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Review

Targeting complement-mediated immunoregulation for cancer immunotherapy

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ABSTRACT

Complement was initially discovered as an assembly of plasma proteins "complementing" the cytolytic activity of antibodies. However, our current knowledge places this complex system of several plasma proteins, receptors, and regulators in the center of innate immunity as a bridge between the initial innate responses and adaptive immune reactions. Consequently, complement appears to be pivotal for elimination of pathogens, not only as an early response defense, but by directing the subsequent adaptive immune response. The discovery of functional intracellular complement and its roles in cellular metabolism opened novel avenues for research and potential therapeutic implications. The recent studies demonstrating immunoregulatory functions of complement in the tumor microenvironment and the premetastatic niche shifted the paradigm on our understanding of functions of the complement system in regulating immunity. Several complement proteins, through their interaction with cells in the tumor microenvironment and in metastasis-targeted organs, contribute to modulating tumor growth, antitumor immunity, angiogenesis, and therefore, the overall progression of malignancy and, perhaps, responsiveness of cancer to different therapies. Here, we focus on recent progress in our understanding of immunostimulatory vs. immunoregulatory functions of complement and potential applications of these findings to the design of novel therapies for cancer patients.

1. Introduction

The presence of cellular infiltrates composed of $CD8^+$ (cytotoxic) effector T cells within malignant tissue, in several types of cancer (T-cell inflamed tumors), suggests efficient spontaneous priming of naïve $CD8^+$ T cells against tumor-associated antigens [1,2]. The type I interferon pathway seems to be pivotal for T cell priming in tumors [2]. In addition, in some patients, there are antibodies against tumor antigens [3]. Therefore, it appears that the human immune system can generate spontaneous adaptive immune responses against malignancy [2]. However, these responses are unable to eliminate tumors, likely, because of the intrinsic immunosuppressive properties of the tumor microenvironment [4]. This notion is further supported by the recent clinical success of the checkpoint inhibitors targeting T cell immunoregulatory mechanisms [5]. Therapeutic targeting of immunosuppressive mechanisms, operating in cancer patients, is more efficient in reducing or reversing cancer progression than attempts to induce de novo antitumor responses (cancer vaccines) [5]. Therefore, it is critical to understand immunoregulatory mechanisms, operating in primary cancer sites and metastasis-targeted organs to advance discovery of novel therapeutic targets or improve already existing forms of cancer immunotherapy. The improvement of checkpoint inhibitors' efficacy is of the highest significance, given that only a fraction of cancer patients responds to this treatment and, in some patients, the clinical benefits are limited [5]. There is growing understanding and appreciation for the concept that only targeting several immunoregulatory mechanisms simultaneously can bring substantial clinical benefits for cancer patients.

The complement system has recently emerged as an important regulator of immunosuppressive mechanisms operating in primary tumor sites [6,7] and metastasis-targeted organs [8,9]. Although the role of complement in cancer remains understudied, several reports point to complement as a recruiter, inducer, and regulator of immunosuppressive cells in the tumor microenvironment and the premetastatic niche [7]. Recent work also demonstrated synergism between programmed cell-death 1 (PD-1) blockade and complement inhibition in reducing progression of tumors in a model of lung cancer [10]. These findings reveal a more practical avenue for ventures

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exploring the complement system as a target in a combined immunotherapy approach in concert with checkpoint inhibitors.

In contrast to T-cell inflamed tumors, in a subset of cancers, tumor tissue commonly lacks infiltrating T cells, suggesting immune exclusion. In these tumors, spontaneous priming of T cells does not occur, therefore, targeting T-cell checkpoints is unlikely to offer substantial clinical benefits [2]. Designing new immunotherapy for these patients seems to be especially challenging [11]. Given a key role of the complement system in regulating innate immunity in infection [12] and possible interconnections of early complement deficiencies with triggering the type I interferon pathway in systemic lupus erythematous (SLE) [13], it is tempting to speculate for a possible role for complement in preventing efficient priming of T cells in non-T cell inflamed tumors.

The complement proteins are abundant throughout the body and are produced in cells involved in immunity. In addition, complement regulates inflammation [14] and antitumor immunity [6,7]. Therefore, it is conceivable that complement may play a central role in orchestrating immunosuppressive mechanisms that overwhelm antitumor immunity in cancer patients.

However, in the absence of malignancy, complement bridges initial innate immune responses with subsequent adaptive immunity by shaping and directing B and $CD4^+$ T cells [15,16], and is pivotal for induction of efficient immunity against pathogens [12,17]. These seemingly contradictory functions of complement in regulating adaptive immunity require further studies and explaining the conflicting results will perhaps remain a challenge for some time in the field of complement biology. Here, we discuss essential functions of the complement system and its dichotomous role in regulating immunity in infection vs. cancer because this dichotomy needs to be cautiously considered when designing cancer immunotherapies targeting complement.

