MINI REVIEW

Title: Dangerous liaisons between interleukin-6 cytokine and toll-like receptor families: a potent combination in inflammation and cancer

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Abstract

The potent pro-inflammatory actions of members of the Interleukin (IL)-6 cytokine and Toll-like receptor (TLR) families have been implicated in numerous inflammatory disorders, as well as inflammation-associated cancers. It is fast becoming apparent that a hallmark of many such inflammatory-related diseases is the overlapping deregulated expression of members of each family, and the consequent augmented activation of shared signaling pathways. Here, we review the molecular basis by which the IL-6 cytokine and TLR family signaling networks are regulated, and integrate recent advances exploring the intimate cross-regulation of these two families which may provide the foundation for the future development of therapeutics to target chronic inflammation-associated diseases, including cancer.

Key-words

IL-6 family cytokines; TLRs; STATs; inflammation; cancer.

1. Introduction

The mammalian immune system has evolved complex defense mechanisms to counteract the myriad of insults such as viral, bacterial, and parasitic infections, as well as tissue injury, presented throughout the life-span of the host. The inflammatory response, governed by both innate and adaptive arms of the immune system, is the primary weapon in the host’s arsenal to rapidly respond to such microbial and non-microbial events and restore tissue homeostasis. Not surprisingly, inflammation represents a tightly controlled and highly orchestrated cellular process, and critical to the successful resolution of inflammation is the coordinated transition from innate to adaptive immunity, aided by the balanced release of a vast array of inflammatory cytokines and chemokines. Among these inflammatory mediators, the Interleukin-6 (IL-6) cytokine family represents one of the most extensively studied groups of cytokines, largely due to the impressive variety of redundant and pleiotropic cellular functions exhibited by its members, including differentiation, maturation, proliferation and survival. These cytokines are defined by their common usage of the widely-expressed gp130 receptor signal-transducing β subunit (1, 2). Their importance to host pathophysiology is evidenced by the wealth of genetic and experimentally-induced animal disease models and clinical data which point to a causal role for IL-6 and gp130 in acute and chronic inflammatory disorders, autoimmune disease and cancer (3-5).
Our understanding of the molecular basis linking microbial recognition by the host to the onset of the inflammatory response has been greatly advanced by the seminal discovery that pattern-recognition receptors (PRRs), of which the Toll-like receptor (TLR) family is the best characterized, act as crucial immune system sensors to trigger the inflammatory response (6). From a traditionalist point of view, it has been widely accepted that TLRs modulate the host defense against infections by inducing many immune-mediated inflammatory processes. These include antimicrobial responses, antigen presentation, leukocyte trafficking, phagocytosis, and maturation and activation of innate (e.g. macrophage) and adaptive (e.g. T cell) immune cells via the regulatory actions of transcription factors such as NF-κB, the prototypic inflammatory transcription factor. It is evident, however, that the broad role of TLRs extends past inducing inflammation to modulating non-immune responses, such as epithelial cell proliferation, survival and migration, which are important for homeostatic processes such as tissue repair and wound healing (7). Many of these TLR-driven processes are key components of the carcinogenesis machinery (7-10), and considering that the inflammatory response can promote carcinogenesis by, for instance, augmenting the release of reactive oxygen species (ROS) leading to oxidative stress and DNA damage, there has been an explosion in research investigating the role of TLRs in promoting the initiation and progression of tumors of both solid (i.e. epithelial) and liquid (i.e. hematopoietic) origins (10-13).

In light of these observations, a major challenge now facing researchers is identifying both the upstream regulators of TLRs and their downstream molecular targets, as well as their roles in promoting the plethora of TLR-driven pathophysiological responses. Additional to the most obvious connection between TLR and IL-6 cytokine families, where the latter is perceived as a downstream transducer of TLR-driven inflammatory responses, a more complex picture is emerging involving cross-talk between these families at the signal transduction, ligand and receptor levels. In this review, we will further explore cross-talk between TLR and IL-6 cytokine families and the subsequent implications for understanding the molecular pathogenesis of inflammatory-related disorders, including cancer.

