Infection-induced IL-10 and Jak-Stat: a review of the molecular circuitry controlling immune hyperactivity in response to pathogenic microbes

Alison J. Carey, Chee K. Tan, and Glen C. Ulett†

School of Medical Sciences, Centre for Medicine and Oral Health, Griffith University
Gold Coast Campus, QLD, Australia 4222

Running Title: Infection-induced IL-10 and Jak-Stat

Conflict of Interest: All authors have no conflict.

Word Count: Abstract 100, Text 4022

Character Count: 48,726 (including all text, spaces, references, and figure legends)

Total Display items: 1 Figure

† Correspondence and Reprints: Glen C Ulett, School of Medical Sciences, Centre for Medicine and Oral Health, Griffith University, Gold Coast Campus, QLD, Australia 4222; phone +61 7 5678 0765; fax +61 7 5678 0795; email g.ulett@griffith.edu.au
Abstract

Generation of effective immune responses against pathogenic microbes depends on a fine balance between pro- and anti-inflammatory responses. Interleukin-10 (IL-10) is essential in regulating this balance and has garnered renewed interest recently as a modulator of the response to infection at the Jak-Stat signaling axis of host responses. Here, we examine how IL-10 functions as the ‘master regulator’ of immune responses through Jak-Stat, and provide a perspective from recent insights on bacterial, protozoan, and viral infection model systems. Pattern recognition and subsequent molecular events that drive activation of IL-10-associated Jak-Stat circuitry are reviewed and the implications for microbial pathogenesis are discussed.
Balanced antimicrobial defense hinges on the IL-10-Jak-Stat module

Innate immune activation in response to microbial pathogens occurs as a result of recognition of foreign microbes or their products by phagocytes via pattern recognition receptor (PRR)-dependent mechanisms. This leads to the activation of crucial phagocyte effector functions to combat microbes including the synthesis of reactive oxygen and nitrogen species, and generation of phagolysosomal proteases that mediate killing of invading microbes. Recognition of microbes by immune surveillance cells such as macrophages using PRRs initiates a signaling cascade that leads to the production of cytokines including interleukins (IL) and chemokines that drive antimicrobial mechanisms and regulate inflammatory responses to clear infection and achieve convalescence.

The transition of macrophages into effector cells for antimicrobial killing typically stems from the classical pathway of activation by T cell- or NK cell-derived interferon (IFN)-γ. This largely occurs via the family of signal transducers and activators of transcription known as the Stat proteins that relay activation messages from ligated cytokine receptors at the cell surface to the nucleus for transcriptional activation. Stat1, after its phosphorylation at the IFN-γ receptor, is a starting point for classical macrophage activation because it induces a broad transcriptional program that includes many antimicrobial effector mechanisms. Stat3, on the other hand, has been labeled ‘the anti-inflammatory Stat’. These two opposing Stat signaling mechanisms exist at the crossroads of immune activation and suppression, and it is here that Stats influence microbial disease pathogenesis. Interruption or interference to normal Stat signaling mechanisms can dramatically alter the host response to infection with
various pathogens and predispose individuals to disease as recently reviewed elsewhere.\textsuperscript{3}

In the last few years, IL-10 has garnered renewed interest as a key modulator of innate immune responses to pathogenic microbes because several studies have revealed novel functions of this cytokine in the control of infectious disease.\textsuperscript{4-8} An emerging theme is to resolve how PRRs such as Toll-like receptors (TLRs) coordinate their actions upon sensing foreign microbes with Janus kinase (Jak)-Stat circuitry to link IL-10 with diverse pathogen recognition events, microbial survival strategies, and downstream effector mechanisms for host defense. Host responses to microbes resulting from the ligation of TLRs such as TLR4 for LPS\textsuperscript{9}, TLR2 for lipoteichoic acid\textsuperscript{10}, TLR5 for flagellin monomers\textsuperscript{11}, and TLR9 for CpG motifs\textsuperscript{12} are carefully regulated to control the degrees of immune activation and suppression during disease.\textsuperscript{13} The role of IL-10 as a major regulator that connects these recognition events with appropriately balanced pro- and anti-inflammatory responses is underscored by these recent studies, which illustrates the complexity of IL10 actions in overall host defense at the axis of infection-immunity.\textsuperscript{4-8}

