Complement: An Unfinished Symphony

Editorial Focus on: The Complement Receptor C5aR1 Contributes to Renal Damage but Protects the Heart in Angiotensin II-Induced Hypertension

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Running Title: A New Era for Complement
The current issue reports that, when hypertension was induced in mice (using infusion of angiotensin II and with NaCl in the drinking water), the absence (knockout) of the first receptor for C5a (C5aR1) resulted in reduced albuminuria but increased amounts of interstitial collagen deposition in the heart. The role of the second C5a receptor (C5aR2) in responses to hypertension was not examined, but should be. Such patterns suggest that mice lacking C5aR1 express different outcomes during hypertension in the heart and in the kidneys. We review different strategies for blockade of complement activation products in order to prevent organ injury due to complement activation. We also emphasize the need to extend the new studies to define the outcomes in hypertensive mice that lack the second C5a receptor, C5aR2. Finally, we describe recent studies demonstrating that in CD4+ T cells intracellular C3 present and is activated (cleaved) by cathepsin enzyme L, resulting in generation of C3a and C3b, which appear to be essential for robust T cell function. Such observations suggest a new pathway of complement activation, namely, intracellular activation of complement, which represents an entirely new aspect of the complement system, with substantial implications.
Introduction

The authors (Wenzel et al (10)) address the role of complement in hypertensive mice infused with angiotensin II and with NaCl in the drinking water. The complement focus was on C5aR1+ cells that also expressed green fluorescent protein (GFP). The authors found GFP+ cells in interstitial areas of both heart and kidneys. These infiltrating cells were CD45+, indicating their origin from the bone marrow. Quite unexpectedly, where C5aR1 KO mice were used, renal damage (quantitated by albuminuria) was reduced, but cardiac buildup of interstitial collagen I, determined by histochemical staining and biochemical quantitation of collagen I, was intensified. These data suggested that C5aR1+ cells amplified renal damage but constrained collagen buildup in the heart in hypertensive mice.

Is There a Role for Complement in Systemic Hypertension?

In general, there are hints that complement may be involved in the vascular remodeling of hypertension (1), but the evidence is quite limited. Over the past two decades attention has been focused on the roles of C5a and its receptors (C5aR1, C5aR2) in inflammatory responses developing after infectious conditions, ischemia-reperfusion, acute lung injury and sepsis (3), to cite just a few examples. It has been known for several decades that C5a binds with high affinity to PMNs via cell surface C5a receptors, triggering a series of signal-transduction pathways that activate the NFκB system with resultant production of proinflammatory mediators. It has become evident that, in addition to phagocytes, other cell types also have binding sites for C5a (fibroblasts, endothelial cells, cardiomyocytes, etc.). C5a binding sites may be transcriptionally upregulated on different cell surfaces in a variety of circumstances such as sepsis. In most cases upregulation of C5aR1 has been associated with enhanced proinflammatory outcomes. Inflammatory damage in the lung, kidneys and liver may be irreversible with resultant fibrosis, or the outcomes may be reversible with conversion of damaged tissues to their preinjury state.
Strategies for Blockade of Complement Activation or Its Activation Products

The most striking translational application of complement research has been seen with a C5 blocking mAb (eculizumab), used in patients with paroxysmal nocturnal hemoglobinuria (PNH) (9). Clinical exacerbations of PNH are due to defective anchoring proteins for CD55 and CD59, which regulate complement activation on cell surfaces (erythrocytes in this case). The same mAb to C5 has been approved for use in pediatric cases of atypical hemolytic-uremic syndrome, a complement-related microthrombotic disorder following a flu-like syndrome and often leading to suppressed renal glomerular function (5). In addition, there are currently phase II clinical trials in Germany involving patients with sepsis. This intervention features the use of a "humanized" neutralizing mAb to human C5a (2). To date, the antibody appears safe for use in humans, although it is too early to know if the antibody intervention will be clinically effective in humans with sepsis.