2. TLRs as critical sensors of the host immune system

There are 11 human and 13 mouse TLRs, and each TLR appears to recognize a distinct set of pathogen-associated molecular patterns (PAMPs) derived from a range of microorganisms including viruses, bacteria, fungi and protozoa (6, 12). TLRs can be further subdivided into two categories dependent on their cellular localization which reflects their ligand specificity: TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10 are localized to the plasma membrane and recognize predominately components of the extracellular matrix of bacteria and viruses, while TLR3, TLR7, TLR8 and TLR9 recognize principally nucleic acids and reside in the intracellular endosomal compartment. Among the TLRs, TLR2 is implicated in the recognition of the widest range of PAMPs including peptidoglycan, lipoproteins from Gram-negative bacteria, fungal zymosan and mycoplasma lipopeptides (14), which is primarily caused by TLR2 forming heterodimers with TLR1 and TLR6. It is also noteworthy that dimerization of TLR2 with TLR1 and TLR6 allows TLR2 to distinguish between triacyl- and diacyl-lipopeptides, respectively, thus demonstrating the specificity of the innate PRRs (15). Further evidence of this diversity and specificity of TLR2 has been shown recently by its cooperation with the related TLR10 to sense bacteria and fungi; the divergent signaling of TLR10 from other TLR2 subfamily members has expanded TLR2’s signaling capabilities (16). The plasma membrane TLR4 is best recognized for its role in detecting the exotoxic component of Gram-negative bacteria lipopolysaccharide (LPS) in conjunction with its extracellular components CD14 and MD2 (17). TLR5, also on the external cell surface, recognizes the protein constituents of bacterial flagellin (18).
Within the cell, TLR3 recognizes double-stranded RNA (19), while TLR7 and TLR8 recognize single-stranded RNA (20, 21), produced by replicating viruses in the cytosol. TLR9 meanwhile recognizes unmethylated CpG-DNA present in both bacterial and viral genomes (22). Similar to human TLR5, mouse and rat TLR11 (which is not expressed in humans) is required for protection against uropathogenic bacteria (23) and recognizes a profilin-like molecule from the protozoan parasite *Toxoplasma gondii* (24).

In addition to a wide variety of microbial ligands, there is an increasing number of endogenous (i.e. host-derived) ligands reported as putative stimulators of TLRs. Endogenous TLR ligands have been identified as proteins and peptides, polysaccharides and proteoglycan, nucleic acids and phospholipids, all of which are cellular components, particularly extracellular matrix degradation products (25). TLR2 and TLR4 in particular appear to play a critical role in the recognition of endogenous ligands and subsequent initiation of the inflammatory response. Given this capacity for TLRs to recognize ‘self’, it is no surprise that TLR activation is involved in pathological conditions such as autoimmune diseases, vascular disease tissue injury, repair and regeneration and tumorigenesis (6, 7, 10, 26, 27). Indeed, increasing evidence from studies of genetic variation in human disease clearly implicates TLRs enacting a critical role in the pathophysiology of inflammation in many diseases traditionally associated with autoimmunity, including atherosclerosis, systemic lupus erythematosus and type 1 diabetes (28). In light of these observations, elucidating how TLRs are activated and regulate the magnitude and duration of the resultant inflammatory response is critical in understanding the role these danger receptors play in the initiation, development and progression of chronic inflammation.

Despite recognizing endogenous ligands, the most potent role of TLRs is in host defense - regulating the innate and adaptive response in epithelial cells as the first line of defense to invading pathogens at mucosal sites, such as skin, and the respiratory, gastrointestinal and genitourinary tracts. TLRs are widely expressed in both hemopoietic and non-hemopoietic lineages (reviewed in (29–31)), however the pattern and levels of expression diverge greatly between tissues and cells and depend largely on cell function and localization in relation to potential ligand availability. The main and best studied cells expressing TLRs, however, are the foot soldiers of innate immunity: dendritic cells and macrophages, which generally express a wide variety of TLRs, commensurate with their role as sentinels of danger. How TLRs recognize such danger signals and trigger complex gene expression programs following their activation have primarily been determined using these two cell lineages and our knowledge on the spectrum of TLR gene and protein expression is fragmentary and reflective of directed research.

