Published in final edited form as:
Trends Immunol. 2010 April ; 31(4): 154–163. doi:10.1016/j.it.2010.01.002.

Crosstalk pathways between Toll-like receptors and the complement system

George Hajishengallis[1,2] and **John D. Lambris**[3]

[1]University of Louisville School of Dentistry, Division of Oral Health and Systemic Disease, Louisville, KY, USA

[2]University of Louisville School of Medicine, Department of Microbiology and Immunology, Louisville, KY, USA

[3]University of Pennsylvania School of Medicine, Department of Pathology and Laboratory Medicine, Philadelphia, PA, USA

Abstract

The Toll-like receptors (TLRs) and complement are key innate defense systems that are rapidly triggered upon infection. Although both systems have been investigated primarily as separate entities, an emerging body of evidence indicates extensive crosstalk between complement and TLR signaling pathways. Analysis of these data suggests that the complement–TLR interplay reinforces innate immunity or regulates excessive inflammation, through synergistic or antagonistic interactions, respectively. However, the facility of complement and TLRs for communication is exploited by certain pathogens as a means to modify the host response in ways that favor the persistence of the pathogens. Further elucidation of regulatory links between complement and TLRs is essential for understanding their complex roles in health and disease.

Toll-like receptors, complement, and potential for crosstalk

In its long co-evolution with microbes, the innate immune system has developed sentinel mechanisms for efficient detection of and prompt response to infection. In this context, the Toll-like receptors (TLRs) (Box 1) and complement (Box 2) are systems that can be rapidly activated; they provide crucial first-line host defense and act as mediators between innate and adaptive immunity [1,2]. It is conceivable that appropriate coordination of the host immune response would necessitate crosstalk between TLR and complement pathways. At least in principle, molecular crosstalk between two signaling pathways can result in emergent properties and unique functional outcomes [3,4]. Accordingly, molecular interplay between TLRs and complement could potentially result in synergistic or even antagonistic interactions. Under normal conditions, these interactions could, respectively, invigorate host defense or regulate it to prevent unwarranted inflammation. However, it is also plausible that certain crosstalk interactions might be instigated by pathogens themselves for modifying the host response in ways that promote the adaptive fitness of the pathogens. The concept of functional cooperation and cross-regulation between TLRs and the complement system is amply supported by an emerging body of recent literature [5-16]. Here, we summarize mechanisms

Corresponding author: Hajishengallis, G. (g0haji01@louisville.edu) and Lambris, J.D. (lambris@upenn.edu).

of TLR–complement communication (Table 1) and discuss their implications in health and disease.

Regulation of inflammatory TLR responses by complement

Certain microbial molecules, including lipopolysaccharide (LPS; a TLR4 agonist), zymosan (a TLR2/6 agonist) and CpG DNA (a TLR9 agonist), activate complement in addition to initiating TLR signaling [12,16]. Systemic administration of any of these molecules to mice lacking decay-accelerating factor (DAF), a major membrane-associated complement inhibitor, induces significantly higher tumor necrosis factor-α (TNF-α), interleukin-1β) IL-1β, and IL-6 but lowers IL-12 responses relative to wild-type mice [16]. $DAF^{-/-}$ mice display enhanced complement activation; therefore, these findings suggest a regulatory role for complement in TLR-induced cytokine responses. This notion was confirmed by a lack of these regulatory effects in dual knockout $DAF^{-/-}/C3^{-/-}$ mice [16]. The observation that complement-TLR crosstalk can be activated by agonists for TLR2, TLR4, and TLR9 [16], suggests involvement of the myeloid differentiation primary-response protein 88 (MyD88) signaling pathway rather than the Toll/IL-1R(TIR)-domain-containing adapter inducing IFN-β (TRIF) pathway. This is because all three TLRs can signal via MyD88, but only TLR4 can additionally signal via the TRIF pathway (Box 1), and it is therefore uncertain whether this pathway is implicated in complement-TLR crosstalk interactions. The observed influence of complement on TLR signaling is exerted predominantly via the C5a receptor (C5aR), and to a much lesser extent via C3aR. This C5aR(C3aR)–TLR crosstalk appears to involve mitogen-activated protein kinases (MAPKs), namely the extracellular signal-regulated kinase (ERK1/2) and the c-Jun N-terminal kinase (JNK), but not the p38 MAPK [16]. Although the complement–TLR synergy for increased TNF-α, IL-1β and IL-6 responses might serve a protective function to control infection, this enhanced proinflammatory response could potentially become detrimental under conditions of excessive complement activation (e.g. in pathological conditions such as sepsis). In fact, the synergistic complement–TLR interaction seen in $DAF^{-/-}$ mice might explain, at least in part, why $DAF^{-/-}$ mice are quite susceptible to inflammatory and autoimmune diseases [17].

These findings from the mouse model [16] are consistent with data from a human whole-blood model. This experimental system was designed to investigate complement interactions with other inflammatory pathways and involved the use of peripheral blood from individuals who are genetically deficient in specific complement components [10]. The study showed that C5 is required for induction of an oxidative burst, phagocytosis and killing of *Escherichia coli*, whereas C5a, in particular, is essential for the first two activities. However, antimicrobial enzyme release is independent of C5a, but highly dependent on C3, although possible C3a involvement was not demonstrated. Interestingly, these complement activities are inhibited to various degrees by CD14 blockade, which indicates complement–CD14 cooperation [10]. Moreover, efficient inhibition of *E. coli*-induced cytokine responses requires combined complement and CD14 inhibition. CD14 does not possess a transmembrane signaling domain, but rather acts as a major co-receptor of TLRs (mostly TLR4 and TLR2) [18]. It is thus possible that the observed cooperative induction of proinflammatory and antimicrobial responses [10] essentially involves crosstalk interactions between complement and TLR signaling pathways (most probably TLR4, because of the strong TLR4 agonistic activity of *E. coli* LPS). The fact that CD14 blockade inhibits complement activities [10] might alternatively be attributed to extracellular interactions between complement receptors and CD14, or other pattern-recognition receptors in general. In this regard, fluorescence resonance energy transfer studies have shown that C5aR co-associates with TLR2 in lipid rafts of activated macrophages [19]. Another intriguing possibility for complement-TLR cooperation that is independent of intracellular crosstalk, is whether active complement fragments could directly stimulate TLR signaling. However, this has yet to be addressed, although, interestingly, TLRs can also be

triggered by self-molecules acting as endogenous ligands (e.g., molecules released from damaged tissues that induce inflammatory responses) [20]. It should be noted that cooperative complement–TLR induction of innate responses might be amplified by TLR-induced cytokines, such as IL-6, that promote the expression of C3aR and C5aR [21].

Another study using the human whole-blood model focused on the complement interplay with TLR9 signaling induced by CpG oligodeoxynucleotides (ODNs), which are candidate vaccine adjuvants [12]. This investigation showed that both DNA-backbone-mediated activation or maturation of antigen-presenting cells and DNA-sequence-specific induction of cytokines are significantly suppressed by complement inhibition at C3. In fact, CpG ODNs by themselves could initiate the classical or the alternative pathway of complement activation, which in turn enhanced the cellular uptake of CpG ODNs [12]. Thus, the immunostimulatory function of CpG ODNs appears to be dependent upon the combined activation of complement and TLR9. This synergy, nevertheless, might also explain instances of toxicity that could be experienced with the use of CpG ODN adjuvants.

