Complement and Adaptive Immunity:
Roles for the Anaphylatoxins C3a and C5a in Regulating Tumor Immunity

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Abstract—Although a relationship between inflammation and some cancers is well-established, the complex role of the complement system in cancer development remains incompletely understood. The studies that have investigated this relationship have primarily reported that the diverse components of the complement system support tumor growth. However, some studies have demonstrated that components of the complement system support adaptive T cell-mediated responses in other diseases and could therefore potentially be beneficial in promoting tumor regression through supporting anti-tumor immune responses. In this review, the most recent evidence illustrating the role of the complement system in adaptive immunity and cancer will be discussed, and some questions will be raised as to whether some complement components can enhance the efficacy of cancer immunotherapy due to their influence on adaptive immunity.

Keywords — cancer, immunotherapy, complement, T cell, decay acceleration factor (DAF).

I. THE COMPLEMENT CASCADE

The complement cascade is a vital component of the innate immune system. Because the innate immune response is able to opsonize bacteria, it was said that this system can 'complement' antibody-mediated antibacterial activity [1]. Consisting of about 25 different proteins, complement factors can be found in the blood, principally as inactive precursors. Upon stimulation, for example by bacteria, complement proteases, known as zymogens, cleave and activate complement proteins through three major pathways – classical, lectin, and alternative. These pathways are extensively reviewed elsewhere [2]. However all three pathways ultimately converge to generate at least two extremely potent pro-inflammatory mediators – the anaphylatoxins C3a and C5a. The major functions of the complement system are 1) to mark, or opsonize, pathogens for targeting and destruction by the membrane attack complex (MAC) or by phagocytes, 2) to serve as chemotactants for the recruitment of immune cells, including macrophages and neutrophils, and 3) to create pores in bacterial membranes for destruction and clearance of infection. Additionally, the complement system promotes effective elimination of pathogens via shaping adaptive immune responses by enhancing T cell and antigen presenting cell (APC) responses.

Due to the potency of complement factors, these proteins have the potential to be very damaging to host tissues. As a result, the complement system contains regulatory proteins that control complement availability and signaling [3]. One of these regulatory proteins is protectin (CD59; also known as MAC-inhibitory protein), which functions to prevent the formation of the membrane attack complex [4]. Another regulatory protein that can protect the host from complement attack is decay accelerating factor (DAF; encoded by the Cd55 gene), which is a ubiquitously expressed transmembrane glycoposphatidylinositol (GPI)-anchored protein. DAF functions to dissociate the complexes of proteases on host cell surfaces that are responsible for the generation of C3a and C5a, thereby reducing the local production and availability of these factors and attenuating the complement response [5]. Not surprisingly, studies have also demonstrated that complement regulatory proteins can also contribute to the regulation of adaptive immune responses via regulating the availability of complement factors within a given microenvironment. This may be critical in understanding the role of complement in the tumor microenvironment, a complex array of cells and their secreted products.

II. COMPLEMENT AND ADAPTIVE IMMUNITY

Complement was originally thought to have a more dominant role in supporting humoral immunity than cellular immunity. However, an increasing amount of data support an equally important role for complement in promoting T cell-mediated immunity. Co-stimulatory signals transmitted during cognate
interactions between T cells and APCs lead to increased expression and secretion of complement components C3 and C5 by APCs and T cells. Activation of both APCs and T cells also up-regulate the G protein-coupled receptors, C3aR and C5aR, which bind to C3a and C5a, respectively [6, 7]. In APCs, signaling via these two complement receptors down-regulates DAF expression, thereby further increasing C3a and C5a levels, along with up-regulation of co-stimulatory molecules and class II MHC [8]. Upon occupancy by their ligands, C3aR and C5aR also signal to activate the phosphatidylinositol 3-kinase (PI3K) and Akt pathways, which induce the activation, proliferation, differentiation, and survival of CD4+ and CD8+ T cells [9]. These findings support the idea that complement-mediated signaling enhances effector T cell responses (Figure 1).

