

Editorial note: ‘Science Letters’ is a new occasional feature in which we offer researchers working in basic science, or in a very specific area of biomedical science, an opportunity to explain their work to a wide clinical anaesthetist readership. We challenge them to outline the nature and clinical implications of their work, and its potential and actual relevance to anaesthesia, in a way that engages and enthuses readers to adapt their own, clinical researches so as to take better account of this science. Researchers willing to take up the challenge are invited to contact the Editor-in-Chief.

Science Letter

Complement takes its Toll: an inflammatory crosstalk between Toll-like receptors and the receptors for the complement anaphylatoxin C5a

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Summary

The innate immune system is responsible for a rapid inflammatory response to pathogens that is essential for the clearance of infections. Although this response is vital, it is nonetheless potentially harmful, and dysregulated inflammation is a feature of many disease states. Thus, the mechanisms that regulate the release of soluble mediators of inflammation are an active focus of investigation. The activation by infections of two key components of the innate immune system, the Toll-like receptors (TLRs) and complement, leading to the release of soluble mediators of inflammation, is critical to microbial killing and clearance. Both TLRs and complement are independently capable of triggering pro-inflammatory responses, but their synergistic interaction resulting from a substantial crosstalk markedly amplifies those responses and may contribute to the pathophysiology of diseases such as sepsis.

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The Toll-like receptors (TLRs) and the complement system are key components of the innate immune response. TLRs are germ line-encoded pattern-recognition receptors whose typical ligands are microbial components, whereas the complement system consists of a series of plasma and cell membrane-bound proteins whose activation is critical to microbial killing and clearance. The concerted activity of TLRs and complement is crucial to a rapid inflammatory response to pathogens, but nevertheless potentially harmful if excessive or disproportionate to the infective insult. A reported signal transduction crosstalk between TLRs and complement may contribute to such an exaggerated inflammatory response. We review the recent findings on this subject and discuss its potential for therapeutic exploitation.

TLRs, complement and the microbial challenge

Ten TLRs (TLR1–TLR10) have been identified in humans [1]. They recognise conserved microbial molecular motifs or patterns (and hence are known as pattern-recognition receptors) present in a variety of microorganisms including bacteria, viruses, fungi and parasites; for example, lipopolysaccharide (LPS, endotoxin) from most Gram-negative bacteria is recognised by TLR4. Activation of TLRs results in the induction of a potent pro-inflammatory response, including the release of pro-inflammatory cytokines and chemokines such as IL-1 β , IL-6, TNF α and IL-8. Microorganisms and their components also activate the complement system, which plays a fundamental role in acute inflamma-

tion and pathogen destruction. The complement system consists of a series of plasma and cell membrane-bound proteins whose activation during infection results in the formation of the lytic membrane attack complex and the generation of opsonins and the anaphylatoxins C3a and C5a [2]. In particular, anaphylatoxin C5a is one of the most potent endogenous pro-inflammatory peptides, inducing a range of effects including granulocyte chemotaxis and the generation of reactive oxygen species as well as stimulating the secretion of inflammatory mediators, albeit less potently than TLR ligands [3]. Given that pro-inflammatory responses resulting from TLR activation or induced by C5a have been implicated in a wide range of pathologies, TLRs and C5a receptors are attractive therapeutic targets [4, 5].

The TLRs and C5a receptors' signal transduction pathways

Microbial recognition by TLRs activates signalling pathways that have been extensively studied [6]. Ligation of all TLRs except TLR3 activates the Myeloid differentiation primary gene product 88 (MyD88) adaptor protein, initiating a signalling cascade that leads to activation of nuclear factor kappa-B and mitogen-activated protein kinase (MAPK) intermediates, resulting in induction of pro-inflammatory gene transcription. By contrast, TLR3 signals via a MyD88-independent pathway, leading to activation of the interferon response factors 3 and resulting in transcription of anti-viral Type I interferons. The C5a induces pro-inflammatory responses through its receptor C5aR (CD88), a G_i protein-coupled receptor that signals via MAPK intermediates. C5aR activation may also signal via a G protein-independent pathway that involves the multifunctional adaptor proteins, β -arrestins. A second, obligately G protein-uncoupled, C5a receptor C5L2, has been described. Its functions are still the subject of controversy, but there is evidence that it may act as an inhibitory regulator of C5aR-mediated pro-inflammatory responses [7–9].