In addition to the classic C5aR (CD88), through which C5a exerts proinflammatory and anaphylactic action, the C5a-like receptor 2 (C5L2; GPR77) functions as an alternative high-affinity receptor for C5a [22]. Owing to its inability to couple to G proteins, C5L2 was originally perceived as simply a non-signaling scavenger receptor that can attenuate C5a-dependent inflammatory responses by competing with C5aR for C5a binding [23,24]. Consistent with this notion, $C5L2^{-/-}$ mice exhibit higher TNF-α and IL-6 responses and higher neutrophil influx into the lung, relative to wild-type controls, after pulmonary immune complex injury [25]. However, as also noted by these authors, these findings did not rule out the possibility that C5L2 might induce anti-inflammatory signaling, albeit in a G-protein-independent way. Indeed, more recent studies by these and other groups suggest that C5L2 might play an active, yet complex role in the regulation of inflammatory responses, which might include crosstalk interactions with the TLR system [14,26,27].

Specifically, $C5L2^{-/-}$ mice were shown to elicit increased IL-1β responses and to exhibit decreased survival upon LPS-induced septic shock, relative to wild-type mice [26]. On the basis of observations that C5L2 is required for optimal C3a-induced signaling in phagocytes and an earlier report that C3a induces anti-inflammatory signaling in LPS-induced sepsis, the authors suggested that C5L2 can proactively downregulate inflammation. The biochemical basis for a C3a–C5L2 interaction, however, is uncertain, and recent evaluation of available evidence suggests that C5L2 is unlikely to serve as a receptor for C3a [28]. Nevertheless, C5L2 might facilitate C3a-induced signaling by forming a heterodimer with C3aR.

An additional active role for C5L2 was proposed by another recent study. In a model of cecal ligation and puncture-induced sepsis, $C5L2^{-/-}$ mice were shown to exhibit increased survival rates compared with wild-type controls [14]. In this model, furthermore, C5aR and C5L2 differentially synergize to cause sepsis, and induction of the high mobility group box 1 (HMGB1) protein is the primary and distinctive contribution of C5L2 [14]. *In vitro*, HMGB1 can be induced by either C5a or LPS, but, intriguingly, induction of HMGB1 by LPS plus C5a or by LPS alone is diminished in $C5L2^{-/-}$ macrophages [14]. These findings strongly indicate C5L2–TLR4 cooperation in the induction of HMGB1, perhaps through a crosstalk mechanism involving mitogen-activated protein kinase kinases 1/2 (MEK1/2), JNK1/2 and phosphatidylinositol-3 kinase (PI3K) [14]. Alternatively, or in addition, this could imply a requirement for C5L2 for optimal TLR4 activation, conceivably by acting as a co-receptor.

More evidence for C5L2 involvement in C5a signaling and TLR4 regulation was provided by an independent group. Specifically, C5L2 was shown to mediate the ability of C5a to suppress induction of cytokines (TNF-α and IL-6) and co-stimulatory molecules (CD86) in LPS-

stimulated mouse macrophages [26]. However, C5a, apparently acting through C5L2, inhibits TNF-α but enhances IL-6 and macrophage-1 antigen (Mac-1) (CR3) expression in LPS-stimulated mouse neutrophils [26]. These data were obtained using $C5L2^{-/-}$ cells and, in a similar way to the study discussed above [14], imply regulatory C5L2–TLR4 crosstalk, which might involve MAPK (ERK1/2, JNK and p38) signaling in neutrophils and ERK1/2 plus Akt signaling pathways in macrophages [26]. However, since the mechanism(s) for induction of signaling downstream of C5L2 are poorly understood, it cannot be excluded that C5L2 could indirectly regulate C5aR or TLR4 signaling, or both. For instance, C5L2 might promote or stabilize the expression or conformation of C5aR (which can definitely regulate TLR4 signaling) or even TLR4, since TLRs are inclined to form complexes with heterotypic receptors [29].

With regard to the possible co-association of C5L2 with anaphylatoxin receptors, a recent study demonstrated C5a-induced co-localization of C5aR with C5L2, at least in human neutrophils [27]. In addressing the biological significance of this interaction, which appears to occur in early endosomes, these investigators showed that activated C5L2 downregulates C5aR-mediated ERK1/2 signaling and chemotaxis without concomitant alterations in C5aR-induced calcium mobilization [27]. Moreover, this study could not demonstrate scavenging activity for C5L2 [27], in contrast to another recent report that C5L2 functions as a recycling scavenging receptor, at least in transfected rat basophilic leukemia (RBL) cells [30]. This apparent discrepancy is probably attributed to differential localization of C5L2, which is localized both on the cell surface and in the cytoplasm of RBL cells [30], but predominantly intracellularly in human neutrophils, regardless of cell activation status [27].

Therefore, accumulating evidence indicates that C5L2 might proactively mediate G protein-independent signaling for the regulation of inflammatory responses in either positive or negative mode [14,26,27]. However, the biological context and associated mechanisms for these differential and versatile roles remain largely uncharacterized, although it might involve species- and/or cell type-specific differences (e.g., C5L2 promotes induction of HMGB1 in mouse macrophages [14] but inhibits ERK1/2 activation in human neutrophils [27]. Further studies are warranted to substantiate the notion that C5L2 crosstalks with TLR4 on macrophages. However, it is firmly established that C5aR and, to a lesser degree, C3aR crosstalk with TLRs, which results in regulation of innate immune and proinflammatory responses (Table 1). Furthermore, as discussed below, the complement–TLR crosstalk regulates adaptive immunity.

Complement–TLR crosstalk and regulation of T-cell immunity

Complement can inhibit TLR-induced production of IL-12. In fact, complement can regulate the expression of additional cytokines of the IL-12 family [9,11,16]. Specifically, activation of C5aR in macrophages inhibits TLR4-induced mRNA expression of IL-12p35, IL-12/IL-23p40, IL-23p19 and IL-27p28, and production of IL-12, IL-23 and IL-27 proteins. The underlying crosstalk mechanism involves induction of PI3K and ERK1/2 signaling, which in turn suppress crucial transcription factors (the interferon regulatory factors 1 and 8; IRF-1 and -8) that preferentially regulate the expression of IL-12 family cytokines [9,11,16] (Figure 1). Similar but considerably attenuated inhibitory effects are seen upon C3aR activation [9,16].

Because IL-12 family members play important regulatory roles in T-cell differentiation and development [31], the C5aR–TLR4 crosstalk might control the nature of T-cell-mediated immunity. In this regard, IL-12 (a heterodimer consisting of p35 and p40 subunits; p35/p40) drives the differentiation of the T helper 1 (Th1) subset from naïve $CD4^+$ T cells, whereas IL-23 (p19/p40) promotes the expansion of the Th17 lineage [31,32]. However, IL-27 (p28/EBI3) appears to regulate the balance between Th1 and Th17, by limiting Th17 development

in favor of Th1 [33]. In addition to its intervention in the TLR pathway, C5aR signaling can inhibit the amplification of IL-12 production induced by CD40–CD154 interaction between antigen-presenting cells and activated $CD4^+$ T cells [9]. The physiological significance of these C5a regulatory effects is probably to attenuate T-cell-mediated inflammatory tissue damage, as occurs in various pathological inflammatory conditions [33].