2. Components of the complement system

The complement system is composed of over 50 blood and lymph circulating, membrane-bound, and intracellular proteins [17]. The functions of some key complement components are summarized in the Table 1. It is an integral part of innate immunity and constitutes the first line of immediate immune defense against invading pathogens. The complement proteins found in serum are mostly secreted by the liver and are comprised of pattern recognition receptors (PRR), involved in detection of pathogens, enzymes that are activated in a cascade like fashion, similar to the coagulation system, and effector molecules. However, the list of immune and non-immune cells that can produce complement proteins has been growing since the discovery of extrahepatic synthesis of complement proteins over three decades ago. For example, bone marrow C3 production accounts for over 9% of all C3 in circulation [18,19]. Other components, such as factor D, are almost exclusively produced by adipocytes [20]. In fact, the majority of cells in the human body can produce at least one or more complement proteins [21]. The importance of this extrahepatic production is underlined by the observation that C4 produced by monocytes can rescue the humoral response against tumor-derived antigens in the absence of serum C4 [22].

Serum-derived complement can be activated via three pathways [17]. The alternative pathway is, evolutionarily, the oldest and is triggered either by bacterial surfaces or spontaneous fluid phase hydrolysis of the complement C3 thioester [23]. The lectin pathway is activated upon engagement of mannose binding lectin (MBL) or the ficolins (termed ficolin-1, ficolin-2, ficolin-3) to specific carbohydrates or *N*-acteyl residues respectively [24,25]. The classical pathway, which was discovered first, is, evolutionarily, the youngest because it is initiated upon binding of C1q to the clusters of at least two IgG molecules (or one IgM), forming a complex with antigen [26]. All three activation pathways result in generation of complement C3 cleaving enzymes, termed C3 convertases (see [16] for more details). The C3 convertases cleave C3 to a small 10 kDa fragment – C3a and a large fragment – C3b. The C3b can attach to bacterial or other nearby surfaces and mark them for phagocytosis in a process known as opsonization [27,28]. Upon the cleavage of approximately ten thousand C3 molecules, another C3b molecule associates with the C3 convertase to give rise of the C5 convertase. The C5 convertase cleaves C5 into a small fragment (C5a) and a large fragment (C5b). Complement proteins C3, C4 and C5 belong to the, evolutionarily, very old α2-macroglobulin family [17] and, as such, they have conserved sequences and structures in different species. The C3 and C5 contain active thioester moieties, which enable them to attach covalently to other molecules. While C4 does not contain active thioester moieties, it is also cleaved into a small fragment (C4a) and a large fragment (C4b), similar to C3 and C5.

The generation of the large fragments C3b and also C4b leads to opsonization of pathogens or cells and phagocytosis by monocytes or macrophages, via engagement of the receptors specific for C3 activation fragments. C3b bound to the cell surface is further processed via action of the soluble and membrane-bound complement regulators (see Table 1 for more details). The large fragment produced from C5 cleavage, C5b, attaches to a target surface (such as bacterial cell wall, or host cell) and forms the backbone for binding of C6, C7, C8 and finally C9 (Membrane Attack Complex-MAC). The C9 polymerizes to form a

Table 1
Non-exhaustive list of complement proteins and their activation products.

Complement protein/s	Receptor	Function	Reference
C3a	C3aR	Anaphylatoxin (can cause smooth muscle contraction, vasodilation and increased histamine release by mast cells), CD4+ T cell activation, monocyte activation (via NLRP3 inflammasome) cancer progression (direct or indirect effect)	[63,65,147]
C4a	PAR1, PAR4	Anaphylatoxin	[169]
C5a	C5aR1, C5aR2	Anaphylatoxin, NLRP3 inflammasome activation in CD4+ T cells and monocytes, regulates cancer progression	[66]
C3b	CD46, FH, CR1	Opsonin (via its receptors), part of the C3 and C5 convertases, activates CD4 T cells in autocrine fashion	[61]
Factor H (FH)	None, binds to Factor I and C3b or cell surface	In complex with the serine protease factor I, cleave and inactivate C3b to iC3b	[170]
CD46	None, binds to C3b, C4b and Factor I	In complex with the serine protease factor I, cleave and inactivate C3b (or C4b) to iC3b, potent costimulatory molecule on CD4+ T cell activation and metabolism	[61,62,73]
Complement receptor 1 (CR1, CD35)	None, binds to C3b, iC3b	Cleaves iC3b to C3dg, mediates inhibitory signals in T cell proliferation	[171]
Complement receptor 2 (CR2, CD21)	None, binds the degradation products of C3b (iC3b, C3dg, C3d)	Potent costimulatory molecule in B cells. Also controls survival (CR1) of germinal center B cells during development	[172–174]
Complement receptor 3 (CR3 or CD11b/CD18, or Mac-1)	None, binds iC3b	Opsonizes iC3b coated bacteria	[28,175]
Complement receptor 4 (CR4 or CD11c/CD18)	None, binds to different part of iC3b than CR3	Opsonizes iC3b coated bacteria	[28,175]