### 3. Induction of inflammation by TLR signaling cascades

While TLRs recognize a divergent range of ligands, most TLRs share a common, canonical signaling pathway leading to induction of a large range of genes consisting cytokines, chemokines, leukotrienes, growth factors, adhesion factors, pro- and anti-apoptotic factors and cell cycle regulators (32-34) in response to pathogen challenge. Following ligand-induced dimerization, TLR signaling relies on homotypic dimerization of the cytoplasmic Toll/IL-1 receptor (TIR) domain, found in all TLRs and TIR-containing cytosolic adapter proteins. With the exception of TLR3, MyD88 is an essential TLR signaling component and is recruited to all TLRs (and members of the IL-1R family) in combination with other TIR-containing adapter proteins: Mal (also known as TIRAP), TRAM/TICAM2 or TRIF/TICAM1 (26). MyD88 further recruits the serine kinases IRAK1 and IRAK4 to the complex which leads to recruitment and activation of TRAF6. TRAF6 in turns activates TAK1 resulting in phosphorylation and activation of the IKK complex. IKKβ subsequently phosphorylates the inhibitor of NF-κB, IκBα, leading to its proteosomal degradation, thus allowing the nuclear translocation of NF-κB and the induction of the inflammatory gene program, characterized by production of...
inflammatory cytokines TNF\(\alpha\), IL-1\(\beta\) and IL-6 (Fig. 1). In the case of TLR3 and TLR4, however, an alternative pathway is also used which further enhances the TLR response through the recruitment of TRIF, which leads to the activation of IRF3 and the secretion of type I IFNs which are required for an effective antiviral response.

Further specificity and fine tuning of the degree, robustness and duration of TLR-induced inflammation is added by the concomitant recruitment of adapter proteins Mal (TLR2 and TLR4) and TRAM (TLR4), and activation of the mitogen-activated protein kinases (MAPK), c-Jun N-terminal kinases (JNK), p38 and extracellular signal-regulated kinase (ERK), which are activated downstream of TRAF6 (35). Activation of MAPKs primarily regulates the post-translational activation of transcription factors like CREB and AP-1, and these pathways synergize the inflammatory gene expression program through the coordinated binding of transcription factors to NF-\(\kappa\)B and AP-1 sites that have been identified in the promoters of inflammatory genes such as \(Il6\) and \(Tnfa\), as well as a plethora of other genes that are up-regulated following TLR activation (36). It should also be said that MAPK activation plays a crucial role in modifying the transcriptional activity of transcription factors such as NF-\(\kappa\)B through controlling the transactivation of factors such as the p65 subunit of NF-\(\kappa\)B (37).

**4. Link between TLRs, inflammation and cancer**

The development of cancer, for which there has been a well-established association with inflammation (38), can be divided into three steps: initiation, promotion and progression (8). The initiating phase of carcinogenesis may be linked by genetic events leading to the activation of proto-oncogenes by mutation, chromosomal rearrangements or gene amplification, and genetic or epigenetic inactivation of tumor-suppressor genes (39). In the context of inflammation-dependent carcinogenesis, the pro-inflammatory ‘rich’ milieu invariably comprises inflammatory cytokines such as IL-6, TNF\(\alpha\) and IL-17 which collectively provide an optimal environment for cancer cells to survive and proliferate rapidly. In such a scenario, it is important to acknowledge that while chronic inflammation may not initiate every tumor, an inflammatory microenvironment is an essential component of every tumor (38, 40). Accordingly, these situations raise a critical question: does tumor initiation (such as from genetic factors) provide the stimuli to drive inflammation by danger receptors, such as TLRs, leading to the promotion and progression of tumorigenesis?

While TLRs play a crucial role in maintaining tissue homeostasis by regulating inflammation and tissue repair, the ‘dark side’ of TLR activity is that excessive TLR-mediated inflammation may contribute to the development of cancer (10), in particular tumor progression and metastasis. Indeed, the notion that TLR-induced inflammation can enhance tumorigenesis was derived from the observation that TLR ligands have the ability to augment the growth of adoptively transferred tumors (41, 42). Since these earlier studies, it has been well documented that TLRs are up-regulated in a variety of tumors, and their stimulation by ligands leads to enhanced secretion of inflammatory cytokines (e.g. IL-6) and other growth stimulatory factors from tumor cells, which feedback in an autocrine and cell autonomous manner to drive tumor cell survival and proliferation (29, 31) (43, 44). In addition to this direct effect on tumor cells, activation of NF-\(\kappa\)B by TLRs may also drive the recruitment of immune cells (e.g. macrophages) which secrete cytokines and chemokines that control the transcriptional programming, and thus growth, of the cellular milieu (including tumor epithelial cells) that constitutes the tumor microenvironment (10).