However, undesirable outcomes might arise when C5a is produced at unwarranted high levels (e.g. in sepsis) or through the uncontrolled action of microbial enzymes that act in a C5-convertase-like manner and generate biologically active C5a [28,34]. Under these conditions, C5a might modify TLR or CD40–CD154 signaling and skew the T-helper response in ways that could interfere with protective immunity. Indeed, *Leishmania major* appears to promote its adaptive fitness via such C5aR-dependent mechanism [9]. This conclusion is based on findings that BALB/c mice, which are normally susceptible to cutaneous leishmaniasis, acquire resistance to *L. major* infection when C5aR is genetically ablated. The protective mechanism was attributed to enhanced Th1 immunity in the absence of C5aR signaling [9].

Despite the ability of C5aR signaling to suppress Th1 polarization *in vivo* [9], activation of the same receptor does not seem to prevent Th17 development [5]. Quite strikingly, in fact, C5a synergizes with TLR signaling for Th17 differentiation, even though production of the pro-Th17 cytokine IL-23 is inhibited *in vivo* [5], as predicted by *in vitro* findings [9,11]. Specifically, concomitant activation of C5aR (but not C3aR) and TLR4 (or TLR2 or TLR9) promotes Th17 development in mice by an IL-6-dependent but IL-23-independent mechanism [5]. Nevertheless, the observed Th17 activity maintains the functional characteristics of the Th17 subset, because it is capable of causing autoimmunity in an adoptive transfer model of experimental autoimmune encephalomyelitis. Replication of the C5aR–TLR synergy for Th17 differentiation *in vitro*, using macrophages and anti-CD3 or antigen-stimulated $CD4^+$ T cells, confirmed that the C5a effect is exerted via C5aR signaling in macrophages rather than in the T cells [5]. This study, therefore, has provided a novel, Th17-associated mechanism for C5a involvement in autoimmunity, which is dependent upon the crosstalk capabilities of complement and TLR signaling pathways.

The C5a–C5aR axis does not constitute the only complement mechanism for regulation of TLR-induced cytokines of the IL-12 family. Indeed, activation of gC1qR, a complement receptor for C1q that initiates the classical complement cascade, downregulates TLR4-induced IL-12 in human monocytes [15]. This regulatory effect is selective for IL-12 (e.g. IL-6, IL-8, and TNF-α are not affected) and is mediated via PI3K signaling (Figure 1). Strikingly, the hepatitis C virus core protein acts as a ligand for gC1qR, and the outcome of this interaction, that is inhibition of IL-12 production and of Th1 immunity, is believed to be exploited by the virus for establishing persisting infections [35]. This evasion mechanism might not be unique to hepatitis C virus because other pathogens (e.g. *Listeria monocytogenes* and *Staphylococcus aureus)* can also interact with qC1qR. Under physiological conditions, however, the gC1qR–TLR crosstalk could function as a homeostatic mechanism that regulates T-cell immunity. This notion is consistent with observations that C1q deficiency in humans and mice causes inflammatory autoimmune pathology [36].

However, additional C1q regulatory mechanisms might contribute to the prevention of immunopathology. Indeed, C1q also downregulates TLR4-induced IL-1β and TNF-α, whereas it enhances TLR4-induced IL-10 [37], possibly through activation of the cAMP response element-binding protein (CREB) and generation of inhibitory NF-κB p50/p50 homodimers [6]. The receptor(s) mediating these effects has not been specified, although it could involve a receptor shared with the mannose-binding lectin (MBL), such as CD35, CD93 or calreticulin [38], because MBL and C1q display almost identical regulatory effects on LPS-stimulated

monocytes [39] that are believed to limit unwarranted inflammation during C1q- and MBL-facilitated clearance of apoptotic cells [39].

CD46, a complement regulatory receptor, also crosstalks with TLR4 and regulates IL-12. Specifically, CD46 inhibits LPS-induced production of IL-12 in monocytes upon binding C3b dimers [40] (Figure 1). Intriguingly, CD46 is utilized as a cellular receptor by the measles virus, which thereby inhibits IL-12 production and causes host immunosuppression (reviewed in [41]). The fact that CD46 is also used as a receptor by other pathogens (*Neisseria gonorrhoeae, Neisseria meningitidis Streptococcus pyogenes*, human herpesvirus-6, and group B and D adenoviruses) raises the possibility that this might be a universal pathway for immune evasion [42,43]. Although the ability of CD46 to regulate TLR4-induced production of bioactive IL-12 is firmly established, the underlying signaling mechanism remains uncertain. However, it is possible that the inhibitory effect involves a post-transcriptional mechanism, as shown in a study with human herpesvirus-6, which similarly causes selective inhibition of TLR4-induced IL-12 after CD46 binding [42] (Figure 1).

Transactivation of complement receptor function by TLR signaling

The complement receptor-3 (CR3; a CD11b/CD18 heterodimeric integrin) can interact with a wide variety of structurally unrelated molecules derived from either the host (e.g. the complement iC3b fragment, fibrinogen and the intercellular adhesion molecule-1) or pathogens (e.g. *Bordetella pertussis* filamentous hemagglutinin, *Porphyromonas gingivalis* fimbriae and mycobacterial lipoarabinomannan) [44]. Because CR3 is involved in leukocyte adhesion and transendothelial migration, its adhesive activity is tightly regulated [45]. Whereas in resting cells CR3 and other integrins display a low-affinity conformation, a rapid and transient shift to a high-affinity binding state can be triggered by inside-out signaling, which is induced by certain other surface receptors, such as chemokine receptors [45].

More recently, it was shown that TLR2 can also transactivate CR3 [46,47]. The TLR2 inside-out signaling pathway proceeds through the Ras-related C3 botulinum toxin substrate 1 (Rac1), PI3K and cytohesin-1 [8,46,47] and is distinct from the myeloid differentiation primary-response protein 88 (MyD88)-dependent TLR2 proinflammatory pathway [7] (Figure 2). Notably, PI3K can be recruited directly to the TLR2 cytoplasmic tail which, unlike TLR4, contains PI3K-binding motifs [48]. The communication of CR3 with the TLR system is bidirectional in that CR3 also regulates TLR signaling [49]. Specifically, CR3 in macrophages promotes the recruitment of the sorting adaptor known as Toll/IL-1R(TIR)-domain-containing adaptor protein (TIRAP) (or MyD88-adaptor like; Mal) to the plasma membrane via its ability to stimulate local phosphatidylinositol 4,5-bisphosphate (PIP2) production. Once TIRAP becomes targeted to membrane-bound PIP2 through its PIP2-binding domain, it can then facilitate the recruitment of the MyD88 signaling adaptor to either TLR2 or TLR4 and thereby initiate TLR signaling [49] (Figure 2).

The ability of a pathogen-sensing receptor such as TLR2 to transmodulate CR3 was initially perceived as a potentially protective mechanism, which could contribute to leukocyte recruitment to sites of infection, a possibility that was supported experimentally in an *in vitro* transmigration system [8]. However, once transactivated, CR3 becomes a target of subversive activity by several pathogens. For example, the oral pathogen *P. gingivalis* instigates a TLR2–CR3 crosstalk that allows it to hijack CR3 for a relatively safe entry into macrophages [50,51]. Indeed, this phagocytic mechanism does not promote the killing of *P. gingivalis*. In contrast, when macrophages phagocytose *P. gingivalis* by alternative receptors (i.e. when CR3 is blocked or genetically ablated), their intracellular killing capacity is markedly potentiated [51]. These findings are explained by the observation that CR3 is not linked to vigorous microbicidal mechanisms [52]. However, this begs another question. Why would

professional killer cells express a phagocytic receptor that is not coupled to efficient intracellular killing? A plausible explanation is that, under physiologic conditions, CR3 is heavily committed with the phagocytosis of iC3b-coated apoptotic cells, which do not constitute a danger that would warrant a vigorous host response (reviewed in [53]). Accordingly, the production of IL-12 is suppressed in macrophages upon phagocytosis of apoptotic cells [54]. This iC3b–CR3 mechanism of IL-12 inhibition is reminiscent of a similar inhibitory effect caused by C3b binding to CD46 [41]. Moreover, similar to observations of CD46 exploitation by pathogens, CR3 is also co-opted as a means of microbial manipulation of IL-12 responses (Figure 2).

