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Is the Complement Activation Product C3a a Proinflammatory Molecule? Re-evaluating the Evidence and the Myth

Liam G. Coulthard and Trent M. Woodruff

The complement activation product C3a is often described as a proinflammatory mediator, alongside its downstream cousin, C5a. However, emerging studies show that C3a has several anti-inflammatory facets in vivo. For example, in the acute inflammatory response, C3a acts in direct opposition to C5a, through preventing the accumulation of neutrophils in inflamed tissues by independently regulating their mobilization. This acute, protective, and opposing activity of C3a to C5a is also illustrated in models of sepsicemia. In this article, we reinvestigate the discovery and original classification of C3a as a proinflammatory mediator and highlight the emerging studies demonstrating anti-inflammatory effects for C3a in the immune response. It is our hope that this review illuminates these apparently contradictory roles for C3a and challenges the general dogma surrounding C3a, which, historically, has ubiquitously been described as a proinflammatory mediator. In light of this, we urge investigators to use “inflammatory modulator” as the descriptor for C3a. The Journal of Immunology, 2015, 194: 3542–3548.

The complement cascade is a key component of the immune system and is essential in providing a balanced response to injury and infection. Complement can be activated through multiple initiating pathways, but all routes lead to the cleavage of the central complement components C3 and C5 to generate the small bioactive fragments C3a and C5a (1). Both C3a and C5a are frequently and consistently described in the literature as proinflammatory mediators, despite a limited number of reports of true proinflammatory activity for C3a in vivo. Furthermore, many reports, in fact, demonstrate an anti-inflammatory role for this major complement cleavage product in infection and disease. To add to the confusion, the only reported in vivo C3aR antagonist (SB290157), which is used frequently in research studies, was, in fact, demonstrated to act as a full agonist (2), thus clouding interpretations of results in disease models in which this agent was used (3). We wish to use this review to draw much-needed attention to the potential anti-inflammatory roles of C3a, so as to refocus the discussion on its revised position in the immune response, allowing new strategies for therapeutic targeting of C3a.

Although the modern literature surrounding C3a and C5a frequently describe divergent functions for these peptides, their discovery and characterization may explain why they are so often portrayed together as proinflammatory mediators. In the late 19th century, it was shown that Ag-exposed serum contained both a heat-labile and a heat-stable bactericidal element. The heat-stable element was demonstrated to be Ab, whereas the heat-labile element was termed “complement” by Paul Ehrlich, as it complemented the Ab response. The anaphylatoxins were discovered through incubation of serum with Ag–Ab complexes, thus activating the classical pathway, and were named for their histamine-releasing ability (4). Although unconfirmed at the time, the origin of the anaphylatoxin activity was suspected to be complement, because of its heat-labile nature (5). This activity was finally ascribed to complement split products with the identification of multiple serum components of complement and the purification of C3a and C5a in the late 1960s. Initial experiments demonstrated that the release of the elusive anaphylatoxin was dependent on the presence of the initiating factors of the complement cascade and that the anaphylatoxin was released at, or shortly after, the involvement of C3 (6). With purification, both C3a and C5a were shown to induce smooth muscle contraction through release of histamine in a mutually redundant manner, both having similar “activity” at the guinea pig ileum (7). This led to the adoption of the term “complement anaphylatoxin” for these peptides, although this label has recently been challenged (8) because it does not truly encompass their widespread functions.

C3a generation, structure, and signaling

Cleavage of C3 results in the formation of two split products: C3a and C3b. C3a is generated at the convergence of all known complement-activation pathways, and its sibling at the point of

Abbriviations used in this article: IR, ischemia-reperfusion; RAGE, receptor for advanced glycosylation end-products; Treg, regulatory T cell.
cleavage, C3b, continues the propagation of the complement cascade (9). Classically, cleavage of C3 results from the action of one of the C3 convertases, which are multiprotein complexes formed from the upstream cleavage events of complement activation. Additionally, C3 can be cleaved by multiple serine proteases present at sites of inflammation, most notably blood proteases, such as thrombin, and immune cell–derived proteases, such as cathepsin (10).