The crucial role of TLRs in the development of tumors as they arise in the native environment has also been demonstrated using genetically-modified mice deficient in the essential TLR signaling adaptor, MyD88. For instance, MyD88-dependent up-regulation of IL-6 was shown to...
be the main driving force behind diethylnitrosamine (DEN)-induced hepatocellular tumorigenesis, which is reliant on a chronic inflammatory environment (45). In addition, Rakoff-Nahoum and Medzhitov demonstrated that MyD88 was critical for tumor promotion in spontaneous (Apcmin/+ ) and carcinogen-induced (azoxymethane; AOM) intestinal tumorigenesis (46). Interestingly, the suppression of intestinal tumorigenesis in the absence of MyD88 was accompanied by elevated tumor cell apoptosis, independent of any changes in the numbers of leukocytes within the tumor microenvironment, further invoking a direct role for MyD88 signaling on tumor cells. MyD88 was further demonstrated as positively mediating tumor development in chemically-induced (using 7,12-dimethylbenz[a]anthracene: DMBA and 12-O-tetradecanoyl-phorbol-13-acetate: TPA or 3’-methylcholanthrene) tumors in both skin and connective tissue (47). An important consideration with the above findings, however, is that MyD88 is also required for IL-1- and IL-18-induced signaling (48), and it is therefore possible that these cytokines, rather than specific TLRs, may contribute to tumorigenesis.

Among the TLR family, TLR2 has been most implicated as a key driver of many cellular processes important for tumorigenesis, including tumor growth, angiogenesis and metastasis (49-52). These studies have invariably been based on ectopic tumor models, and have proposed numerous divergent mechanisms by which TLR2 contributes to tumorigenesis, ranging from direct growth-promoting actions on the tumor cells themselves (52, 53), to the suppression of anti-tumor immunity to promote tumor metastasis (51). Interestingly, such properties of TLR2 have previously been assigned to the oncogenicity of STAT3 (54), suggesting that TLRs may also use cytokine signaling pathways (i.e. STAT3). The notion of such cross-talk between TLRs and cytokines as a key stepping stone in the molecular pathogenesis of the carcinogenesis cascade has been strengthened by a recent discovery: in a pre-clinical mouse model of spontaneous gastric inflammation-associated tumorigenesis, hyper-activation of STAT3 in the gastric department, via the IL-6 cytokine family member IL-11, leads to the transcriptional up-regulation of endogenous TLR2 (55). Moreover, in this model TLR2 augments the survival and proliferation of gastric epithelial tumor cells independent of inflammation (55). While the implications of such cross-talk between IL-6 cytokines and TLRs will be discussed in more detail below, collectively these above observations highlight the challenge we now face to fully comprehend the complex role of TLR2 (and other TLRs for that matter) in innate and adaptive immunity, as well as non-immune (e.g. epithelial) cellular responses during disease pathogenesis.

5. The IL-6 cytokine family; a brief overview of biology and signal transduction

A biological hallmark of members of the IL-6 cytokine family, which includes IL-6, IL-11, IL-27, IL-31, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiostrophin (CT-1), and cardiostrophin-like cytokine (CLC), is the often diverse and redundant functions affecting a vast array of cellular processes, including apoptosis/cell survival, proliferation, differentiation and maturation. In light of this functional pleiotropy, it is not surprising that IL-6 family cytokines have been implicated in numerous physiological responses (e.g. multi-organ homeostasis) as well as the pathogenesis of inflammatory and autoimmune diseases, and various types of cancer (3, 56-58). As a consequence, a wealth of studies over the last two or more decades has been dedicated to understanding the mechanisms by which this cytokine family elicits intracellular signaling cascades within target cells to trigger such a plethora of biological activities. Such information has been already extensively detailed in the literature (for example, see reviews by Heinrich et al. (2) and Garbers et al. (59)), and for the purpose of this current review, will only be summarized.