Indeed, direct CR3 binding by *P. gingivalis* fimbriae inhibits TLR2-induced IL-12 through outside-in signaling that activates ERK1/2, which in turn selectively inhibits mRNA expression of IL-12p35 and IL-12/IL-23p40 [53] (Figure 2). This allows *P. gingivalis* to promote its survival in wild-type mice, whereas CR3-deficient mice are superior in controlling *P. gingivalis* infections because of increased induction of IL-12 and, secondarily, IFN-γ, a potent activator of intracellular killing [53]. The CR3-dependent inhibition of IL-12 is not specific for TLR2 (TLR4-induced IL-12 is also inhibited) but is selective for this cytokine, because the expression of other proinflammatory cytokines is either unaltered or increased [53,55]. The TLR2–CR3 crosstalk pathway could be an Achilles' heel that confers host susceptibility to a number of other pathogens. Indeed, mycobacteria and spores of *Bacillus anthracis* can both activate TLR2 inside-out signaling and bind transactivated CR3, which results in enhanced CR3-mediated uptake of these organisms [13]. In a similar way to earlier observations with *P. gingivalis*, CR3-deficient mice display enhanced resistance to infection with *B. anthracis* spores, attributable to possible spore carriage by the macrophages to sites of spore germination and bacterial growth [13]. Likewise, the ability of *Mycobacterium tuberculosis* to parasitize macrophages might depend, at least in part, on its capacity to stimulate TLR2-induced CR3 uptake [47].

Although the TLR2–CR3 pro-adhesive pathway can be activated by bacteria in monocytes–macrophages or neutrophils [46], a similar pathway is not operational in dendritic cells, which appear to be relatively inert with regard to induction of inside-out signaling for CR3 activation [56]. Additional functional differences between dendritic cells and macrophages in the context of complement–TLR crosstalk are discussed in the following section.

Cell-type-specific regulatory effects of complement on TLR responses

In contrast to its effects on monocytes–macrophages, C5a does not inhibit TLR- or CD40/CD154-induced IL-12 production in human or mouse dendritic cells, even though C5aR is readily activated in those cells [41]. In fact, C5aR (or C3aR) signaling promotes microbially induced IL-12 and IL-23 in dendritic cells, and $C5aR^{-/-}$ (or $C3aR^{-/-}$) mice fail to stimulate T-cell immunity *in vitro* and *in vivo* [57]. The underlying mechanism for this cell-type-specific difference in IL-12 regulation is unclear. However, it is of interest that whereas ERK1/2 activation inhibits IL-12 in macrophages [9,11], it exerts the opposite effect in dendritic cells [58].

Another possible mechanism contributing to C5a-mediated upregulation of TLR4-induced IL-12 production in dendritic cells involves the capacity of C5aR to inhibit immunosuppressive cAMP-dependent protein kinase A signaling [59]. This mechanism, which is consistent with the ability of C5aR to couple to Gαi-protein-coupled receptors, moreover upregulates TNF-α and downregulates IL-10 production [59]. However, C5a does not inhibit cAMP production in macrophages or neutrophils, and actually C5aR signaling stimulates cAMP-dependent protein kinase A activity in neutrophils [60]. Strikingly, although C5aR activation by itself does not stimulate cAMP production in macrophages, it synergizes with TLR2 for induction

of high and sustained cAMP levels. This C5aR–TLR2 crosstalk is exploited by *P. gingivalis*, which thereby causes macrophage immunosuppression in the presence of C5a [19].

The molecular basis of these cell-type-specific C5a effects on cAMP induction is uncertain. However, upon activation of the Gαi subunit, the released Giβγ subunits can potently regulate adenylate cyclase synthesis of cAMP, either positively or negatively, depending on the enzyme isoform [61]. Strikingly, the adenylate cyclase isoforms that are positively regulated by Giβγ are not those that are sensitive to the inhibitory action of Gαi [61]. Thus, it is possible that dendritic cells and macrophages or neutrophils or both types of cell express distinct isoforms of adenylate cyclase, which in turn display differential regulation in response to C5aR-induced Gi signaling.

In a similar fashion to dendritic cell C5aR, activation of CD46 signaling in LPS-stimulated dendritic cells promotes the expression of IL-12p35, IL-23p19 and IL-12/IL-23p40. Although no significant production of bioactive IL-12 is detected under these conditions, functional IL-23 is readily produced and enhances IL-17 production by activated T cells [62]. Importantly, LPS- or CD46-stimulated dendritic cells isolated from patients with multiple sclerosis express higher mRNA levels of IL-23p19 compared with similarly treated dendritic cells from healthy controls [62]. These data provide further evidence for the involvement of complement in Th17-dependent autoimmune diseases, such as multiple sclerosis, through crosstalk with TLR4 signaling. However, the reason why dendritic cells and monocytes–macrophages behave disparately in terms of regulating the expression of IL-12 family cytokines in response to activation of the same receptors (C5aR or CD46) remains poorly characterized.

Unlike C5aR and CD46, however, gC1qR signaling inhibits TLR4-induced IL-12 in dendritic cells too [15,35]. This finding might be attributed to differences in the activated signaling pathways downstream of these receptors. Specifically, although both C5aR and gC1qR activate PI3K, only C5aR can activate ERK1/2 [15] which, in dendritic cells, upregulates IL-12 [58]. Regarding the regulatory effects of C1q, another layer of complexity is introduced by the presence of multiple receptors for this complement component [38]. Two of these receptors, gC1qR and cC1qR (calreticulin), interact with the globular and collagenous regions of C1q, respectively [38,63]. In contrast to gC1qR, activation of cC1qR by C1q actually potentiates IL-12 production by TLR-activated dendritic cells, which thereby foster the differentiation of Th1 cells [63]. This notion was demonstrated using the collagenous region of C1q as stimulus, whereas results obtained in similar experiments using the C1q globular fragment were probably inconclusive because of the presence of residual collagen-like triplets [63]. It can thus be speculated that the relative expression of these C1q receptors, which could depend on the cell type or the stage of cell maturation or differentiation, could determine the outcome of C1q-induced crosstalk signaling with TLRs. In summary, C1q might have multifunctional roles in immunity, ranging from proinflammatory actions (e.g. activation of the classical complement cascade [2] or stimulation of Th1 responses [58,63]) to immunoregulatory functions [6,15, 37]; the latter are consistent with the association of C1q deficiency with autoimmune conditions such as systemic lupus erythematosus [36].