Structurally, C3a is a 77-aa peptide consisting of three to four helical regions and a series of nonregular residues at the C-terminal responsible for binding at C3aR (11, 12). Short portions of the C terminus were demonstrated to be necessary both for agonist activity at C3aR and intrinsic antimicrobial activity (12, 13). The activity at C3aR of the terminal residues in isolation is far lower than that of the native molecule, and there is evidence that these flexible C-terminal residues are stabilized in the conformation necessary to bind C3aR by the upstream α helix (14). Hence, this C-terminal region of C3a has been a nidus for drug-design research, both in terms of therapies capable of modulating C3aR activity and those that take advantage of the intrinsic antimicrobial abilities of the molecule (15, 16). After its generation, C3a is cleaved quickly at the C-terminal arginine to form C3a-desArg. This molecule has no detectable activity at C3aR, but it was shown by some groups to bind the second receptor for C5a, C5aR2 (C5L2) (17, 18). The interactions with C5aR2 exhibit effects on metabolism and, in this sphere, C3a-desArg is homologous to acetylation-stimulating protein (19).

However, this review focuses on the interaction of C3a with its canonical receptor C3aR. It should be noted though, that there are additional factors emerging in the literature that also intersect with the C3a axis (detailed below and summarized in Fig. 1). C3aR is a classical seven-transmembrane, G protein–coupled receptor that shares close homology to the receptors for C5a, C5aR1, and C5aR2. C3aR couples to heterotrimeric G proteins and demonstrate a promiscuity of G protein interaction that is dependent on cell type (20–22). Intriguingly, the internalization and recycling of C3aR, which are dependent on G protein–coupled receptor kinase–mediated receptor phosphorylation (23), are inhibited by the presence of C5a in human granulocytes (24). Studies in this area were perhaps the first reports of functional antagonism between C5a and C5aR at the molecular level. Until recently, C3a was thought to be the only ligand for C3aR; however, the neuropeptide TLQP-21, a cleavage fragment of the VGL propeptide, was shown to bind and activate murine C3aR through conformational change of the ligand upon receptor binding (25, 26).

Additionally, the actions of C3a need not be mediated solely through its canonical receptor, C3aR. In a model of experimental autoimmune encephalitis, ectopic CNS C3a expression worsened disease outcome in C3ar1−/− mice. However, in mice lacking C3aR, the induced C3a expression improved pathology, suggesting that the actions of C3a in CNS disease may not be confined solely to its interaction with C3aR (27). This finding was corroborated in a second paper from the same group, demonstrating a protective effect for CNS C3a expression in LPS-induced endotoxic shock that was not diminished in the absence of C3aR (28).

C3a was also shown to bind to the receptor for advanced glycosylation end-products (RAGE) with a high affinity, but this interaction is more complex than a simple ligand–receptor interaction (29, 30). Ruan et al. (29) demonstrated that C3a is able to form a complex with CpG oligonucleotides to enhance IFN-α release from mononuclear leukocytes. Intriguingly, there is no demonstrable effect of a C3a–RAGE interaction alone, despite strong and specific binding between the two.

**C3a activity on immune cells**

C3aR is expressed by all leukocytes of myeloid (and several nonmyeloid) lineages; however, the functional characteristics of the receptor, as well as the response to C3a, are dependent on cell type. In the past few decades the literature concerning C3a and its actions at the cellular level demonstrated a bipolar role for this molecule in different immune cell types (Fig. 2). For example, despite a canonical proinflammatory histamine degranulation response for C3a on mast cells (31), our laboratory recently showed a potent anti-inflammatory response for C3a on neutrophils, by attenuating their mobilization into the circulation following injury (32). Uninhibited mobilization can

![Diagrammatic representation of receptor-ligand interactions surrounding C3a. C3a, generated from complement pathway activation, interacts with its canonical receptor, C3aR, and RAGE. Functional interaction with RAGE is dependent on formation of a C3a-CpG oligonucleotide (oligo) complex. The VGF cleavage product, TLQP-21, is also a ligand of C3aR. The terminal arginine of C3a is cleaved by serum carboxypeptidases (CPase) to form C3a-desArg (acetylation stimulating protein). C3a-desArg may bind and signal through C5aR2.](http://www.jimmunol.org/)

*FIGURE 1.* Diagrammatic representation of receptor–ligand interactions surrounding C3a. C3a, generated from complement pathway activation, interacts with its canonical receptor, C3aR, and RAGE. Functional interaction with RAGE is dependent on formation of a C3a-CpG oligonucleotide (oligo) complex. The VGF cleavage product, TLQP-21, is also a ligand of C3aR. The terminal arginine of C3a is cleaved by serum carboxypeptidases (CPase) to form C3a-desArg (acetylation stimulating protein). C3a-desArg may bind and signal through C5aR2.
lead to an increase in neutrophil numbers at the site of injury, which can significantly worsen disease progression.