Synergism between TLRs and C5a receptors, and the modulatory effects of C5aR on TLRs

A synergistic interaction between TLRs and C5a receptors has long been recognised. Okusawa et al. [10] found that the level of IL-1 β released from human monocytes stimulated with a combination of LPS and C5a was greater than additive compared with that induced by either alone. Other investigators have reported similar findings using a range of experimental models, TLR ligands and pro-inflammatory cytokine outputs [3, 11–15]. More recently, Zhang et al. [16] demonstrated a positive modulatory effect of C5aR activa-

tion on TLR responses using mice deficient in the complement inhibitory protein Decay Accelerating Factor (DAF). Compared with wild-type littermates, DAF $^{-/-}$ mice proved to be hypersensitive to TLR-mediated stimulation with a range of ligands, as measured by IL-1 β , IL-6 and TNF α release. The authors concluded that the increased TLR sensitivity in the DAF $^{-/-}$ mice was a result of a positive regulatory effect of complement – overactivated due to the absence of DAF – on TLR-induced cytokine production, and was mediated by C5aR and, to a much lesser extent, C3aR activation. The synergistic enhancement of the TLR- and C5aR-mediated pro-inflammatory cytokine response to infection, and the positive effect of C5aR activation on TLR-mediated responses, have obvious relevance for the overwhelming cytokine response that is a feature of both sepsis and the sterile inflammatory syndromes that cause shock (such as trauma, haemorrhage and burns); in all these conditions, elevated systemic levels of pro-inflammatory cytokines have been implicated in the pathophysiology of the disease and many of them (IL-1 β , TNF- α and IL-6, for example) shown to correlate positively with mortality [17–19].

A further immunomodulatory effect of C5a on TLR-mediated cytokine release has also been reported. The IL-12p70 is a pro-inflammatory cytokine that is secreted by activated monocytes/macrophages and dendritic cells in response to stimulation with TLR ligands (but not C5a). Its functions are complex, but include induction of IFN γ -dependent proliferation of NK cells, differentiation of CD8 $^{+}$ T cells and of particular note, polarisation of naive CD4 $^{+}$ T cells towards a pro-inflammatory T-helper 1 (Th1) profile. Wittmann et al. [20] and subsequent investigators [21–24] reported that monocytes/macrophages stimulated with a combination of C5a and TLR ligands showed a markedly decreased production of IL-12-family cytokines compared with those stimulated with TLR ligands alone. This correlated with an inhibition of Th1-mediated immunity and involved increased production of the IL-12 inhibitory cytokine IL-10 [16]. Whereas the synergistic enhancement of the initial pro-inflammatory cytokine response to infection may contribute to the development of the shock state early in sepsis, it is possible that this inhibitory effect of C5a on TLR-induced IL-12 production contributes to the immunosuppression observed in late-phase sepsis, of which the loss of a Th1 response is a feature [25].

Recently, Wang et al. [26] described a mechanism by which pathogens may exploit the putative crosstalk between TLRs and C5aR to evade immune destruction by macrophages. *Porphyromonas gingivalis* is an oral gram-negative anaerobe that causes periodontitis and has been implicated in ventilator-associated pneumonia [27]. This bacillus

interacts with cells predominantly via TLR2 and expresses cysteine proteases with C5 convertase activity, which generate C5a from C5 in saliva. This C5a paradoxically enhances the ability of *P. gingivalis* to survive intracellular killing after ingestion by macrophages by increasing TLR2-mediated production of intracellular cAMP, which inhibits the nitric oxide-dependent killing of the pathogen.

A bi-directional TLR-C5a receptor crosstalk

These findings provided substantial evidence for synergism and point at a possible crosstalk between complement and TLRs that may influence the extent of the pro-inflammatory and immunomodulatory responses to infection. Previous investigations have demonstrated that the observed TLR-C5aR synergism involves an enhancing effect of C5a on a dominant TLR-driven inflammation. Our recent work, however, addressed the question of whether or not TLR activation can modulate C5a receptor-mediated pro-inflammatory responses. This would indicate the existence of a genuine, bi-directional, TLR-C5a receptor crosstalk. We observed a marked positive effect of TLR activation on cell sensitivity to C5a, both in vitro and ex vivo [28], thus confirming a bi-directional TLR-C5a receptor crosstalk. Indeed, human peripheral blood mononuclear cells or anticoagulated whole blood, pre-exposed to a range of TLR ligands or whole bacteria, showed a dramatic enhancement in the release of the neutrophil chemoattractant IL-8 when stimulated with human C5a, compared with cells not pre-exposed to the TLR ligands; similar enhancing effects were observed on the release of other inflammatory mediators. This indicated that TLR activation can 'prime' cells for enhanced responses to C5a. This effect, however, was not observed in whole blood from TLR4 signalling-deficient mice pre-exposed in vivo to LPS, confirming the critical role of TLR signalling. The fact that hypersensitivity to C5a was observed in cells primed with a wide range of TLR ligands indicates that 'TLRs' modulatory capacity may not be restricted by the nature of the pathogen. Notably, this effect was largely independent of the concentration of ligand used, as pre-exposure to even 10 pg.ml^{-1} LPS, a concentration well below the median concentration reported in patients with sepsis ($\sim 300 \text{ pg.ml}^{-1}$) [29], resulted in hypersensitivity to C5a. This observation suggests that TLR-induced enhancement of cell responses to C5a is an extremely sensitive mechanism that might operate during mild as well as severe infections.