General conclusions and future perspectives

The recent literature has provided documented examples of cross-regulation between complement and the TLRs, in several *in vitro* and *in vivo* experimental systems in mice and humans (Table 1). The physiological significance of the complement–TLR interplay is probably to coordinate host defense, both at the innate and the adaptive level. Despite the proinflammatory nature of both systems, some of the crosstalk pathways lead to antagonistic effects, ostensibly for homeostatic reasons. However, the same complement receptors that can regulate TLR signaling (e.g. CR3, C5aR, gC1qR and CD46) can be hijacked by bacterial or

viral pathogens (Figures 1 and 2) to skew the host response in ways that interfere with protective immunity. Future research is expected to elucidate additional regulatory links between the complement and TLR systems, which is essential for understanding their precise roles in health and disease. This, in turn, should lead to improved therapeutic interventions to enhance protective immunity, attenuate immunopathology or neutralize sophisticated mechanisms of microbial immune subversion.

Box 1. Toll-like receptors

The TLR family of pattern-recognition receptors plays a central role as sensors of infection. Different TLRs recognize distinct types of conserved microbial structure, thus endowing the innate response with a degree of specificity [1,64]. For example, TLR2 responds to lipoteichoic acid (LTA), TLR3 to viral double-stranded RNA, TLR4 to LPS, TLR5 to flagellin and TLR9 to bacterial CpG DNA (Figure I). With the exception of TLR3, TLRs induce nuclear factor (NF)-κB-dependent expression of proinflammatory and immunoregulatory genes via a common signaling adaptor, the myeloid differentiation primary-response protein 88 (MyD88). However, TLR3, as well as TLR4 upon endocytosis into endosomes, can signal through the Toll/IL-1R(TIR)-domain-containing adapter inducing IFN-β (TRIF) pathway, which triggers activation of the interferon regulatory factor-3 (IRF-3) and type I interferon production [65]. Additional complexities, which might serve to tailor the host response according to the nature of the invading pathogen, include the exclusive use by TLR2 and TLR4 of the sorting adapter known as MyD88-adapter like (Mal) or TIR-domain-containing adaptor protein (TIRAP), and the compartmentalization of TLRs. In this regard, TLRs that predominantly detect extracellular microbial structures are expressed on the host cell surface (TLR-1, -2, -4, -5 and -6), whereas TLRs that specialize in detecting viral or bacterial nucleic acids are aptly located intracellularly on endocytic vesicles or organelles (TLR-3, -7, -8 and -9) [64] (Figure I). Furthermore, TLR heterodimerization within the family (e.g. TLR2 uses either TLR1 or TLR6 as signaling partners) or outside of it (e.g. TLR2 co-associates with CD14 or CD36) might create a combinatorial repertoire to discriminate better among the abundant and diverse microbial structures [18].

Box 2. Complement and pathogen recognition

The integrated complement system includes serum proteins, membrane-bound regulators and receptors for interactions with various immune mediators. The complement cascade can be triggered via three distinct pathways (classical, lectin or alternative) which converge at the third component of complement (C3) [2]. C3 activation by pathway-specific C3 convertases leads to the generation of effector molecules involved in the (i) recruitment and activation of inflammatory cells (e.g. the C3a and C5a anaphylatoxins, which activate specific G-protein-coupled receptors, C3aR and C5aR, respectively), (ii) microbial opsonization and phagocytosis (e.g. the iC3b opsonin which interacts with CR3) and (iii) direct lysis of targeted pathogens (by means of the C5b–9 membrane attack complex [MAC]) [2] (Figure I). In contrast to the classic C5aR (CD88), an alternative but quite enigmatic receptor for C5a, the C5a-like receptor 2 (C5L2; GPR77), does not couple to G proteins and cannot induce mobilization of intracellular Ca^{2+} [22]. However, there is substantial evidence that C5L2 can induce G protein-independent intracellular signaling to promote or attenuate inflammatory responses in an apparently cell-type specific way [14, 26,27], although it might also have functions that are not related to signaling (e.g. serving as a recycling scavenger receptor, at least in transfected cell lines [30]).

Pathogen recognition by complement predominantly involves the 'missing-self recognition' strategy, because pathogens normally lack complement regulatory proteins that would prevent complement activation. However, in a similar way to TLRs, complement also uses the 'pattern recognition' strategy, because important complement components, such as C1q, properdin, and the mannose-binding lectin, can recognize conserved microbial structures such as cell wall components and carbohydrate motifs [28]. TLRs and complement receptors also interact with microbial virulence proteins which, far from being conserved, are unique to specific pathogens (e.g. the early secreted antigenic target protein-6 of *Mycobacterium tuberculosis* interacts with TLR2 [66], the chemotaxis inhibitory protein of *Staphylococcus aureus* recognizes C5aR [67], and the fimbriae of *Porphyromonas gingivalis* bind CR3 [53]). In these cases, it is the virulence proteins that have probably evolved to recognize these innate immune receptors (quite possibly for immune subversion) rather than vice versa.

Acknowledgments

Studies performed in the authors' laboratories and cited in this paper were supported by US Public Health Service Grants DE015254 and DE018292 (to G.H.) and AI068730 and GM062134 (to J.D.L.). The authors regret that a number of important studies could only be cited indirectly through comprehensive reviews, because of space and reference number limitations.

References

1. Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature 2007;449:819–826. [PubMed: 17943118]
2. Markiewski MM, Lambris JD. The role of complement in inflammatory diseases from behind the scenes into the spotlight. Am J Pathol 2007;171:715–727. [PubMed: 17640961]
3. Zak DE, Aderem A. Systems biology of innate immunity. Immunol Rev 2009;227:264–282. [PubMed: 19120490]
4. Natarajan M, et al. A global analysis of cross-talk in a mammalian cellular signalling network. Nat Cell Biol 2006;8:571–580. [PubMed: 16699502]
5. Fang C, et al. Complement promotes the development of inflammatory T-helper 17 cells through synergistic interaction with Toll-like receptor signaling and interleukin-6 production. Blood 2009;114:1005–1015. [PubMed: 19491392]
6. Fraser DA, et al. Generation of inhibitory NFκB complexes and phosphorylated cAMP response element-binding protein correlates with the anti-inflammatory activity of complement protein C1q in human monocytes. J Biol Chem 2007;282:7360–7367. [PubMed: 17209050]
7. Hajishengallis G, et al. Induction of distinct TLR2-mediated proinflammatory and proadhesive signaling pathways in response to *Porphyromonas gingivalis* fimbriae. J Immunol 2009;182:6690–6696. [PubMed: 19454663]
8. Harokopakis E, et al. TLR2 transmodulates monocyte adhesion and transmigration via Rac1- and PI3K-mediated inside-out signaling in response to *Porphyromonas gingivalis* fimbriae. J Immunol 2006;176:7645–7656. [PubMed: 16751412]
9. Hawlisch H, et al. C5a negatively regulates toll-like receptor 4-induced immune responses. Immunity 2005;22:415–426. [PubMed: 15845447]
10. Lappegård KT, et al. Human genetic deficiencies reveal the roles of complement in the inflammatory network: lessons from nature. Proc Natl Acad Sci U S A 2009;106:15861–15866. [PubMed: 19717455]
11. la Sala A, et al. G(i)-protein-dependent inhibition of IL-12 production is mediated by activation of the phosphatidylinositol 3-kinase-protein 3 kinase B/Akt pathway and JNK. J Immunol 2005;175:2994–2999. [PubMed: 16116186]
12. Mangsbo SM, et al. Complement activation by CpG in a human whole blood loop system: Mechanisms and immunomodulatory effects. J Immunol 2009;183:6724–6732. [PubMed: 19864604]

