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Review

# New therapeutic and diagnostic opportunities for injured tissue-specific targeting of complement inhibitors and imaging modalities

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ABSTRACT

Despite substantial opportunity and commercial interest in developing drugs that modulate the complement system in a broad range of non-orphan indications, several obstacles remain to be overcome. Among these issues is the biophysical nature of complement proteins, whose circulating levels are typically very high and whose turnover rates are relatively rapid, especially in the setting of chronic inflammatory conditions. This situation necessitates the use of very high levels of therapeutic compounds in order to achieve both multi-pathway and multiple effector mechanism inhibition. In addition, one must avoid infectious complications or the systemic impairment of the other important physiological functions of complement. Herein we focus on the development of a novel therapeutic strategy based on injured tissue-specific targeting of complement inhibitors using the antigen-combining domains of a small subset of natural IgM antibodies, which as endogenous antibodies specifically recognize sites of local damage across a broad range of tissues and locally activate complement C3, resulting in C3 fragment covalent fixation. Because the use of such recombinant tissue-targeting inhibitors precludes the utility of measuring systemic levels of complement biomarkers or function, since a goal of this targeting strategy is to leave those processes intact and unimpeded, we also briefly describe a new method designed to quantitatively measure using imaging modalities the inhibition of generation of fixed C3 fragments at sites of inflammation/injury. In addition to the ability to determine whether complement activation is locally constrained with the use of inhibitors, there is also a broader application of this imaging approach to inflammatory and autoimmune diseases characterized by local complement activation.



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## 1. Overview – dysregulation of local complement activation is of paramount importance in human diseases

The complement system consists of soluble activation pathway proteins as well as membrane-bound receptors and both soluble and membrane-bound regulatory proteins [1]. Importantly, the outcomes of complement system activation depend on the specific context in which the process occurs, and the number, localization and levels of the effector molecules generated. There are also many beneficial effects of complement activation that must be accommodated and allowed to function normally in any attempt to modulate the system. Beneficial effects include recognition and clearance of foreign and non-self-antigens and pathogens [2], enhancement of humoral [3] and cellular [4] immune responses, non-inflammatory clearance of apoptotic bodies containing self-antigens [5], transport of immune complexes for disposal in the reticuloendothelial system [6], tissue regeneration following injury [7,8], appropriate pruning of neurons during development [9], and shaping the composition of the endogenous natural antibody (NatAb) repertoire [10]. There is also an increasing appreciation that intracellular complement activation may play an important role in modulating T cell responses [11].

A key concept that members of this author group have focused on for nearly two decades is that, despite the near universal therapeutic focus on systemic inhibition of the complement system, the effects of pathogenic dysregulation of complement are almost exclusively manifest locally by tissue-specific injury and impaired function. That is especially evident in diseases such as atypical hemolytic uremic system (aHUS) and age-related macular degeneration (AMD), where germ line mutations of complement factors with potential systemic effects result in very localized tissue injury [12,13]. Additionally, even in diseases such as systemic lupus erythematosus (SLE) in which complement is characterized as "systemically activated", the actual damage in individual patients is most often focused on a subset of tissues such as the kidney [14]. Thus, in a disease context when one desires to block complement activation locally, for example to protect the retina, brain or kidney, it is only logical to design a strategy in which one would maintain elsewhere the positive protective functions that are listed above, and focus inhibition to the site where it is needed.

Another important consideration with regard to therapeutic development in the complement system is that it is activated by three interacting recognition mechanisms through the classical, alternative and lectin pathways. Essential issues with regard to the choice of inhibitors for each pathway relative to the disease of interest are addressed elsewhere [15,16]. Importantly, though, each pathway converges on the centrally important C3 protein when multi-component C3 convertases are formed and C3 is cleaved. In this activation process, a thioester bond in C3 allows the covalent attachment through ester or amide linkages to other molecules of the C3 protein in the C3b form [17]. The activation of C3 is associated with the soluble anaphylatoxin C3a release and is followed by C5 cleavage and activation, the coincident release of C5a [18] and formation of the pore-like membrane attack complex (MAC) [19]. Because the generated C3b forms additional C3 convertase, the fixation of C3b to tissues creates a favorable microenvironment for further complement activation. Subsequent cleavage of C3b generates the iC3b/C3dg/C3d forms and binding of the specific molecules to their cognate C3 fragment receptors [20]. This process effectively "marks" the C3 fragment-bound targets as immunologically different. It is the deposition of tissue-localized C3 fragments and their subsequent processing to fragments with altered neoepitope presentation that underlies the imaging of local C3 activation described below in this review.

## 2. Rationale, history and previous strategies for development of local tissue targeted complement inhibitors

The vast majority of historical and even contemporary approaches to complement inhibition focus on systemic blocking activity of the compound. That could be considered the case even in the context of "local" delivery in an organ such as the eye, where the injurious complement activation is occurring at the posterior pole (RPE, Bruch's membrane, choriocapillaris) and not in the vitreous at the site of injection of an inhibitor such as anti-Factor D monoclonal antibody [21]. The basic rationale behind the development of site-targeted complement inhibitors, however, is the supposition that localized delivery will not only increase inhibitor bioavailability and durability at sites of disease, but will minimize disruption of the normal physiological and protective mechanisms of complement. In addition, although advantages of targeted inhibition are most relevant for the treatment of chronic diseases, avoidance of short-term disruption of host defense mechanisms is desirable even during acute conditions, especially so if the patient is immunocompromised. The choice of which linked inhibitor to use (Crry, DAF, FH, CR1, CD59) is also important and is dependent upon several factors, including the need in the particular indication being studied to block at the point of terminal MAC formation (CD59), or the desire to impair C3 and C5 convertase function in order to obtain either alternative pathway/amplification inhibition modulation alone (FH) or inhibitory effects on all three pathways (Crry, CR1, DAF).

The first publications focusing on targeted complement inhibition to sites of injury appeared in 1999 and described two basic approaches. In one approach, cell specific targeting of CD59 was achieved by linking the inhibitor to an antibody or antibody fragment (Fig. 1). The fusion proteins specifically targeted cells expressing cognate antigen and provided targeted, but not untargeted cells, with effective protection from complement-mediated lysis and injury [22]. In another approach, it was shown that the decoration of soluble CR1 (sCR1), an inhibitor of the C3 and C5 convertases derived from all three pathways, with sialyl Lewis$^x$ (sLe$^x$) moieties significantly enhanced the protective effect of the inhibitor in a rat model of selectin-dependent lung injury [23] and in a mouse model of ischemic stroke [24]. The sLe$^x$ carbohydrate moiety binds to both P and E selectin, adhesion molecules that are upregulated on activated endothelial cells. In the lung injury model, the enhanced protective effect of sCR1 sLe$^x$ correlated with increased binding of sCR1 sLe$^x$ to the lung vasculature when compared to binding of sCR1. Thus, decoration with the sLe$^x$ moiety represents a strategy to increase the efficacy of complement inhibitors by targeting them to selectin-expressing activated endothelial surfaces. Importantly, however, the efficacy of sCR1 sLe$^x$ was still dependent on the ability to maintain systemic complement inhibition at the same time.

A short form of sCR1, designated APT070, has also been targeted to cell membranes, albeit nonspecifically, by incorporation of a membrane targeting myristoylated peptide to the 3 N-terminal SCRs of CR1. APT70 was shown to be 100-fold more active than its parent protein *in vitro* complement inhibition assays, although