The reports on the function of C3a on neutrophils are complicated, owing to past difficulty in obtaining pure neutrophil cell isolates. Indeed, many of the earliest reports indicating that C3a induced neutrophil activation were later refuted because of contamination with nonneutrophil granulocytes (33). What is now clear is that, despite high expression of functional cell surface C3aR on neutrophils, C3a does not chemoattract or stimulate degranulation of neutrophils, despite inducing downstream signaling through Erk1/2 and Akt (33, 34). Interestingly, the signaling produced by C3a stimulation of neutrophils was shown in one report to be dependent on the presence of C5aR2, suggesting that C5aR2 may contribute to C3a signaling through interactions with C3aR (34). However, our recent studies indicate that, at the level of the bone marrow, C3a prevents migration of neutrophils into the circulation by acting in direct opposition to neutrophil-mobilizing factors, such as G-CSF, in a manner reminiscent of stromal cell–derived factor-1 (32).

C3a also can induce signaling in human monocytes and monocyte-derived macrophages; however, this interaction, with TLR-4 costimulation, induces the production of proinflammatory mediators, such as IL-1β, TNF-α, IL-6, and PGE2 (35–39). This suggests that, in the chronic phase of inflammation, where monocyte/macrophage responses become more predominant over neutrophils, C3a may indeed act as a classical proinflammatory mediator. This view is supported by evidence that C3ar1−/− mice exhibit reduced responses in atopic diseases, such as allergic asthma and allergic dermatitis (45), again suggesting a proinflammatory role for C3a in these situations.

Administration of C3a also was demonstrated to augment the T cell response, promote T cell proliferation, and prolong the inflammatory response through suppressing regulatory T cell (Treg) production (46, 47). Additionally, T cells generate intracellular C3a that contributes to their survival (48); accordingly, T cell populations are reduced in C3ar1−/− mice in several models of disease (46, 47). Although broad T cell expression of C3ar remains controversial (49), TCR stimulation upregulates C3aR mRNA expression in isolated T cell populations (50). Additionally, altered Treg responses occur with adoptive transfer of C5ar1−/−/C3ar1−/− T cells into a wild-type animal (47). C5ar1−/−/C3ar1−/− Tregs also demonstrate prolonged survival and enhanced function, suggesting that C3aR/C5aR1 stimulation of T cells is also important in...
regulating inflammatory responses (51). C3aR signaling in APCs suppresses IL-4 production, inhibiting a Th2-polarized response (52). C3aR signaling also acts in concert with C5aR1 signaling to suppress the production of TGF-β1 from dendritic cells, reducing the stimulus for differentiation to Tregs and removing the inhibition on the Th1 response (47). However, in vivo models of allergic asthma demonstrate that the type of experimental Ag used can alter the T cell response promoted by C3a, suggesting a complicated role for this molecule in T cell biology (46, 53).

C3a in disease

Ischemia-reperfusion injury. Intestinal ischemia-reperfusion (IR) injury, through transient occlusion of the mesenteric arterial tree, causes an increase in circulating neutrophils upon reperfusion that are critical to the generation and promulgation of tissue injury (54). Tissue injury also directly activates the complement system, leading to the generation of the peptides C5a and C3a (31). The role for C5a in the progression of this pathology is both very well understood and unambiguous; C5a functions at the level of injury to cause extravasation and degranulation of the circulating neutrophils (55). By direct contrast, we demonstrated in a model of intestinal IR that the role for C3a in this acute pathology is overwhelmingly anti-inflammatory (32). Thus, the historical model for C3a as being a “weaker” form of C5a as a proinflammatory mediator is no longer uncomplicated. C3a levels in both blood and intestinal tissues increase after a period of ischemia, likely from activation of the complement system in response to damage-associated molecular patterns (32). Additionally, at the site of acute inflammation, carboxypeptidases preferentially degrade C5a over C3a, perhaps allowing for C3a to escape degradation and exert systemic actions (8). We recently showed that one function of this C3a release is to attenuate the neutrophilia associated with injury by confining unmobilized neutrophils to the bone marrow reservoir (32). Mice lacking C3aR have a significantly greater increase in circulating neutrophils post-IR than do their wild-type counterparts. This correlates with increased neutrophil migration into ischemic tissue and worsened histopathological outcomes. The effect of the C3a–C3aR axis in this acute injury also appears to be restricted solely to an effect on neutrophils, because circulating levels of other leukocytes remained similar to wild-type animals. Additionally, the wild-type phenotypic response to IR could be fully restored through wild-type bone marrow transfer to C3aR-deficient animals, indicating that the anti-inflammatory actions of C3a take place at the level of the bone marrow rather than at the site of injury (32). Finally, we showed a direct requirement for C3aR to restrict G-CSF-mediated neutrophil mobilization from the bone marrow, in the absence of tissue injury (32).