13. Oliva C, et al. CD14-Mac-1 interactions in *Bacillus anthracis* spore internalization by macrophages. Proc Natl Acad Sci U S A 2009;106:13957–13962. [PubMed: 19666536]
14. Rittirsch D, et al. Functional roles for C5a receptors in sepsis. Nat Med 2008;14:551–557. [PubMed: 18454156]
15. Waggoner SN, et al. gC1q receptor ligation selectively down-regulates human IL-12 production through activation of the phosphoinositide 3-kinase pathway. J Immunol 2005;175:4706–4714. [PubMed: 16177118]
16. Zhang X, et al. Regulation of Toll-like receptor-mediated inflammatory response by complement in vivo. Blood 2007;110:228–236. [PubMed: 17363730]
17. Kim DD, Song WC. Membrane complement regulatory proteins. Clin Immunol 2006;118:127–136. [PubMed: 16338172]
18. Beutler B. Microbe sensing, positive feedback loops, and the pathogenesis of inflammatory diseases. Immunol Rev 2009;227:248–263. [PubMed: 19120489]
19. Wang M, et al. Microbial hijacking of complement-Toll-like receptor crosstalk. Sci Signal. 2010 revised version under review.
20. Rifkin IR, et al. Toll-like receptors, endogenous ligands, and systemic autoimmune disease. Immunological Reviews 2005;204:27–42. [PubMed: 15790348]
21. Rittirsch D, et al. Harmful molecular mechanisms in sepsis. Nat Rev Immunol 2008;8:776–787. [PubMed: 18802444]
22. Monk PN, et al. Function, structure and therapeutic potential of complement C5a receptors. Br J Pharmacol 2007;152:429–448. [PubMed: 17603557]
23. Okinaga S, et al. C5L2, a nonsignaling C5a binding Protein. Biochemistry 2003;42:9406–9415. [PubMed: 12899627]
24. Gao H, et al. Evidence for a functional role of the second C5a receptor C5L2. Faseb J 2005;19:1003–1005. [PubMed: 15784721]
25. Gerard NP, et al. An anti-inflammatory function for the complement anaphylatoxin C5a-binding protein, C5L2. J Biol Chem 2005;280:39677–39680. [PubMed: 16204243]
26. Chen NJ, et al. C5L2 is critical for the biological activities of the anaphylatoxins C5a and C3a. Nature 2007;446:203–207. [PubMed: 17322907]
27. Bamberg CE, et al. The C5a receptor C5L2 is a negative modulator of C5aR mediated signal transduction. J Biol Chem. 2009 Epub ahead of print. 10.1074/jbc.M109.092106
28. Klos A, et al. The role of the anaphylatoxins in health and disease. Mol Immunol. 2009
29. Triantafilou M, Triantafilou K. The dynamics of LPS recognition: complex orchestration of multiple receptors. J Endotoxin Res 2005;11:5–11. [PubMed: 15826372]
30. Scola AM, et al. The human complement fragment receptor, C5L2, is a recycling decoy receptor. Mol Immunol 2009;46:1149–1162. [PubMed: 19100624]
31. Goriely S, et al. How microorganisms tip the balance between interleukin-12 family members. Nat Rev Immunol 2008;8:81–86. [PubMed: 18084185]
32. Gaffen SL, et al. Hajishengallis G. A new inflammatory cytokine on the block: re-thinking periodontal disease and the Th1/Th2 paradigm in the context of Th17 cells and IL-17. J Dent Res 2008;87:817–828. [PubMed: 18719207]
33. Weaver CT, et al. IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu Rev Immunol 2007;25:821–852. [PubMed: 17201677]
34. Hajishengallis G. *Porphyromonas gingivalis*-host interactions: open war or intelligent guerilla tactics? Microbes Infect 2009;11:637–645. [PubMed: 19348960]
35. Waggoner SN, et al. HCV core protein interaction with gC1q receptor inhibits Th1 differentiation of CD4+ T cells via suppression of dendritic cell IL-12 production. J Leukoc Biol 2007;82:1407–1419. [PubMed: 17881511]
36. Manderson AP, et al. The role of complement in the development of systemic lupus erythematosus. Annu Rev Immunol 2004;22:431–456. [PubMed: 15032584]
37. Fraser DA, et al. C1q differentially modulates phagocytosis and cytokine responses during ingestion of apoptotic cells by human monocytes, macrophages, and dendritic Cells. J Immunol 2009;183:6175–6185. [PubMed: 19864605]

38. Lu JH, et al. The classical and regulatory functions of C1q in immunity and autoimmunity. Cell Mol Immunol 2008;5:9–21. [PubMed: 18318990]
39. Fraser DA, et al. C1q and MBL, components of the innate immune system, influence monocyte cytokine expression. J Leukoc Biol 2006;80:107–116. [PubMed: 16617157]
40. Karp CL, et al. Mechanism of suppression of cell-mediated immunity by measles virus. Science 1996;273:228–231. [PubMed: 8662504]
41. Kohl J. The role of complement in danger sensing and transmission. Immunol Res 2006;34:157–176. [PubMed: 16760575]
42. Smith A, et al. Selective suppression of IL-12 production by human herpesvirus 6. Blood 2003;102:2877–2884. [PubMed: 12829600]
43. Gill DB, Atkinson JP. CD46 in *Neisseria* pathogenesis. Trends in Molecular Medicine 2004;10:459–465. [PubMed: 15350899]
44. Hajishengallis G, et al. Subversion of innate immunity by periodontopathic bacteria via exploitation of complement receptor-3. Adv Exp Med Biol 2008;632:203–219. [PubMed: 19025124]
45. Abram CL, Lowell CA. The ins and outs of leukocyte integrin signaling. Annu Rev Immunol 2009;27:339–362. [PubMed: 19302044]
46. Harokopakis E, Hajishengallis G. Integrin activation by bacterial fimbriae through a pathway involving CD14, Toll-like receptor 2, and phosphatidylinositol-3-kinase. Eur J Immunol 2005;35:1201–1210. [PubMed: 15739163]
47. Sendide K, et al. Cross-talk between CD14 and complement receptor 3 promotes phagocytosis of mycobacteria: regulation by phosphatidylinositol 3-kinase and cytohesin-1. J Immunol 2005;174:4210–4219. [PubMed: 15778383]
48. Arbibe L, et al. Toll-like receptor 2-mediated NF-κB activation requires a Rac1-dependent pathway. Nat Immunol 2000;1:533–540. [PubMed: 11101877]
49. Kagan JC, Medzhitov R. Phosphoinositide-mediated adaptor recruitment controls Toll-like receptor signaling. Cell 2006;125:943–955. [PubMed: 16751103]
50. Hajishengallis G, et al. *Porphyromonas gingivalis* fimbriae proactively modulate β_2 integrin adhesive activity and promote binding to and internalization by macrophages. Infect Immun 2006;74:5658–5666. [PubMed: 16988241]
51. Wang M, et al. Fimbrial proteins of *Porphyromonas gingivalis* mediate in vivo virulence and exploit TLR2 and complement receptor 3 to persist in macrophages. J Immunol 2007;179:2349–2358. [PubMed: 17675496]
52. Lowell CA. Rewiring phagocytic signal transduction. Immunity 2006;24:243–245. [PubMed: 16546092]
53. Hajishengallis G, et al. Complement receptor 3 blockade promotes IL-12-mediated clearance of *Porphyromonas gingivalis* and negates its virulence in vivo. J Immunol 2007;179:2359–2367. [PubMed: 17675497]
54. Kim S, et al. Transcriptional suppression of interleukin-12 gene expression following phagocytosis of apoptotic cells. Immunity 2004;21:643–653. [PubMed: 15539151]
55. Hajishengallis G, et al. Peptide mapping of bacterial fimbrial epitopes interacting with pattern recognition receptors. J Biol Chem 2005;280:38902–38913. [PubMed: 16129673]
56. Varga G, et al. Active MAC-1 (CD11b/CD18) on DCs inhibits full T-cell activation. Blood 2007;109:661–669. [PubMed: 17003381]
57. Strainic MG, et al. Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4+ T cells. Immunity 2008;28:425–435. [PubMed: 18328742]
58. Baruah P, et al. C1q enhances IFN-γ production by antigen-specific T cells via the CD40 costimulatory pathway on dendritic cells. Blood 2009;113:3485–3493. [PubMed: 19171874]
59. Peng Q, et al. Dendritic cell function in allostimulation is modulated by C5aR signaling. J Immunol 2009;183:6058–6068. [PubMed: 19864610]
60. Ward PA. The dark side of C5a in sepsis. Nat Rev Immunol 2004;4:133–142. [PubMed: 15040586]
61. Sunahara RK, Taussig R. Isoforms of mammalian adenylyl cyclase: Multiplicities of signaling. Mol Interv 2002;2:168–184. [PubMed: 14993377]