Molecular pathways of IL-10 signaling through Jak-Stat are not only associated with antimicrobial defense against potential pathogens but evidence is accumulating that these pathways are also involved in tolerance to commensal flora. \textit{Lactobacillus rhamnosus} for example, as a normal microbe inhabitant of the placental mucosa, triggers signaling pathways involving IL-10 and Jak-Stat to control tumor necrosis factor (TNF)-\textalpha{} production, which prevents pre-term birth.\textsuperscript{14} This example of moderation of local inflammatory conditions by lactobacilli during pregnancy is not the
only commensal-host interaction that relies on the IL-10-Jak-Stat circuitry for good human health outcomes. The role of IL-10 and Stat3 in maintenance of tolerance and homeostasis in the gut, for example, is evident from seminal papers describing the development of chronic enterocolitis in gene-deficient mice.\textsuperscript{15, 16} More recently, the identification of pediatric patients with mutations in the IL-10 receptor who develop enterocolitis shows the relevance of IL-10 for tolerance to gut commensals in the human system.\textsuperscript{17} These observations show that commensals interact with local immune surveillance mechanisms and IL-10 and its related Jak-Stat signaling module serves to safeguard against potentially tissue-damaging inflammation. Importantly, the underlying molecular mechanisms of immune signaling that occur subsequent to commensal or pathogen detection and IL-10 production, including how IL-10 affects Jak-Stat circuitry, how it deactivates pathogen-sensing cells, and how this influences microbial clearance during infection is an area of intense current research. Here, we examine recent studies of IL-10 at the nexus of infection immunity in the context of immune suppression through Jak-Stat and consider the consequences of downstream signaling through this module for microbial pathogenesis.

**Diverse pathogens induce IL-10 and activate the IL-10 receptor complex**

IL-10 is a prototypic anti-inflammatory cytokine that is produced in response to a multitude of pathogens\textsuperscript{18} and acts as the master regulator of immunity to infection as recently reviewed elsewhere.\textsuperscript{19} In acute infection, one of the central roles for IL-10 is to deactivate macrophages and terminate inflammatory responses in order to limit excessive release of tissue-damaging, pro-inflammatory mediators that are synthesized by cells such as macrophages to kill microbes. IL-10 is released from various cells including macrophages, dendritic cells, subsets of CD4\textsuperscript{+} and CD8\textsuperscript{+} T
cells, and B cells, and therefore functions as a vital immune modulator at various stages of infection.\textsuperscript{19} The role of IL-10 in limiting collateral tissue damage that arises from acute inflammation in both infectious and non-infectious disease has been increasingly characterized over the past 5 years.\textsuperscript{20-24} In addition to acute infectious conditions and the aforementioned effects mediated by commensal flora, the influence of IL-10 on microbial pathogenesis is nuanced in states of chronic infection such as with mycobacteria, for example, where the immune suppressive effects of IL-10 can promote the survival of microorganisms and contribute to persistent disease. In this regard, some pathogens appear to pro-actively induce IL-10 as a virulence strategy to interfere with inflammation and pro-actively abrogate antimicrobial effector functions. \textit{Mycobacterium avium}, for example, is one of several pathogenic bacteria that induces IL-10 which, following ligation of the IL-10 receptor complex and the triggering of subsequent Jak-Stat signaling cascades, influences the progression of infection (Fig 1).\textsuperscript{25} Similarly, \textit{M. avium}-induced IL-10 prevents TNF-\(\alpha\) production and macrophage apoptosis, and this may represent one possible mechanism for increased pathogen survival by providing an intracellular niche.\textsuperscript{25} Other examples of IL-10 induction by pathogens leading to chronic infection include \textit{Leishmania major}\textsuperscript{26}, and \textit{M. tuberculosis}.\textsuperscript{27,28}