With the exception of IL-31, signal transduction by IL-6 family cytokines is absolutely dependent on the ubiquitously-expressed glycoprotein 130kD (gp130) receptor signaling β
subunit. In the case of IL-6 and IL-11, engagement with their ligand-binding receptor α-subunits leads to homodimerization of gp130, whereas most other IL-6 family cytokines signal via heterodimers of gp130 associated with distinct receptor signaling β-subunits, namely the LIF receptor (LIF, CNTF, CLC, CT-1, OSM), OSM receptor (OSM) and WSX-1 (IL-27) (2, 3). As inferred above, IL-31 goes against trend by signaling via a heterodimer of the OSM receptor with the gp130-like (GPL) receptor subunit (60). Irrespective of the mode of these gp130-related dimerization events, an essential consequence of ligand-induced gp130 dimerization is activation (i.e. tyrosine phosphorylation) of the receptor-associated Janus kinase (JAK) non-receptor tyrosine kinases, leading to tyrosine phosphorylation of the gp130 cytoplasmic domain. The four C-terminal phospho-tyrosine residues (pY) in gp130 facilitate recruitment of the latent transcription factors STAT 1 and 3 (61) (Fig. 2), whereas the membrane-proximal pY757/pY759 of mouse/human gp130 serve as binding sites for the tyrosine phosphatase src homology phosphatase (SHP) 2 which facilitates activation of the ERK MAPK and phosphatidylinositol-3-kinase (PI3K)/Akt pathways (62). Importantly, pY757 also plays a crucial role in controlling the magnitude and duration of gp130 signaling by recruiting suppressor of cytokine signaling (SOCS)3 to gp130 (63) where it inhibits JAK activity and targets the receptor complex to the proteasome for degradation (64).

Over the last decade it has become apparent that this cytokine family has evolved several elegant strategies to broaden its actions on many different cell types, despite the often restricted expression of the ligand-binding receptor subunits. The best characterized case of this is for IL-6, which can signal by two distinct modes, classical and trans, a phenomenon which has been extensively reviewed elsewhere (4, 56). While IL-6 classical signaling via the membrane-bound (m) IL-6Rα is restricted to those limited cell types expressing mIL-6Rα, IL-6 can also broaden it biological effects on a plethora of gp130+IL-6Rα+ cells via the naturally-occurring soluble (s) IL-6Rα subunit which is primarily generated by proteolytic cleavage of mIL-6Rα. Not only does this mode of signaling confer IL-6 responsiveness to otherwise IL-6 non-responding cells, but it can also further amplify the magnitude and duration of a classical IL-6 response by a gp130+IL-6Rα+cell. Another strategy employed by members of this cytokine family to promote both redundancy and pleiotropy involves cross-talk at the ligand-receptor interaction level. For instance, in humans OSM can elicit biological activities on cells via heterodimers of gp130 with either the LIF or OSM receptor, thus increasing the spectrum of responsive cells to OSM (65). Another cytokine which can also use multiple receptor combinations to enhance its biological repertoire is CNTF, which in addition to binding the CNTF receptor α subunit can also bind the mIL-6Rα, leading to the formation of LIF receptor and gp130 signaling β subunit heterodimers (66). As will be discussed in the next section, it has recently become apparent that the IL-6 family cytokines, in particular IL-6 and IL-11, have also developed ways to “tap into” the TLR signaling network to enhance their repertoire of biological activities.

6. Cross-regulation of IL-6 family cytokines and TLRs; mechanisms and their role in inflammatory-related diseases

An overwhelming number of studies based on clinical data and mouse disease models have implicated the IL-6 family, and IL-6 as the principal family member, in many acute and chronic inflammatory disorders (e.g. peritonitis, inflammatory bowel disease, sepsis, atherosclerosis), autoimmune diseases (e.g. rheumatoid arthritis), and cancers (57, 67). Regarding the latter, it is of interest that cancers of the liver, colon, lung and pancreas also have a chronic inflammatory component and are causally linked with TLR signaling (9, 45, 46, 68). In addition to IL-6, it should also be noted that the related cytokine IL-11 has gained much attention in recent years, especially in the gastro-intestinal tract, where its over-production has been linked to a causal role in both inflammation-associated gastric and colon tumorigenesis (69-71), and more recently in skin carcinogenesis (72).
While the complex molecular basis by which inflammation contributes to tumorigenesis remains unresolved, it is likely to involve intimate cross-talk between oncogenic and inflammatory pathways. In this regard, in many of the above-mentioned cancer types, a recurrent theme is the deregulated co-activation of STAT3 and NF-κB, the archetypal downstream transcription factors of the TLR and IL-6 cytokine families, respectively. These transcription factors have taken center stage over recent years due to their ability to propagate inflammation that is a key component in the promotion of carcinogenesis (73, 74) (Figs. 1 and 2). At face value, NF-κB and STAT3 share strikingly similar properties in the context of promoting carcinogenesis, often in a cell-type dependent manner. For instance, activation of either transcription factor in tumor (i.e. epithelial) cells leads to the up-regulation of genes involved in cell survival/anti-apoptosis, cell cycle progression and angiogenesis, while their activation in immune cells of the tumor microenvironment augments gene expression for inflammation-enhancing factors, which can lead to the excessive production of reactive oxygen and nitrogen species cells, causing DNA damage and cellular transformation (74, 75). Although the intricacies of the molecular basis for their concurrent activation remain to be established, a plausible explanation, at least in part, is inter-family cross-talk whereby IL-6 family cytokines feed into the TLR receptor system to activate “non-gp130” signaling cascades. While this will be discussed in more detail below, strong evidence for the interplay of NF-κB and STAT3 came from the elegant demonstration by Stark and co-workers that non-phosphorylated versions of STAT3 and the p65 subunit of NF-κB accumulated over time in response to IL-6, forming a transcriptional complex which induced the expression of a unique set of genes not induced individually by phosphorylated STAT3 (76). Although yet to be formally proven in an in vivo disease setting, it is conceivable that the formation of such a transcriptional complex to integrate signals from IL-6 cytokines and TLRs would lead to a potent pro-inflammatory and/or oncogenic response. Nonetheless, it is evident that cross-talk or cross-regulation of either pathway is likely to have significant effects on the initiation and/or subsequent severity of tumorigenesis, progression and metastasis.

