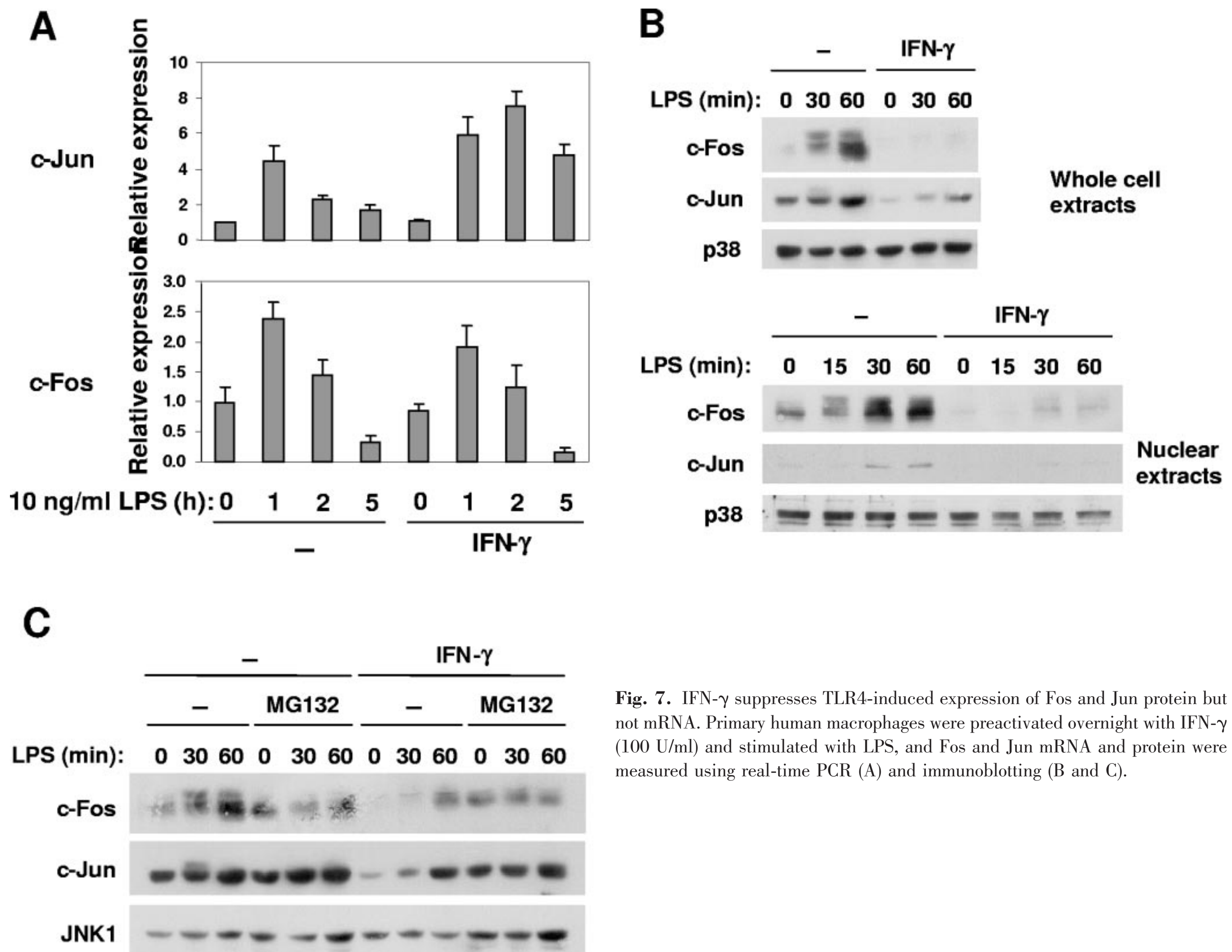

Fig. 7. IFN-γ suppresses TLR4-induced expression of Fos and Jun protein but not mRNA. Primary human macrophages were preactivated overnight with IFN-γ (100 U/ml) and stimulated with LPS, and Fos and Jun mRNA and protein were measured using real-time PCR (A) and immunoblotting (B and C).

helpful during acute infection, will likely be deleterious in chronic inflammatory diseases such as RA.

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