Additional studies have demonstrated the influence of both systemic and local production of complement on T cell responses in models of transplantation, autoimmune, and viral infection. In experimental models of transplantation, C3aR- and C5aR-mediated signals up-regulated allo-stimulatory effects in dendritic cells, whereas deficiency in these receptors resulted in reduced levels of MHC II and co-stimulatory molecules, which further dampened Th1 responses and increased IL-10 production [10]. Similarly, macrophages from C3-deficient mice also showed a reduced ability to stimulate allo-reactive T cells [11]. Additional studies using mouse models of multiple sclerosis have shown that APC-derived C3a and C5a signal through C3aR and C5aR on T cells, inducing IFN-gamma and IL-17 production and exacerbating disease severity [12] (Figure 1). However, a very recent study brings these findings into question: using a C5aR-gfp reporter, Song and colleagues showed that C5aR expression is restricted to myeloid cells [13]. Therefore, careful re-evaluation of the direct effect of C5a on T cells is needed.

Recent studies have demonstrated that natural CD4+Foxp3+ T regulatory (nTreg) cells express both C3aR and C5aR [8, 9]. Signaling through these receptors diminished the suppressive function of nTreg cells. Genetic deletion or blockade of C3aR and/or C5aR signaling via pharmacological inhibition on nTreg cells resulted in the abrogation of autoimmune colitis, and promoted the survival of skin allografts [10]. Further mechanistic studies revealed that phosphorylation and activation of Akt via C3aR/C5aR signaling inactivated Foxo1 following phosphorylation, which contributed to lowered Foxp3 expression levels in nTreg cells and reduced suppressive activity of these cells [10]. Signaling through receptors for C3a and C5a may also lead to reduced Foxo1 expression levels, with a concomitant downregulation of Foxp3 and loss of suppressive activity [8]. Taken together, these data suggest that complement also alters adaptive immunity by diminishing the suppressive capacity of T regulatory cells (Figure 1).

A role for complement components in the generation of induced regulatory T (iTreg) cells also exists. A recent report demonstrated that in the absence of C5aR signaling, CD4+Foxp3+ T cells can become CD4+Foxp3+ iTreg cells and develop a suppressive phenotype both in vitro and in vivo [9]. Treatment of CD4+Foxp3+ cells with a C5aR antagonist resulted in reduced IL-6 production and increased production of TGF-β and IL-10, as well as elevated levels of Foxp3 expression. iTreg cells that were induced via C5aR antagonism and adoptively transferred into mouse models of experimental autoimmune encephalomyelitis (EAE) led to diminished disease, suggesting a novel role of complement signaling in the suppression of autoreactive T cells, induction of Treg cells, and regression of autoimmune disease [9]. More importantly, these findings reveal a novel therapeutic intervention for treating autoimmune disease as well as a possible explanation for immune suppression in cancer, where elevated levels of Treg cells in cancer patients may be due to down-regulation of C5aR in the presence of other immune-suppressive cytokines like TGF-β. Further studies will be required to test this possibility.

**Figure 1. Interactions between complement and the adaptive immune response.** The anaphylatoxins C3a and C5a have diverse functions. Binding of C3a or C5a to their respective receptors leads to activation of the PI3K/Akt pathway in effector T cells and promotes pro-inflammatory function. In antigen presenting cells (APCs), these signals lead to increased stimulatory capacity through increased expression of costimulatory ligands and MHC. In regulatory T cells, ligation of C5aR and C3aR leads to down-regulation of Foxp3 expression and reduced suppressive activity. This may lead to accelerated autoimmune disease or graft rejection. DAF reduces levels of C5a and C3a, reducing the effects described above.

The complement regulatory protein DAF (CD55) has also been studied for its role in regulating T cell responses. Not surprisingly, Cd55-deficient dendritic cells produce significantly higher levels of C3a and C5a compared to wild-type DCs, resulting in a more robust expansion of antigen-specific effector CD4+ and CD8+ T cells, enhanced T cell responsiveness, and diminished function of natural and induced regulatory T cells via down-regulation of Foxp3 [14, 15]. Furthermore, Cd55 deficiency in dendritic cells correlated with increased expression of CD40 and down-regulation of PD-L1 [16]. The lack of DAF expression by T cells, however,
had no affect on their ability to respond to antigen [16]. These studies further underscore the complexity of the regulation of complement factors on adaptive immune responses (Figure 1).