Because neutrophils are the major contributing cell type responsible for propagation of the acute inflammatory response, this emerging evidence of anti-inflammatory activity for C3a through the retention of neutrophils in the bone marrow forms the crux of the thesis for this review: that C3a should not be grouped with C5a as a purely proinflammatory anaphylatoxin. Indeed, several other in vivo studies in the literature demonstrate a more complex role for C3a in disease models, particularly in the acute setting.

Sepsis. In sepsis, it was noted previously that elevated C3a and C5a correlated with fatal outcomes in hospital patients, but determining the cause and effect in these studies is not possible (56). The significant difficulty with research into the effects of the complement activation peptides is that their genesis also amplifies the terminal elements of the complement cascade. In the past, without the use of knockout mice or specific antagonists, it has been difficult to tease these aspects apart.

C3-deficient animals have been used for investigation of complement-mediated pathogen clearance during sepsis; it was demonstrated, perhaps unsurprisingly, that the lack of C3 reduces survival rates and increases pathogen load in experimental models (57). It might be expected that the increased pathogen load due to the loss of the terminal elements of the complement system explains the premature death of C3-deficient animals. It remains to be seen whether any of the direct antimicrobial actions of C3a are of sufficient magnitude to contribute to this effect (13).

Attributing the effects of C3 deficiency to an attenuation of complement action is complicated by the fact that C3 is not an absolute requisite for the formation of the terminal elements of the complement cascade, because C5 can be cleaved by serum proteases (10). These proteases are upregulated in C3−/− animals, leading to pathophysiologically significant generation of C5a and C5b-9 (58). In keeping with this, the differences demonstrated in bacterial load between C3-deficient and C3-supplemented animals are not as dramatic as the survival curves suggest (59). It is difficult to appreciate from this evidence whether it is the bactericidal or the C3aR-mediated actions of C3 supplementation that contribute to improved survival in septic animals. However, the initial report of a C3ar1−/− mouse included an investigation into sepsis survival and concluded that C3aR signaling alone was key to improved survival (60). Additionally, this study demonstrated elevated circulating levels of the proinflammatory cytokine IL-1β in C3ar1−/− animals. Further studies by the same group used heat-inactivated Escherichia coli administration as a model of Gram-negative bacteremic sepsis. This research also demonstrated a protective effect for C3aR, because C3ar1−/− animals had significantly elevated mortality compared with their wild-type counterparts (61). Conversely, C5ar1−/− animals were protected from the bacteremia, again demonstrating the divergent roles of these two molecules (61).

Although these investigators did not include measurements of circulating neutrophils in the LPS- or E. coli-challenged animals, recent studies surrounding the actions of C3a in inflammatory disease suggest that a reactive neutrophilia may have been the cause of the reduced survival in C3ar1−/− animals. In support of this, the investigators noted that the elevated IL-1β previously attributed to high C3ar1−/− mortality in the LPS model was unlikely to be the cause of death in bacteremia; instead, they suggested that the role of C3a is to regulate the inflammatory response generated through C5a signaling (60, 61).