62. Vaknin-Dembinsky A, et al. Increased IL-23 secretion and altered chemokine production by dendritic cells upon CD46 activation in patients with multiple sclerosis. Journal of Neuroimmunology 2008;195:140–145. [PubMed: 18403025]

63. Csomor E, et al. Complement protein C1q induces maturation of human dendritic cells. Mol Immunol 2007;44:3389–3397. [PubMed: 17383729]

64. Ishii KJ, et al. Host innate immune receptors and beyond: making sense of microbial infections. Cell Host Microbe 2008;3:352–363. [PubMed: 18541212]

65. O'Neill LA. When signaling pathways collide: positive and negative regulation of toll-like receptor signal transduction. Immunity 2008;29:12–20. [PubMed: 18631453]

66. Pathak SK, et al. Direct extracellular interaction between the early secreted antigen ESAT-6 of *Mycobacterium tuberculosis* and TLR2 inhibits TLR signaling in macrophages. Nat Immunol 2007;8:610–618. [PubMed: 17486091]

67. Postma B, et al. Chemotaxis inhibitory protein of *Staphylococcus aureus* binds specifically to the C5a and formylated peptide receptor. J Immunol 2004;172:6994–7001. [PubMed: 15153520]

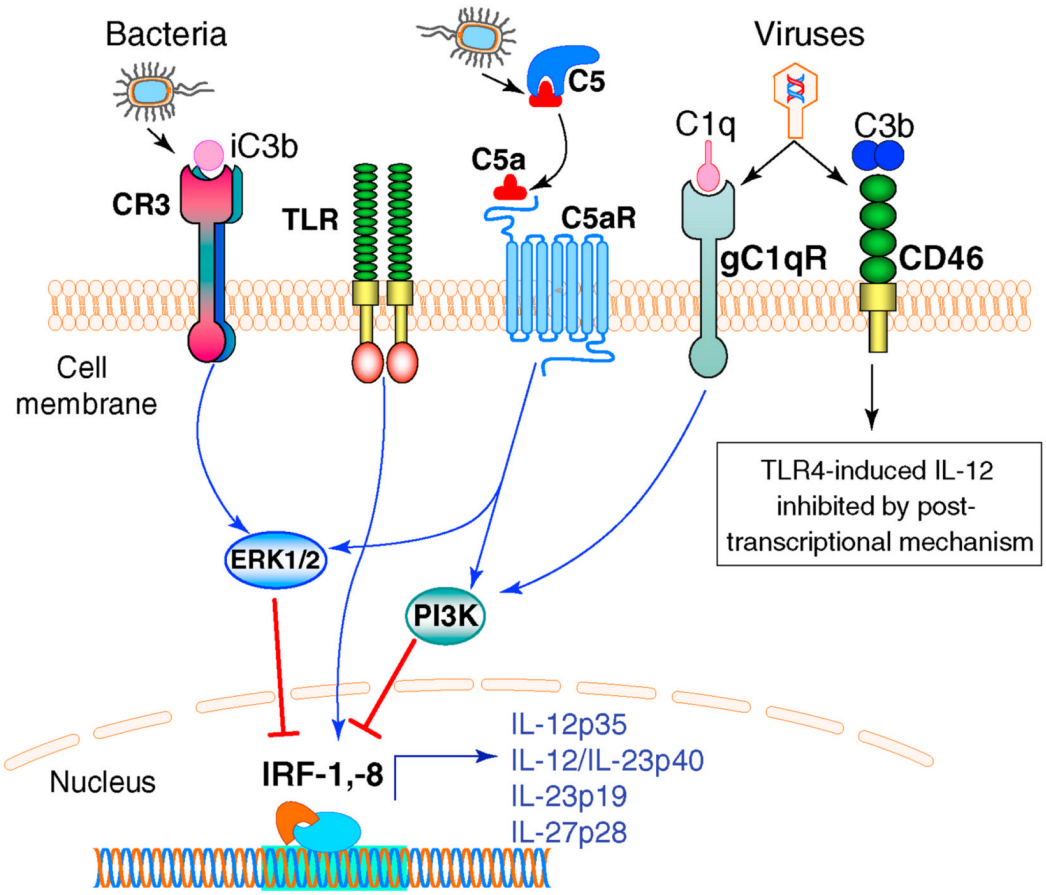

Figure 1.

Complement regulation of TLR-induced cytokines of the IL-12 family. Activation of these complement receptors by their natural ligands downregulates TLR-induced mRNA expression of IL-12p35, IL-12/IL-23p40, IL-23p19 and IL-27p28 and production of bioactive IL-12, IL-23 and IL-27 [9,11,15,16,41,53]. The underlying signaling mechanisms involve induction of PI3K or ERK1/2 signaling, which in turn suppresses crucial transcription factors (IRF-1 and IRF-8), although the CD46 mechanism appears to be exerted at the post-transcriptional level. However, these receptors can also be activated by bacterial or viral pathogens, which thereby can manipulate TLR-induced IL-12 cytokines and associated T-helper responses in ways that interfere with protective immunity [35,41,53]. Although C5aR cannot be directly activated by pathogens, C5aR can come under pathogen control through microbial C5 convertase-like enzymes, which generate biologically active C5a [34]. The regulatory mechanisms shown appear to be cell-type specific and have been documented in human or mouse monocytes–macrophages.

Figure I. TLRs and their major ligands.

Figure I. Simplified representation of the complement cascade.