Functionally, IL-10 exerts its immune suppressive and other effects by interacting with the IL-10-specific receptors, IL-10 receptor-\(\alpha\) (IL-10R1) and -\(\beta\) (IL-10R2). These receptors partner as a complex and are expressed only on hematopoietic cells including B cells, T cells, NK cells, macrophages and monocytes.\textsuperscript{29} Both are members of the class II cytokine receptor family.\textsuperscript{30} IL-10R1 acts as the ligand binding chain while IL-10R2 functions as the accessory chain that
recruits Jaks to the intracellular domain. Activation of the IL-10 receptor complex necessitates a tetramer consisting of two IL-10R1 and two IL-10R2 chains, which bind homodimeric IL-10 to the extracellular domains of IL-10R1 (Fig 1). IL-10R2 does not bind to IL-10 directly and binding of IL-10 to IL-10R1 without the co-presence of IL-10R2 fails to initiate signal transduction and relay of the immune regulatory message from IL-10. Successful engagement of the IL-10 receptor complex subsequently activates distinct Jak-Stat pathways and downstream signaling events that converge through various mechanisms to influence nuclear transcriptional events such as those mediated by NF-κB (Fig 1).

**IL-10 biological effects during infection occur via Jak-Stat signal relay**

Engagement of the Jak-Stat signaling circuit by ligation of the IL-10 receptor complex occurs principally through Stat3. This is most likely to involve mediator genes induced by Stat3 transcriptional activity because the inhibitory effects of IL-10 require new protein synthesis. Recently, activated Stat3 was shown to initiate the biological effects of IL-10 in a tristetraprolin (TTP)-dependent manner. TTP removes certain cis-acting A+U rich (ARE)-containing unstable mRNAs such as TNF-α but is not the sole major mediator of IL-10-inhibitory effects because of the emerging picture that there is no single master mediator of IL-10-induced deactivation. Rather, multiple IL-10-induced genes control certain aspects of macrophage activation/deactivation and mediate distinct parts of the anti-inflammatory effects of IL-10. These include, for example, Bcl-3, which controls TNF-α but not IL-6, Dusp1, which blocks p38 mitogen-activated protein kinase (MAPK) and inhibits IL-6 and several chemokines (but not IL-12p40) and Nfil3, which inhibits IL-12p40 expression. Stat3-induced genes appear to act as transcriptional suppressors, inhibiting the recruitment of activating factors to
target promoters, and may even induce secondary mediators.\(^1\) Products from Stat3 target genes can impede signal transduction from cell surface receptors and activate NF-κB and MAPK pathways that directly control pro-suppression and pro-inflammatory transcriptional events in the nucleus (Fig 1). While some studies have shown that IL-10 can activate Stat1 directly\(^{36,37}\) this pathway of signaling has not been associated with the anti-inflammatory effects of IL-10, and the precise outcomes of IL-10 signal relay through Stat1 remain unclear. Moreover, while IL-10 mediates its suppressive effects via the activation of Stat3, it is not the only cytokine to do so. IL-6 family cytokines, including IL-6 and IL-11 also activate Stat3 and, to a lesser extent, Stat1 as well as MAPK and phosphatidylinositol 3-kinase (PI3K) cascades through the gp130 receptor to bring about its pleiotropic actions including anti-inflammatory effects, as recently reviewed elsewhere.\(^{38,39}\)

Upon binding of IL-10 to its receptor complex, phosphorylation of the receptor-associated protein tyrosine kinases, Jak1 and Tyk2 occurs (Fig 1). Jak1 is recruited to the intracellular domain by the IL-10R1 chain, while Tyk2 is recruited to the receptor complex by IL-10R2.\(^{40}\) These kinases serve as a temporary docking site for inactive cytosolic Stat1 and/or Stat3\(^{41}\), which are recruited by Jak1 and Tyk2 to the site upon phosphorylation of the IL-10R1 chain at two tyrosine residues.\(^{29}\) The Stats bind to the IL-10R1 chain via the Src homology 2 (SH2) domain and are tyrosine-phosphorylated by the receptor-associated Jaks. Activation of Stat3 leads to its homodimerization (similar to Stat1) and, although Stat1:Stat3 heterodimers have been described, evidence for these in IL-10 signal transduction is lacking.\(^{37,41}\) Translocation of activated Stats to the nucleus enacts high affinity binding with Stat-binding elements (SBEs), which promotes transcription of IL-10 responsive genes, including, for
example, transcriptional regulators (Bcl-3, Nfil3), signaling modulators (Dusp1), and those that can contribute to alternative macrophage activation.\textsuperscript{42}