Mechanistically, TLR-induced hypersensitivity to C5a did not result from up-regulation of the canonical C5a receptor C5aR, as monocyte and neutrophil C5aR cell-surface expression was in fact lower after exposure to LPS. Rather, it appears to involve negative modulation of the

second C5a receptor, C5L2, acting as an inhibitor of C5aR, since the sensitivity to C5a of blood cells from C5L2-deficient mice was not enhanced by pre-exposure to LPS. Furthermore, pre-exposure of human mononuclear cells to TLR ligands reduced the expression of C5L2 and this reduction correlated with reduced release of high-mobility group box-1 (HMGB1), a late mediator of inflammation whose release upon C5a stimulation depends on C5L2, but not C5aR. In addition, the modulatory effects of TLR activation on both C5a-induced HMGB1 release (inhibitory) and IL-8 release (enhancing) were reproduced by specific blockade of C5L2 with a monoclonal antibody. Hence, it appears that TLR activation may enhance pro-inflammatory responses to C5a by 'inhibiting the inhibitor' of those responses, C5L2 (Fig. 1). Thus, these studies demonstrated the existence of a complex immunomodulatory network that amplifies innate host defence involving two major components of the innate immune system, TLRs and complement.

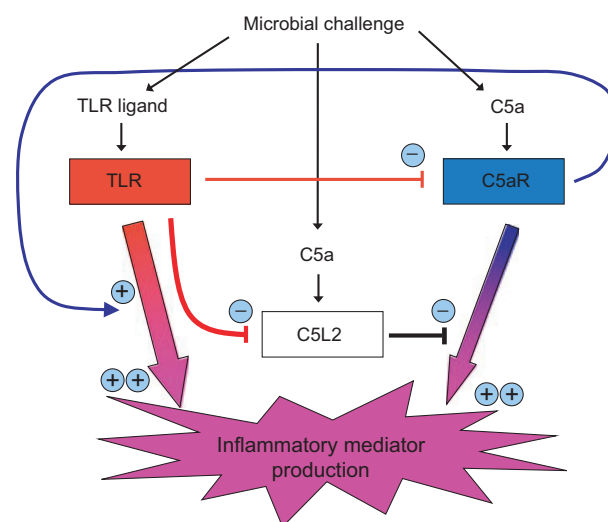


Figure 1. Proposed model of Toll-like receptor (TLR)-C5aR crosstalk. Microbial challenge results in generation of C5a and release of TLR ligands (black arrows). Signalling pathways are activated following ligation of TLRs (red gradient arrow) and C5aR (blue gradient arrow) that directly induce inflammatory cytokine release, but the C5aR-induced pro-inflammatory signalling pathway is negatively modulated by C5L2 ligation (black blunt arrow). The C5aR ligation enhances TLR-mediated cytokine production (blue arrow), whereas TLR ligation down-regulates expression of both C5aR and C5L2 (red blunt arrows). The enhancing effect resulting from the TLR-induced C5L2 down-regulation outweighs the inhibitory effect of TLRs on C5aR expression, thus resulting in a net positive effect on C5a-induced cytokine production.

Future perspectives

In view of the harmful consequences of an over-robust cytokine response to pathogens in sepsis, and the role that C5L2 plays in the pro-inflammatory TLR-C5a receptor crosstalk, the second C5a receptor is an attractive therapeutic target. Over the last two decades, much attention has been given to immunomodulatory therapies in sepsis, but with very limited success [30]. However, it is likely that a 'one size fits all' approach has contributed to this failure. Diverse pathological mechanisms doubtless underlie the clinical presentation, and giving the same immunomodulatory treatment to all who meet the clinical criteria for sepsis is probably inappropriate. A better understanding of the pathophysiology of sepsis may permit the identification of patient subgroups on the basis of their immunological response, with specific therapies tailored to different groups. In this connection, the TLR-C5a receptor crosstalk is a promising avenue of enquiry. We observed that the extent of TLRs' modulatory effect on cell sensitivity to C5a varies dramatically between individuals [28]. Indeed, the increase in C5a-induced IL-8 release by whole blood as a result of pre-exposure to LPS ranged from a 60-fold enhancement in one subject to almost none in others. Sensitivity to TLR-mediated modulation of cell responses to C5a may depend on variations in the constitutive levels of expression of TLRs, C5aR and C5L2 and/or their signal transduction intermediates in each individual. This variability may determine the differing susceptibilities of different individuals to an excessive, systemic inflammatory response to a given infective insult. Thus, a detailed characterisation of the immunophenotypes of TLR priming 'responders' and 'non-responders' may allow identification of prognostic biomarker signatures and a sub-population of sepsis patients that would benefit from a therapy aimed at interrupting the TLR-C5L2-C5aR crosstalk.

Competing interests

No other external funding or competing interests declared.

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