Renal disease. Several studies outlined the pathogenic role that complement plays in the development of autoimmune lupus nephritis. High concentrations of C3aR and C3 are present in the glomeruli, correlating with disease severity (62), and seemingly suggesting a pathophysiologial role for the C3a–C3aR axis and, perhaps, a therapeutic target for treatment of lupus nephritis. It was demonstrated previously that infusion of the C3aR antagonist SB290157 in the MRL/lpr mouse model of lupus nephritis resulted in increased survival and...
reduced renal inflammation (63). However, the same mouse model backcrossed with C3aR-knockout mice indicates that the loss of C3aR accelerates renal injury and increases mortality (64). This contradictory result was attributed to the ill-defined pharmacological activity of the widely used C3aR antagonist, SB290157, which was reported to have agonist activity upon binding C3aR (2). However, although the pharmacology of SB290157 may be controversial, the conclusion drawn by the investigators could be reinterpreted to take into account the acute “antineutrophil” effects of C3aR that were discovered recently (32). It is uncontroversial that, at least at the level of the nephron, C3aR is involved in the pathogenesis of renal disease, including the induction of metaplasia in nephron epithelial cells central to the disease (65, 66). These actions appear synergistic to the role of C5aR1 at the tissue level, but they may be redundant in the presence of elevated C5a concentrations, which is a more potent proinflammatory mediator than C3a, at the receptor level. Contrary to reports of intestinal IR, there is a slight improvement in disease outcomes for C3ar1−/− animals subjected to renal IR injury (65). In this study, the investigators demonstrated that C3a administered directly to renal tubule epithelial cells increased the production of chemotactic factors directing leukocytes to the site of injury. Interestingly, a decrease in global leukocytes was demonstrated in the diseased C3ar1−/− kidney isolates, as indicated by CD45+ flow cytometry. However, there was no difference from wild-type mice with regard to granulocyte infiltration, suggesting that the dynamics of this model may be more complex than presented. Quantification of circulating granulocytes was not recorded in this model, but it is tempting to hypothesize that C3ar1−/− animals exhibited an injury-induced neutrophilia, as previously reported (32), which is tempered by the decreased cytokine production at the tissue level, as noted by these investigators.

Conclusions
The terminology surrounding the effects of C3a in the literature warrants revisitation. C3a is frequently and consistently described as proinflammatory in nature in the literature and is grouped conceptually with its cousin, C5a. The reality is rather more subtle and nuanced. It is true that C3a has several proinflammatory facets, but in this review we highlighted numerous studies from a wide variety of independent laboratories that consistently point to significant anti-inflammatory functions for C3a, in direct functional opposition to C5a. It also should be noted that different mediators that arise from the same metabolic pathway, as do C3a and C5a, are often physiological antagonists in vivo. A good example is the prostanoids: individual mediators are well recognized to act on their cognate receptor and, with opposing effects, are physiological antagonists of each other. Therefore, we should not be surprised by the concept that C3a and C5a may also function as physiological antagonists of each other. Indeed, C3a and C5a have directly opposing effects on blood pressure mediated entirely through prostanoid activity (67). Collectively, the studies presented in this review contravene the current dogma and suggest a change to the nomenclature or at least a wide-ranging recognition of the unforeseen complexity of the pathophysiological roles of C3a. At the very least, there should be earnest attention and debate to leaven our understanding of what is clearly a complex and multifaceted issue.

The response of C3a to injury is a dichotomy. In the acute setting, C3a prevents mobilization of neutrophils, limits their accumulation into tissues, and, therefore, reduces the inflammatory response at the tissue level. However, in certain chronic disease models, such as asthma and rheumatoid arthritis, C3a clearly demonstrates certain proinflammatory actions and contributes to disease progression (8). The difference in the response of inflamed tissues to C3a, between the acute and chronic phases of inflammation, may well be due to the differing cell types involved (e.g., neutrophils versus monocyte/macrophages; Fig. 2). However, the different facets of C3a in disease are not mutually exclusive but instead depend upon the balance between the pro- and anti-inflammatory effects of the molecule. Even in models of disease that demonstrate a worse outcome with disruption of the C3a–C3aR axis, there are also measurable benefits, such as reduced tissue proinflammatory cytokines in the intestinal IR model (32). It is the balance of
these actions in disease that determines the ultimate outcome (Fig. 3).

This dual role of C3a in inflammation also poses significant opportunities for the development of therapeutics. It may be possible to harness the anti-inflammatory activity of C3a in the acute inflammatory phase through the use of C3aR agonists, of which there are emerging several promising candidates (68). We recently demonstrated the validity of this approach in an intestinal IR model (32). Equally, the proinflammatory aspects of C3a in chronic disease may warrant the targeting of C3aR using selective antagonists to reduce the cytokine-mediated proinflammatory response. Future rational design of therapeutics targeting C3aR should take the dual roles of this receptor into account.

In this review we aimed to revisit the historical and recent literature on the pathophysiological role of C3a in inflammation. In our view, it is time for a rethink and a careful, more nuanced consideration of the roles of C3a in inflammation, which seem far more catholic than those of its more famous and celebrated cousin, C5a. We suggest that future investigators recognize this detail through the description of C3a as an inflammatory mediator, rather than as a purely proinflammatory peptide.

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Disclosures

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References


BRIEF REVIEWS: C3a AS A PRO-/ANTI-INFLAMMATORY MOLECULE


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