Regulation of Jak-Stat signal transduction downstream of the IL-10 receptor complex occurs at both the extracellular and intracellular interface to prevent excessive immune suppression or alternative macrophage activation that can impede efficient antimicrobial activity. Several regulators antagonize the translocation of signal transducers that induce the transcription of various IL-10 responsive genes within the nucleus such as those aforementioned. The most studied regulators in this regard are the suppressors of cytokine signaling (Socs) proteins. Socs1 and Socs3 are both produced in response to IL-10, and both function to suppress Jak-Stat signaling, but via different mechanisms.\textsuperscript{43} In this manner, Socs1 and Socs3 regulate pro-inflammatory responses including TNF-\(\alpha\), IFN-\(\gamma\), IL-6, and nitric oxide production via negative feedback loops that operate alongside IL-10 signaling.\textsuperscript{43-48} Where Socs1 suppresses Jak activity directly by binding of its SH2 domain to tyrosine-phosphorylated Jak, Socs3 necessarily binds to the activated receptor to inhibit Jak activity.\textsuperscript{43} Both Socs also indirectly affect IL-10-associated Jak-Stat signaling through the MAPK system.\textsuperscript{49, 50} With regard to pro-IL-10 responses, Socs3 is produced in an LPS-dependent manner in macrophages as a result of TLR4 signaling, which activates the MAPK signaling cascade of ERK1/2, p38 and JNK.\textsuperscript{51} This induces IL-10, which drives Jak-Stat by activating Stat3, and this triggers further Socs3 production.\textsuperscript{51} IL-10 induces Stat3 activation in monocytes\textsuperscript{52} thereby suppressing pro-inflammatory responses including TNF-\(\alpha\) and NO production.\textsuperscript{45, 46} Socs3 is also the main negative feedback regulator for IL-6-mediated activation of the Jak-Stat pathway.\textsuperscript{53} However, it is important to note that Socs3 is not a mediator of macrophage deactivation \textit{per se},
although it is induced strongly by IL-10. Instead, Socs3 controls Stat activation by IL-6.\textsuperscript{54-56} In the absence of Socs3, IL-6 causes persistent Stat3 activation that correlates with an anti-inflammatory effect similar to the one induced by IL-10. IL-10 signaling appears to be insensitive to Socs3 as a feedback inhibitor probably because the IL-10 receptor complex has no phosphotyrosine motifs to act as a Socs3 recruitment site.\textsuperscript{53} Socs1, the main function of which is the negative control of IFN-γ signaling\textsuperscript{57}, is also produced in an IL-10-dependent manner, and is thought to be responsible for the negative feedback inhibition of IL-10.\textsuperscript{53} Thus, both Socs are involved in negative feedback inhibition of Jak-Stat signaling through distinct mechanisms, which enables them to contribute to fine-tuning IL-10 signaling and aid the balance of pro-inflammatory responses during infection.

Another regulatory mechanism in place to control the IL-10-Jak-Stat circuit is post-translational modification of Stats (including Stat3) by acetylation and serine phosphorylation. These effects provide some late-stage signal control to modulate the circuit after initiation of signaling, but also, some early control since, for example, Stat3 serine727 phosphorylation occurs rapidly after IL-10 or IL-6 stimulation and can influence Stat activity and cell differentiation.\textsuperscript{58} Other late-stage regulatory mechanisms include the protein inhibitors of activated Stats (PIAS), such as Stat3-specific PIAS3.\textsuperscript{59} The extent to which these late-stage mechanisms exert control over IL-10 signaling subsequent to Stat3 activation, however, remains unclear.\textsuperscript{60} One other class of negative regulators of cytokine secretion also impacts IL-10 activity: the so-called SH2-containing protein tyrosine phosphatases (SHPs).\textsuperscript{61} While PIAS proteins inhibit Stat dimerization and prevent Stats interacting with DNA, which restricts their availability\textsuperscript{62}, SHPs, in contrast, inhibit signaling by recruitment to phosphorylated
tyrosine residues after Jak activation and dephosphorylate signaling components essential to kinase activation. Together, these composites of late-stage regulatory mechanisms illustrate the balance of IL-10-Jak-Stat signaling that is required to manage successful antimicrobial responses.