III. COMPLEMENT AND CANCER

Recent advances in complement research have demonstrated a role for complement in several types of cancer, including prostate, colon, and ovarian cancers, as well as lymphoma [17, 18]. Although complement activation usually takes place in the circulation through cleavage of precursor proteins, complement factors are not biologically relevant until deposited on microbial or other cell surfaces. Thus, detecting complement deposition in tumor tissues indicates activation within a tumor. Complement factors have been shown to influence nearly every step of neoplastic progression including cellular proliferation, survival, angiogenesis, and invasion and migration.

Complement factor C4 aids in proliferation of the TC-1 syngeneic model of murine cervical cancer [19], and the membrane attack complex (MAC), which is composed of complement factors C5b, C6, C7, C8, and C9, was demonstrated to activate the cell cycle and oncogenic pathways. Increases in cytosolic calcium via activation of the MAC leads to the activation of various downstream mediators including protein kinase C and diacylglycerol, which are important regulators of the cell cycle, as well as activation of members of the mitogenic-activated protein kinase (MAPK) family [20, 21]. Additionally, engagement of C5aR and C3aR on tumor cells was coupled to the activation of MAPK proteins including PI3K, Akt, and mechanistic target of rapamycin (mTOR), all of which are associated with increased tumor cell proliferation when over-expressed or activated [22].

The formation of new blood vessels in tumors are critical for perfusion and the acquisition of migratory and invasive properties in tumor cells. Complement factors were shown to directly and indirectly promote both neovascularization and epithelial-mesenchymal transition (EMT). In C3ar- and C5ar-deficient mice, vascular endothelial growth factor (VEGF) levels were significantly decreased. Additionally, wild-type mice treated with antagonists of C3aR and C5aR, or with neutralizing antibodies against C3a and C5a, showed reduced levels of VEGF and blood vessel formation [23]. C3a/C3aR interaction on tumor cells also induces EMT via the down-regulation of E-cadherin [24]. C5a enhances endothelial migration, as well [25]. Moreover, complement has been demonstrated to have an indirect role in the reduction of E-cadherin expression through C5-induced insulin-like growth factor (IGF) expression. Increased IGF expression is associated with the down-regulation of E-cadherin and subsequent EMT and metastasis [26]. Therefore, complement can modify the tumor microenvironment by promoting angiogenesis and altering the invasive properties of tumor cells through induction of EMT.

IV. COMPLEMENT AND TUMOR IMMUNITY

A role for complement in regulating tumor immunity remains an area of on-going research. C3a and C5a were reported to inhibit antigen-specific CD8+ T cell-mediated anti-tumor immune responses [27]. In that study, C5a was also demonstrated to serve as chemoattractants for myeloid-derived suppressor cell (MDSCs). Pharmacologic blockade of C5aR led to tumor regression, which was attributed to a reduction in MDSC recruitment and reactive oxygen species (ROS) production. This, in turn, enabled CD8+ T cell-mediated killing of tumor cells [27, 28]. This study suggested an immune suppressive role for C5a in tumor progression (Figure 2).

Despite these findings, a recent study suggested that, depending on the local concentration, complement proteins may also have opposing roles within the tumor microenvironment [29]. In that study, immunodeficient mice that were challenged with C5a-transfected SKOV-3 human ovarian tumor cells displayed significantly smaller tumors compared to control vector-transfected tumors. The C5a-expressing tumors were heavily infiltrated by natural killer (NK) cells and macrophages, and lower levels of VEGF, arginine, and TNF-α were detected in the C5a-expressing tumors. In a parallel study, accelerated tumor growth was noted in mice challenged with the syngeneic lymphoma cell line.
RMA transfected to produce high levels of C5a. This was accompanied by an increased frequency of Gr-1+CD11b+ MDSCs in the spleen and a reduction in tumor-infiltrating CD4+ and CD8+ cells. Conversely, mice that received RMA cells transfected to produce low levels of C5a had a significantly smaller tumor burden and increased levels of IFN-γ-producing CD4+ and CD8+ T cells in both the tumor-draining lymph nodes and spleen. These findings suggest that tumor progression is altered as a function of the local concentration of complement factors like C5a within the tumor microenvironment [29] (Figure 2).