Historically, cross-regulation of TLR-induced inflammatory pathways by STAT transcription factors centered on the role of IL-10 in down-modulating inflammation. This mechanism involves STAT3 competing with the pro-inflammatory IFNγ/STAT1-dominated response, which may play a key role in deciding cellular fate by biasing the inflammatory microenvironment via competing feed-back and feed-forward inflammatory loops (77). Emerging data, however, provides tantalizing evidence towards a closer relationship between TLR signaling and STATs. Intriguingly, it has been demonstrated that IL-1β (78), TLR2 and TLR4 (79) could induce rapid serine 727 (but not tyrosine 701) phosphorylation of STAT1 via IRAK1 and MyD88 in epithelial and macrophage cell lines, suggesting a direct involvement of STAT1 in IL-1/TLR signaling and activation of the inflammatory response. A further study demonstrated differential serine phosphorylation of STAT1 by TLR9 in dendritic cells and macrophages that divergently modulated inflammation (80). Another important consideration is that several TLRs and components of the IL-1/TLR signaling pathway are IFN-regulated genes (81) and thus that deregulated STAT expression and/or activation may play a key role in augmenting TLR expression, which may in turn lead to a hyper-inflammatory state (Fig. 3).

Despite the observation that IL-6 family cytokines and TLRs, and their respective signaling pathways, are often up-regulated concurrently in inflammatory diseases and cancer, the role of these family members in disease pathogenesis has invariably been investigated with either family in isolation. However, the realization that both TLR and IL-6 cytokine family members synergize to promote disease outcomes was best highlighted by the obligate requirement for IL-6 by MyD88 signaling in the pathogenesis of the DEN-induced liver cancer model, discussed above (45). While this finding is consistent with the generalized notion that the induction of IL-6 family cytokines, and in particular IL-6 due to its potent pro-inflammatory actions, are a
downstream effector of TLR-induced cellular responses, it has recently emerged that IL-6 family cytokines can themselves modulate the TLR response by different mechanisms. In particular, two separate studies by our laboratory have exploited a mouse model (gp130F/F) for deregulated systemic gp130/STAT3 hyper-activation caused as a consequence of a “knock-in” mutation at Y757F in the cytoplasmic domain of gp130, which abolishes binding of the negative regulator SOCS3 (82). The gp130F/F mice display potentiated LPS/TLR4-induced lethality characterized by increased STAT3-dependent IL-6 production (83) which is independent of elevated hemopoietic TLR4 signaling (84), implying that augmented TLR4 signaling in non-immune cells, as a consequence of IL-6/STAT3 hyper-activation, was responsible for driving inflammation. Further evidence implicating a non-essential role for hemopoietic TLR signaling in STAT3-driven disease pathogenesis comes from the recent description of the IL-11/STAT3 signaling axis directly up-regulating epithelial expression of TLR2 in gastric tumors (55). Targeting of TLR2 inhibited gastric tumorigenesis, but not inflammation, characterized by reduced proliferation and increased apoptosis of the gastric epithelium. This transcriptional regulation of the TLR2 gene by STAT3 in the gastric epithelium was specific, since the gastric epithelial gene expression of other TLR family members, for instance TLR4, was not affected (55).