Finally, several accessory molecules influence the modulation of antimicrobial function in response to IL-10 through Stat3. One pathway that influences the IL-10 Jak-Stat circuit is through the kinases PI3K–Akt-glycogen synthase kinase 3 (GSK3) (PI3K-Akt-GSK3). This module was recently shown to positively regulate Stat3 signaling in macrophages. Socs1 and Socs3 positively regulate κB-containing promoter activity in a NF-κB subunit p65/RelA-dependent manner in macrophages, leading to modulation of NF-κB signaling and transcriptional responses. The exact mechanism behind this is not yet clear however it may be that the Socs proteins inhibit the activation of Stats, which, in turn alleviates the competition between Stats and RelA for binding p300 in the nucleus, leading to activation of RelA-dependent transcription. IL-10 also induces Bcl-3, which interacts with the p50 subunit of NF-κB, to bind to the TNF-α promoter and inhibits its production in response to LPS. Bcl-3 can boost NF-κB-dependent gene transcriptional activation, so it may contribute to the inhibition of cytokines by up-regulating IL-10-induced genes.

**Microbes influence pathogenesis through the IL-10-Jak-Stat circuit**

The best-studied microbial product in relation to IL-10 synthesis and Jak-Stat signal transduction is LPS. Overall, IL-10 causes the down-regulation of a number of LPS-inducible genes that encode pro-inflammatory mediators including IL-1 and IL-6, IFN-γ-inducible genes including the nitric oxide synthase gene, and IL-4-inducible
genes including those encoding MHC class II.\textsuperscript{70} The products of these genes contribute to effective antimicrobial responses against several pathogens, which helps to explain the deleterious effects of IL-10 on immune control of these infections.\textsuperscript{71} In contrast, IL-10 is essential to the control of fatal hyperactive immune stimulation caused by the over-production of these mediators during systemic LPS challenge.\textsuperscript{72} This is illustrated in Figure 1 where systemic LPS drives excessive Stat1-triggered pro-inflammatory responses after initial signaling through TLR4, resulting in responses that cannot be controlled by Socs-regulated immune suppression. Here, IL-10 determines the transition from reversible sepsis to irreversible shock by counterbalancing pro-inflammation with pro-suppression.\textsuperscript{73}

For some pathogens, the LPS affects on IL-10 responses play an important role in pathogenesis. In \textit{Bordetella pertussis} infection, for example, innate resistance hinges on TLR4 recognition of LPS and subsequent synthesis of IL-10, which inhibits inflammatory pathology that contributes to disease.\textsuperscript{74} Additional protective roles for IL-10 in reversing the lethal effects of LPS were recently shown in a lung injury model that examined IL-18 supplementation and the requirement of IL-10 in immune protection.\textsuperscript{75} Further evidence of the beneficial biological impact of IL-10 in specific infection involving LPS comes from studies in conditional Stat3\textsuperscript{-/-} mice, where mice develop endotoxemia from excessive TNF-\textalpha, IL-1\textbeta and IFN-\gamma and succumb to septic peritonitis and multiple organ failure during systemic infection.\textsuperscript{16,76} These observations demonstrate the essential link between IL-10 and Stat3 for moderation of immune hyperactivity in response to Gram-negative pathogens and control of acute disease. Finally, a recent study showed that mice are protected from lethal endotoxic shock by liposomal delivery of Socs3 plasmid DNA, which inhibits the development of
macrophage LPS tolerance, although these observations need to be confirmed. Such endotoxin tolerance and refractory phenotypes in monocytes, that are associated with systemic Gram-negative infection and the progression of sepsis, are associated with a failure to up-regulate inflammatory cytokines as a result of IL-10 synthesis. Thus, liposomal delivery of Socs3 might represent an attractive means of controlling hyperactive immune responses at the level of systemic infection. In models of immune protection, prevention of LPS-induced TNF-α through IL-10-mediated Jak-Stat signaling via Stat3 probably represents the major mechanism of counter-acting brutal inflammatory responses that mediate collateral tissue pathology. Numerous other bacterial factors influence the production of Jak-stat-mediated IL-10 through TLRs other than the highly studied TLR4. For example lipoteichoic acid, which signals through TLR2 and CpG-ODN, which signals through TLR9 are both able to inhibit the ability of IL-10 to induce the phosphorylation of Stat3 in macrophages through suppression of IL-10R function.