There has been interest in strategies that block convertases for C3 and C5. These convertases cleave either C3 into C3a and C3b or C5 into C5a and C5b. C3a and C5a are powerful proinflammatory anaphylatoxins, while C3b is an important opsonic-promoting factor. C5b is interactive with C6-C9 to produce the membrane attack complex (MAC) which can cause lysis of cells and infectious agents such as bacteria, viruses and protozoa. The group at the University of Pennsylvania over several years has developed a cyclic peptide (compstatin) that blocks C3 convertase and may be useful in humans with complement-mediated disorders (8). A concern about this approach is that the amount of blockade of complement will have to be carefully calibrated in order to avoid excessive reduction in C3b generation, which could significantly interfere with the opsonic innate immune pathways. One strategy would be to use the drug in a manner that does not result in dissemination of the drug throughout the body. Accordingly, the drug might find application in age-associated macular degeneration in the eye, in which it could be slowly released near the retina. Currently, the only FDA-approved drug for macular degeneration involves monthly injections of antibody to vascular endothelial growth
factor into the vitreous (4). This intervention appears to be effective in reducing the progression of macular degeneration.

Possible Role of C5aR2 in Events of Ischemia-reperfusion or Hypertension

In the report by Wenzel et al (10), the focus has been exclusively on the role of C5aR1 which is the more common C5a receptor reacting with C5a. C5aR2 (originally designated as C5L2) was described as a “C5a default receptor”, and was thought to be more reactive with C5a des arg. C5a des arg is the product of C5a reacting with carboxy peptidase N, resulting in loss of most of the chemotactic activity. Early on it was thought that C5aR2 regulated the amount of C5a so as to prevent excessive buildup of C5a. Accordingly, C5aR2 reacting with C5a would attenuate inflammatory damage in tissues (6). Another vexing issue is the finding that C5aR1 is chiefly on the surface of cells (especially phagocytes). Some recent studies of C5aR2 have suggested it is chiefly located in the cytosol (6), raising the question of how C5a could gain access to cytosolic C5aR2. Disputes continue as to whether, on balance, C5aR2 is proinflammatory or antiinflammatory. For these reasons, designing therapies that would target either C5aR1 or C5aR2 for blockade, or both, have been complicated because of the conflicting data related to the biological activities of the two receptors.

Complement Activation Inside the Cell

Complement has been defined as a series of many proteins, which, when sequentially activated, result in protein/peptide products in the extracellular compartment. These products have biological activities resulting in proinflammatory responses, cell lysis and activation of the innate immune system. We are now challenged by a recent report describing intracellular activation of C3 in CD4+ T cells, due to activation of the protease, cathepsin L, which generates intracellular C3a and C3b from C3 (7). It has been suggested that C3a is necessary for T cell survival and autocrine production of proinflammatory cytokines by T cells. Accordingly, the theory would suggest that intracellular activation (cleavage) of C3 in T cells promotes important T cell responses but, in certain situations, this may lead to harmful proinflammatory outcomes.
It will be important to define further the details of biological pathways and outcomes of intracellular activation of complement, including if there is a parallel pathway for intracellular activation of C5, as well as determining what other types of cells possess similar intracellular pathways of complement activation.

**Concluding Remarks**

The Wenzel report (10) underscores the potential dichotomy in the roles of C5aR1+ cells in hypertension-induced changes in the kidney and the heart. This would suggest the likelihood of different pathophysiological pathways related to C5aR1, depending on the organ under study. If such conclusions are to be extended, this represents an important problem in therapeutic interventions aimed at blockade of C5a or its receptors. Without additional data, it is impossible to predict if blockade of C5a or its receptors would be therapeutically advantageous or possibly harmful.

The other important message is that the report by Liszewski et al (7) suggests that a new direction for complement research is emerging, namely, intracellular complement activation which has not been previously described. This could have enormous implications related to our understanding about the roles of extracellular and intracellular activation of complement.

To an extent, over 50 years biochemical details have emerged for complement proteins, complement activation pathways, complement regulatory proteins and molecular structural details of complement activation products and receptors. Many scientists felt after so much progress that there was little more to be learned about complement. How wrong they were!
References


4. **Foundation AMD.** Macular Degeneration Treatments [https://www.macular.org/treatments.](https://www.macular.org/treatments.) [18 April, 2016].