In contrast to the above finding, and thus highlighting the specificity (including cell-type) by which TLR family members can be regulated by STAT3, is the recent demonstration that IL-6-induced STAT3 activation up-regulates TLR4 gene expression in vastus lateralis muscle biopsied from human patients presenting with impaired glucose tolerance, as compared to control subjects (85). Importantly, this STAT3-mediated up-regulation of TLR4 and the associated inflammation has been proposed as being a primary mechanism underlying insulin resistance, and links TLR signaling to metabolic syndrome (86). A further illustration of the emerging broad ability of IL-6 family cytokines, via the JAK/STAT pathway, to regulate TLR gene expression is the finding that IL-27, an IL-6 family member produced by activated antigen-presenting cells, up-regulates TLR4 in a STAT3- and NF-κB-dependent manner, enhancing LPS-induced inflammation (87). While these above observations pertain to the up-regulation of TLR family members by IL-6 family cytokines, TLR2 and TLR4 expression has also been observed to increase in the absence of IL-6. In a model of LPS-induced hemorrhagic lung inflammation, TLR2 and TLR4 expression was increased in IL-6 deficient mice following LPS challenge (88), suggesting IL-6 was protective in this model of inflammation via the down-modulation of TLR2 and TLR4 expression. However, the cell type in which this decrease in TLR expression was evident was not formally identified, and thus, the absolute relationship to disease protection was not determined.

Nonetheless, the above-mentioned studies collectively provide positive evidence that IL-6 family cytokines via the JAK/STAT pathway can influence TLR-driven responses by modulating TLR gene expression. Furthermore, this notion also suggests a divergence between the role of JAK/STAT signaling in hemopoietic and non-hemopoietic cell lineages that may dictate either pro- or anti-inflammatory outcomes, which may be particularly important in the context of tumorigenesis. In this regard, the tumor microenvironment is comprised not only of tumor cells, but also supporting stromal epithelia and fibroblasts, along with resident and infiltrating leukocytes which produce a milieu of cytokines, growth factors, proteases and bioactive molecules (89) (Fig. 3). In such a heterogeneous cellular environment, it has been proposed that it may not be the relative abundance of each cell type but rather their respective activation and polarization profile that determines whether the effect will suppress or promote tumor growth (71, 89). Thus, the interplay and balance of JAK/STAT signaling and thus TLR-induced inflammation in specific cellular environments may play a critical role in regulating the inflammatory microenvironment conducive to either promoting or suppressing tumor growth and progression.
7. Conclusions
Traditionally we discuss, perceive and illustrate signaling pathways as sequentially activating downstream factors in a linear and autocrine fashion. However, accumulating data exemplifies this simplistic version of signaling as being atypical, with cross-talk or cross-modulation becoming more apparent in ever increasing instances, never more so than in inflammatory pathways, as discussed in this review. The discovery and characterization of TLRs has fundamentally altered our perception of immunology, as well as the role of innate immunity and inflammation in a wide variety of diseases. Indeed, accepting inflammation as the basis of most disease pathologies has placed understanding how TLRs induce, modulate and drive inflammation as a cornerstone to informed therapeutic intervention. While there is abundant information as to how TLRs initiate inflammation via the canonical signaling pathways leading to activation of NF-κB and production of a myriad of inflammatory cytokines and chemokines, there is a critical need to understand how TLRs are regulated themselves, how these pathways are ‘fine-tuned’ and what the pathological outcomes of deregulated inflammation are. Modulation by signaling regulators traditionally considered external to the TLR pathways, such as the JAK/STAT pathway (via IL-6 family cytokines), appears to play an ever increasing role in influencing TLR-mediated inflammation. Conversely, we little understand what direct role, if any, TLR signaling may impart on IL-6 family/JAK/STAT signaling.

Cross-talk between these two key inflammatory and oncogenic pathways, either directly or indirectly, may hold the key to understanding how diseases such as cancer progress through the various stages of initiation, promotion and progression. We can no longer perceive such pathways as acting inconsequentially with one another, but rather that they act as an orchestrated cacophony of signals leading to a controlled homeostatic immune response, or upon deregulation, to perturbations of homeostasis. Regarding the latter, deregulation or mutation of factors such as STAT3 may lead to a hyper-inflammatory state which could lead to progression of disease and severe clinical outcomes, as described above.