Beyond observations on bacterial LPS, infectious diseases like Q-fever and leishmaniasis, which involve strict intracellular pathogens, are dramatically influenced by the dynamics of IL-10 and downstream Jak-Stat signaling through Stat3. In Q fever, for example, the genes for both IL-10 and Stat3 are highly up-regulated by Coxiella burnetii in males. This response has been correlated with IL-10-associated bacterial survival in monocytes where the immune suppressive effects of Stat3 activation may dampen cellular antimicrobial effector mechanisms and lead to poor microbe killing. In macrophages engineered to over-express IL-10, C. burnetii survival has been associated with a non-microbicidal transcriptional program consisting of increased expression of arginase-1, mannose receptor, and Ym1/2. This contrasts with a
phenotype of inducible NO synthase and inflammatory cytokines needed to kill the bacteria, which occurs in the absence of high level IL-10 synthesis.\textsuperscript{82} These findings illustrate the importance of the level of activation of the IL-10-Jak-Stat circuitry based on engineered cell lines, which are impossible to gauge in IL-10-deficient or Stat3-/- mice. Similarly, transgenic mice overexpressing IL-10 can be good models with which to study the effects of IL-10 in chronic infections.\textsuperscript{42,83-85} Analogous to the pathogenesis of \textit{C. burnetii} infection, IL-10 blocks bacterial killing in \textit{Mycobacterium tuberculosis}-infected human macrophages by inhibiting phagosome maturation. In this case, the effect of IL-10 was shown to be Stat3-dependent, but independent of MAPKp38 and ERK1/2 activity in a recent study.\textsuperscript{86} In contrast to the pathogenesis of Q-fever, the production of Socs3 in certain protozoan diseases appears to represent an essential negative feedback mechanism for driving immunity against intracellular parasites. For example, during \textit{Leishmania major} infection, the production of Socs3 diminishes IL-10 synthesis, which contributes to effective protozoan clearance (Fig 1).\textsuperscript{87,88} \textit{Leishmania donovani}, on the other hand, induces IL-10, and the subsequent Stat3 activation that drives the expression of IL-4Rα and arginase 1 enables these intracellular pathogens to circumvent NO-dependent killing by macrophages.\textsuperscript{89} The differential effects of IL-10 during infection with intracellular pathogens such as these may depend on the cellular source of IL-10 during infection and its interaction with infected versus non-infected cells, as recently reviewed.\textsuperscript{49}

Recent studies on classical extracellular pathogens that display intracellular lifestyle traits such as \textit{E. coli}\textsuperscript{90-92} and streptococci\textsuperscript{93,94} have also revealed roles for IL-10-Jak-Stat signaling in disease pathogenesis. Uropathogenic \textit{E. coli} (UPEC) are a primary cause of urinary tract infections whereas \textit{Streptococcus agalactiae} mediates
infections during pregnancy, and in neonates and elderly individuals.\textsuperscript{95} Observations that UPEC can invade and replicate within epithelial cells suggest that this organism may occupy an intracellular niche within the host. UPEC is also able to survive within primary mouse bone marrow-derived macrophages and a recent study suggested that some UPEC might subvert macrophage antimicrobial pathways similar to intracellular pathogens.\textsuperscript{90} \textit{S. agalactiae} also displays some intracellular lifestyle traits such as survival in macrophages and induction of apoptosis in host cells.\textsuperscript{96} Both of these microbes trigger up-regulation of IL-10 during infection of the urinary tract, and for UPEC this is associated with Jak-Stat signaling and Socs3.\textsuperscript{7} In fact, Jak-Stat signaling was among the most highly activated canonical pathways triggered by UPEC during cystitis\textsuperscript{7}. Thus, IL-10 and related Jak-Stat signaling appears to be important in early immune responses in the bladder to these pathogens that display some traits of intracellular lifestyles within the host.