V. FUTURE STUDIES AND PROSPECTS

The studies described above suggest that there is a complex relationship between complement, adaptive immunity, and tumor immunity. Additional research is needed to determine whether the source of complement dictates a given response. For example, a recent study suggested that APC-derived C3a and C5a was solely responsible for the observed shift in the frequency of CD4+ and CD8+ T cells and T regulatory cells [10]. Moreover, the factors that regulate complement and complement receptor expression within the tumor remain elusive. Understanding the role of tumor cell-derived complement versus APC- or T cell-derived complement and how these factors are regulated within the tumor microenvironment could give additional insight into how complement components differentially modulate anti-tumor immune responses. This, in turn, could lead to more targeted therapies to improve tumor immunity. However, we would hypothesize that within the tumor microenvironment, the immune stimulatory effects of C5a on T cells are ‘over-powered’ by the suppressive effects on myeloid cells, creating a more immunosuppressive microenvironment.

Furthermore, the role of DAF in the context of cancer remains poorly understood. DAF expression correlates with poor survival in patients with breast and colorectal cancer, and is associated with tumorigenesis of blood cancers [18, 30-32]. Correspondingly, DAF expression was elevated in patients with advanced prostate cancer, and in vitro studies revealed that DAF was functionally active and capable of preventing complement-mediated lysis of the prostate cancer cell lines PC-3 and DU145. Silencing DAF using small interfering RNA (siRNA) reduced prostate tumor burden as well as metastasis, suggesting that DAF expression promotes tumor development and progression by protecting prostate cancer cells from complement-mediated lysis [33]. Elevated DAF expression would indicate lower levels of C3a and C5a within the tumors, although this aspect was not investigated. Therefore, the findings also support the idea that complement factors have an anti-tumoral role, however, this remains under-explored. In addition, DAF is proposed as a receptor for CD97, a G protein-coupled receptor which is expressed by a variety of immune and inflammatory cells. Therefore, it may also function outside its role as a complement regulatory protein.

Cd55 deficiency also leads to increased expression of co-stimulatory ligands and down-regulation of immune suppressive ligands such as PD-L1 as well as a reduction in T regulatory cell populations. Cd55 deficiency and elevated levels of C3a and C5a were also shown to exacerbate models of autoimmunity due to enhanced T cell responses. However, this heightened immune responsiveness could be beneficial in the tumor setting. Studies using tumor-bearing Cd55-deficient mice, and specifically cell type-specific Cd55-deficient mice could further elucidate the role of DAF in sustaining tumor-specific T cell responses. We would predict that DAF deficiency, leading to elevated levels of C3a and C5a, leads to increased immune suppression and loss of T cell responsiveness.

While the effect of complement on antibody-based cancer therapy has been explored [34], the impact that it could have on cellular therapy for cancer remains unclear. Adoptive T cell therapy for cancer has the potential to be a very promising therapeutic strategy. However, its success is limited by T cell tolerization within the tumor microenvironment. We would propose that complement proteins may have adjuvant-like effects during sensitization to tumor antigens and could be used to reverse the immune suppressive effects of the tumor microenvironment that lead to T cell tolerance. However, care would need to be taken to avoid the ability of C5a to recruit suppressive cells like MDSC. Understanding the balance between these immune stimulatory and suppressive effects of complement will be critical to exploit them for clinical application. As a result, more effective strategies that target complement and complement regulatory proteins may enable the development of more durable anti-tumor immune responses.

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References