Critically, there appears to be a divergence in the cause and effect of cross-talk between TLR and IL-6 cytokine family signaling in different cell types. Given that our understanding of the roles of the IL-6 cytokine and TLR families in inflammation and cancer are based on systemically gene-deficient and chemically-induced mouse disease models, however, future studies will be necessary: firstly to analyze the contributions of specific cell types in initiating disease, particularly cancer, and secondly to address how cross-talk between TLR/NF-κB and IL-6 cytokine family/STAT3 may alter the microenvironment of tissues and organs to allow disease to progress. Cross-talk between such apparently divergent inflammatory pathways may provide a greater awareness of how these pathways are not only ‘fine-tuned’ in the context of positive innate immune responses following infection, but also, how deregulation of either pathway may have significant implications upon the other.

While there have been monumental developments in understanding and appreciating the role of inflammation as underlying a plethora of diseases, how we translate the results of predominantly mouse-related studies into treating human disease will be challenging. How difficult this may be is suggested by a recent study which identified that LPS-mediated activation of TLR4 induced regulation of approximately 2,500 genes in both human and mouse macrophages, but that there was extensive divergence in gene expression between the two species (24% of orthologues were identified as "divergently regulated") (90). Such observations yet again re-iterate the need for caution in interpreting and digesting findings in murine models of hyper-inflammatory disease and how results may translate to human disease. Overall, such studies emphasize that genetic differences between humans and murine models of inflammation underlie the phenotypic
differences observed in the innate immunity between the species, and thus explain the variation in success of human therapeutic interventions based upon targeting in mouse models of disease (91-94). How TLR and IL-6 cytokine family signaling networks are integrated both transcriptionally and dynamically between species, and the cross-talk between these families, would appear to have a profound impact upon disease outcome. There is an urgent need for further studies to better appreciate both the conservation and divergence among these pathways in both different diseases and species.

Finally, inflammation is characterized by the five cardinal signs: Dolor (pain), Calor (heat), Rubor (redness) Tumor (swelling), and Functio (loss of function). Perhaps a sixth cardinal sign (or sin) of inflammation should be added, Fidēlis (fidelity), because the dangerous liaison of inflammatory pathways such as TLRs and IL-6 appears to engender a potent mix akin to Dante’s Inferno.

Acknowledgments
We thank Rebecca Smith for administrative assistance and critical reading of the manuscript. This work was supported by grants from the Association for International Cancer Research (U.K.), Cancer Council of Victoria (Australia), and the National Health and Medical Research Council (Australia), as well as the Victorian Government’s Operational Infrastructure Support Program (Australia). Brendan Jenkins is supported by a Senior Medical Research Fellowship awarded by the Sylvia and Charles Viertel Foundation.

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**Fig. 1.** Schematic illustration of TLR and IL-1R signaling cascades. Ligand engagement of both TLR and IL-1R family members leads to activation of the key signaling adaptor MyD88. TLR- and IL-1R-induced activation of MyD88 leads to the activation of the NF-κB transcription factor, as well as MAPK and PI3K signaling pathways, which collectively promote cellular proliferation and survival. TLR4 can also signal in a MyD88-independent manner, utilizing the TRIF signaling adaptor, which in turn up-regulates IRF3, leading to induction of type I IFNs which signal via the JAK/STAT pathway. DAMP, danger-associated molecular pattern.

**Fig. 2.** Opposing pro- and anti-inflammatory actions of IL-6 and IL-10 cytokines, respectively, via the differential activation of STAT3 transcriptional complexes. IL-6 signaling predominantly utilizes STAT3 heterodimers associated with other pro-inflammatory transcription factors, such as STAT1 and NF-κB, leading to a pro-inflammatory response. Conversely, IL-10 induces the formation of STAT3 homodimers, resulting in an anti-inflammatory response.

**Fig. 3.** Cell-type specificity of the cross-regulation between TLR and IL-6 cytokine family members during inflammation-associated tumorigenesis. Hyper-activation of STATs, such as STAT3 by IL-6 and/or IL-11, in normal tissue epithelia up-regulates the gene expression of TLRs. Deregulated epithelial TLR signaling consequently promotes tumorigenesis by further augmenting the inflammatory response (i.e. secretion of pro-inflammatory cytokines and chemokines to recruit leukocytes/immune cells), and/or directly acting on tumor cells to drive cell survival and proliferation. Additionally, in leukocytes, STAT-mediated up-regulation of TLRs leads to their sustained recruitment and activation, thus promoting a chronic inflammatory microenvironment.