Viral-host pathogen studies have also provided important insights into the role of IL-10 in antimicrobial responses at the Jak-Stat axis. In co-infection models of HIV, for example, the virus stimulates infected cells to produce IL-10, which activates Stat3 to impede autophagy of bystander macrophages and monocytes (Fig 1). The inhibition of phagocytic cell death has a direct impact of the pathogenesis of co-infection with other intracellular pathogens, whereby macrophages are prevented from normal killing of co-infecting \textit{M. tuberculosis} and \textit{Toxoplasma gondii}.\textsuperscript{97} Cytomegalovirus (CMV), which is typically associated with disease in the immune-compromised such as HIV-infected persons, synthesizes its own IL-10 homologues. These viral-derived IL-10 homologues interact with the IL-10 receptor complex on human cells, and thereby, directly compete with human IL-10 for receptor binding. As a result, CMV IL-10
homologues interfere with downstream signaling events stemming from ligation of the IL-10 receptor complex during immune responses to the virus (Fig 1), which circumvents elimination of the virus and leads to chronic infection. In the pathogenesis of acute CMV infection, however, IL-10R signaling has been implicated in promoting the survival of NK cells, which contribute to innate responses and effective clearance of the virus. Together, the nuanced effects of IL-10 signaling through Jak-Stat during HIV and CMV infection emphasize the varied outcomes that can result from viral interference with this circuit in acute and chronic disease depending on the virus and circumstances of infection.

**Pathogens can activate Stat3 independent of IL-10**

Many immune suppression signals in response to microbes occur as above through engagement of the IL-10 receptor complex and activation of the cognate downstream Jak-Stat module. However, not all mechanisms of immune suppression during infection are absolutely dependent on these signaling pathways through the IL-10-Jak-Stat module. For example, the intracellular pathogen *T. gondii* activates Jak-Stat signaling including anti-inflammatory Stat3 in an IL-10-independent manner to bring about suppression of TNF-α synthesis during infection and macrophage apoptosis (Fig 1). Interference with host cell death is important in pathogenesis since enhanced microbe clearance related to the induction of apoptosis is a known effector mechanism in combating some intracellular pathogens. The suppressive effects of Toxoplasma on LPS-induced cytokine synthesis and IFN-γ-induced nitric oxide are mediated by the microbes’ rhoptry kinase, ROP16, which is injected into the host cell. Here, the enzyme activates Stat3, as well as Stat6 to bring about immune suppression that includes direct effects on arginase-1. Mice harboring a deletion of Socs3 in
macrophages succumb to toxoplasmosis, but their resistance is restored by anti-IL-6 administration, suggesting that in the absence of Socs3, macrophages are hypersensitive to the anti-inflammatory properties of IL-6. These signaling events involving Stat3 and Socs3 during *T. gondii* infection directly impact the pathogenesis of disease but do so independently of IL-10. Similar to *T. gondii*, *Salmonella enterica* induces IL-10-independent Stat3 activation in macrophages via an unknown mechanism. Immune suppression prevents severe inflammatory consequences at the gut epithelium associated with this infection. Thus, both of these organisms provide examples of microbes that induce immune suppression via Stat3 activation in an IL-10-independent manner, which influences microbe survival and pathogenesis. It will be important to determine the mechanisms of how these pathogens induce Stat3-driven immune suppressive effects independent of IL-10. For example, are there homologues of ROP16 that can activate Stat3 as observed for the protozoan *T. gondii* in other bacterial pathogens? What role, if any, do other cytokines such as IL-6 have in IL-10-independent activation of Stat3 during infection for immune suppression in response to pathogens like *T. gondii* and *S. enterica*?