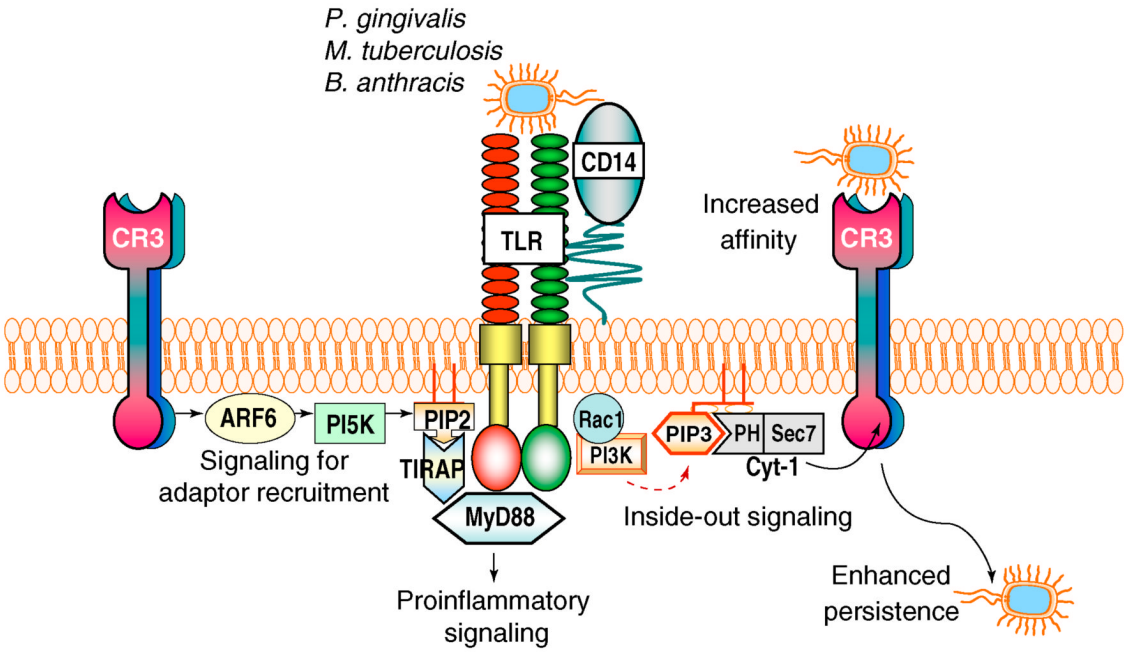

Figure 2.

TLR–CR3 crosstalk pathways. Induction of TLR2 inside-out signaling, mediated by Rac-1, PI3K and cytohesin-1 (Cyt-1), upregulates the high-affinity state of CR3. The terminal component, Cyt-1, contains a pleckstrin homology (PH) domain, which binds and uses phosphatidylinositol-(3,4,5)-triphosphate (PIP3) as a docking site to interact (via its Sec7 domain) with the CD18 cytoplasmic tail of CR3. Certain bacteria (*P. gingivalis, M. tuberculosis, B. anthracis*) bind CD14 and induce TLR2 inside-out signaling for activating and binding macrophage CR3, which leads to relatively safe uptake of these organisms, thus enhancing their intracellular persistence [13,34,46,47]. In a reciprocal manner, CR3 can regulate the signaling activity of TLRs that utilize TIRAP as an adaptor, i.e. TLR2 and TLR4 [49]. Specifically, outside-in signaling by CR3 leads to activation of ADP ribosylation factor 6 (ARF6) and induction of phosphatidylinositol-(4,5)-bisphosphate (PIP2) production by phosphatidylinositol 5-kinase (PI5K). This activity promotes the targeting of TIRAP to membrane-bound PIP2 through its PIP2-binding domain. TIRAP subsequently acts as a sorting adaptor, which facilitates the recruitment of the signaling adaptor MyD88 to either TLR2 or TLR4 for initiation of downstream signaling [49].

Table 1

Crosstalk between complement and Toll-like receptor signaling pathways

Receptors involved		Outcomes	Possible crosstalk point(s)	Experimental system	Refs
Complement	**TLRs**				
C3aR[a]	TLR2	Enhanced TNF-α, IL-1β and IL-6 responses; lower IL-12 and IL-23	MAPK (ERK1/2, JNK) and PI3K	Mouse macrophages, human monocytes, *in vivo* mouse models	[5,9,11,16]
C5aR	TLR4				
	TLR9	Inhibition of Th1; promotion of Th17			
Receptors downstream of C3 (C5aR and perhaps C3aR)	CD14/TLR4	Enhanced induction of oxidative burst, proinflammatory cytokines (TNF-α, IL-1β, IL-8), antimicrobial responses, phagocytosis and killing of *E. coli*	Not addressed	Human whole-blood model	[10]
Receptors of and downstream of C3 (C3aR, C5aR, CR3)	TLR9	Enhanced expression of activation/ maturation markers (CD40, CD83) and cytokines (TNF-α, IL-6, IL-8) in antigen-presenting cells CpG ODN-induced complement activation leading to enhanced CR3 uptake of CpG ODN	Not addressed	Human whole-blood model	[12]
C5aR	TLR2	Synergistic induction of cAMP and suppression of nitric oxide-dependent bacterial killing	Adenylate cyclase	Mouse macrophages, in vivo mouse model	[19]
C3aR C5aR	TLR4	Increased IL-12 and IL-23 production	ERK1/2	Human or mouse dendritic cells, *in vivo* mouse model	[41,57,59]
CR3	TLR2 TLR4	Selective inhibition of IL-12; other proinflammatory cytokines unaltered or enhanced	ERK1/2	Human monocytes, mouse macrophages, *in vivo* mouse models	[41,53,54]
CR3	TLR2 TLR4	TIRAP recruitment to activated TLRs	PIP2	Mouse macrophages	[49]

Receptors involved		Outcomes	Possible crosstalk point(s)	Experimental system	Refs
Complement	**TLRs**				
CR3	TLR2	Activation of the CR3 ligand-binding capacity via TLR2 inside-out signaling	Rac1, PI3K and cytohesin-1	Human monocytes or neutrophils, mouse macrophages	[8,46,47]
C5L2[b]	TLR4	Enhanced HMGB1 induction	MAPK (MEK1/2 and JNK1/2) and PI3K/Akt	Mouse macrophages, cecal ligation and puncture-induced sepsis model in mice	[14]
C5L2	TLR4	Inhibition of TNF-α; enhancement of IL-6 and CR3 (Mac-1) expression	MAPK (ERK1/2, JNK, p38)	Mouse neutrophils	[26]
C5L2	TLR4	Suppressed induction of TNF-α, IL-6 and CD86	ERK1/2 and Akt	Mouse macrophages	[26]
gC1qR	TLR4	Selective suppression of IL-12	PI3K	Human monocytes and dendritic cells	[15,35]
cC1qR	TLR4	Enhanced expression of co-stimulatory molecules, TNF-α, IL-12, and promotion of Th1 (no evidence for cC1qR–TLR4 synergy, possible additive effects)	NF-κB	Human dendritic cells	[63]
Unspecified C1q/MBL receptor	TLR4	Inhibition of IL-1β and TNF-α and enhancement of IL-10	CREB and NF-κB	Human monocytes (also macrophages and dendritic cells but without effect on IL-10)	[6,37]
CD46	TLR4	Selective suppression of IL-12	Post-transcriptional mechanism	Human monocytes–macrophages	[41,42]
CD46	TLR4	Enhanced expression of IL-12p35, IL-23p19 and IL-12/IL-23p40 Promotion of Th17	Not addressed	Human dendritic cells	[62]

[a] C5aR considerably more potent than C3aR in regulating TLR-induced cytokine responses; C3aR not involved in Th17 development.

[b] An alternative to C5L2–TLR4 crosstalk is that C5L2 might be required for optimal TLR4 signaling (e.g. co-receptor function).