**Conclusions**

The interplay that occurs between signaling pathways in response to IL-10 and antimicrobial outputs has emerged as a complex series of activating and inhibitory regulatory molecules that function through Jak-Stat. How pathogens hijack the Jak-Stat module through IL-10-dependent and -independent mechanisms in a manner that benefits their survival within the host is an intriguing area of current research. There are many other questions in addition to those above related to what gene targets are activated by pathogens that utilize the IL-10-Jak-Stat module for subversion of host
immune responses. How such targets might be exploited for therapeutic benefit will be an important area for future exploration. In light of the recent studies these areas of research are primed for investigation.
Acknowledgements

This work was supported by a grant from the Australian National Health and Medical Research Council (569674). GCU is supported by a Future Fellowship (FT110101048) from the Australian Research Council. CKT is supported by a Prime Ministers Endeavour Fellowship from the Australian Government. We thank Roland Lang for critical review of the manuscript.


35. Smith AM, Qualls JE, O’Brien K, Balouzian L, Johnson PF, Schultz-Cherry S, Smale ST, Murray PJ. A distal enhancer in Il12b is the target of transcriptional repression by the STAT3 pathway and requires the basic leucine zipper (B-ZIP) protein NFIL3. The Journal of biological chemistry 2011; 286:23582-90.


45. Prele CM, Keith-Magee AL, Yerkovich ST, Murcha M, Hart PH. Suppressor of cytokine signalling-3 at pathological levels does not regulate lipopolysaccharide or interleukin-10 control of tumour necrosis factor-alpha production by human monocytes. Immunology 2006; 119:8-17.


62. Antoniv TT, Ivashkiv LB. Interleukin-10-induced gene expression and suppressive function are selectively modulated by the PI3K-Akt-GSK3 pathway. Immunology 2011; 132:567-77.


**Figure 1: Interactions between microbial pathogens and IL-10-Jak-Stat signaling pathway elements.** Recent studies (reference shown in parentheses) have shown *C. burnetii, M. avium, S. agalactiae,* and other Gram-negative pathogens induce IL-10 synthesis in contrast to protozoa such as *L. major* and commensal *L. rhamnosus* that inhibit its production. Stat3, normally recruited and phosphorylated at the IL-10 receptor complex, are directly engaged by pathogens including *S. enteria* and *T. gondii*. Viruses such as HIV also affect Stat3 directly, or indirectly by producing homologues that compete with IL-10 for receptor complex docking sites, as shown for CMV. Nuclear translocation of active Stat3 for contact with Stat binding elements is a potential pathway element for pathogen-driven effects that has not yet been described. Socs1 and Socs3, induced as a result of IL-10 signaling through MAPK and AKT/PKB, suppress LPS/TLR4/CD14-induced IL-10, IL-6 biological activity from gp130 receptors, MHC class II, and Stat1-induced mediators including NO, TNF-α and IFN-γ. Microbes such as *L. major* hijack this element within the pathway to abrogate suppressive effects of Socs towards active Stat3 as a mechanism of interfering with antimicrobial responses in macrophages.

**Abbreviations:** IL-10R: IL-10 receptor; JAK1: Janus kinase 1; Tyk2: Tyrosine kinase 2; MHC II: Major histocompatibility complex class II; Stat: Signal transducer and activator of transcription; PI3K: phosphatidylinositol 3-kinase; AKT/PKB: Ak-t/protein kinase B; MAPK: Mitogen-activated protein kinase; Socs: suppressor of cytokine signaling; iNOS: inducible nitric oxide synthase; NO: nitric oxide; TNF-α: tumor necrosis factor-α; IFN-γ: interferon-γ; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; IκB-α: Inhibitory subunit of NF-κB-α; P: phosphorylation; R: receptor; HIV: Human Immunodeficiency Virus; CMV: Cytomegalovirus; ROP16: rhoptry kinase of *T. gondii.*