



Inhibiting C5a/C5aR axis reduces myeloid-derived suppressor cells and enhances PD-1 blockade therapy in lung cancer

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PD-1 immune checkpoint blockade therapy in lung cancer

In the US (1), and the world, lung cancer remains the leading cause of cancer death. Lung cancer causes more deaths than the next three most common cancers of the colon, breast and prostate combined. In the US an estimated 160,000 Americans and worldwide more than 1.1 million people die from lung cancer annually (2). Approximately 220,000 new cases occur every year in the US. Although previously thought to be non-immunogenic and non-responsive to immune-based therapies, recent groundbreaking studies in lung cancer with immune checkpoint blockade therapy reveal robust anti-tumor activity and durable responses in previously treated patients with progressive locally advanced or metastatic non-small cell lung cancer (NSCLC). This has also changed the treatment paradigm leading to immune checkpoint blockade as a frontline therapy for advanced stage lung cancer. Studies in NSCLC and melanoma patient-derived tumor specimens reveal that responses to immune checkpoint blockade therapy rely on tumor infiltration of activated T effector cells (3-7). Immune inhibitory molecules are upregulated on activated T cells in tumors causing a down regulation of anti-tumor activity. The programmed cell death protein 1 (PD-1; also known as CD279) is an inhibitory receptor that

regulates immune responses. The PD-1 receptor interaction with the PD-L1 and PD-L2 ligands deliver inhibitory signals that regulate the balance between T cell activation and tolerance. Recent studies reveal responses in approximately 20% of NSCLC patients treated with inhibitors of the PD-1 checkpoint (3-6,8,9). Progress in our understanding of immune biology provide insights on the multiple pathways that modulate T cell and antigen presenting cell activities, hence PD-1/PD-L1 blockade alone is insufficient to address and mitigate all the deficiencies that contribute to the down regulation of anti-tumor immune responses in the tumor microenvironment (TME). This has led to concerted efforts into evaluating rational combinations with radiation therapy, chemotherapy, targeted therapy or vaccines to improve durable complete responses in cancer patients treated with PD-1/PD-L1 blockade therapy. Early investigations are encouraging suggesting that some of the combinations are more beneficial than monotherapy alone. With continued support of these research endeavors it is anticipated that increased immune based treatment options will be available for lung cancer. A number of combinations with PD-1/PD-L1 are under investigation for various cancer types. In this editorial we will focus on the combined efficacy of complement peptide C5a signaling inhibition and PD-1 blockade in preclinical lung cancer models based on the observations of Ajona *et al.* (10).

The complement system

The complement system is part of innate immunity consisting of at least 30 distinct plasma proteins that when activated opsonize pathogens, induce a series of inflammatory responses and form the membrane attack complex (MAC) that culminate in the elimination of pathogens. Much of the understanding of complement activation is derived from studies on pathogen clearance that defined three distinct mechanisms of complement activation on the pathogen surface that include the classical, alternative and the lectin pathways. Although these pathways are initiated by different molecules, they converge to generate the same set of effector molecules. The classical pathway is initiated during an adaptive immune response by the binding of C1q to Fc portion of the antibody-antigen complexes on targets, which links the effector mechanisms of innate and adaptive immunity. The alternative pathway is initiated by a spontaneously activated complement component C3 that binds to the surface of a pathogen. Finally the mannan-binding lectin pathway is initiated by binding of the mannan-binding lectin, a serum protein, to mannose-containing carbohydrates on bacteria or viruses. Each pathway follows a sequence of reactions to generate a protease called C3 convertase. These reactions are known as the 'early' events of complement activation, and consist of triggered-enzyme cascades in which inactive complement proteins are successively cleaved to yield two fragments, the larger of which is an active serine protease. The active protease is retained at the pathogen surface, and this ensures that the next complement protein in the pathway is also cleaved and activated at the pathogen surface. Hence C3b acts as an opsonin that enhances the phagocytosis of the C3b bound targets. By contrast, the small peptide fragments C3a, C4a and C5a are released from the site of the reaction and act as soluble mediators of inflammation. The small complement fragments C3a, C4a and C5a are differentially active: C5a is more active than C3a, which is more active than C4a. C3a, C4a and C5a cause local inflammatory responses by acting directly on local blood vessels, stimulating increased: blood flow, vascular permeability, and binding of phagocytes to endothelial cells. In addition C5a and C3a activate mast cells to release histamine and TNF- α that contribute to the inflammatory response. C5a directly increases the activity of the phagocytes through the G protein C5a receptor expressed by macrophages and neutrophils. On the other hand, the membrane bound complement proteins C5b-C9 form the MAC on targets

that lead to their lysis.

Complement activation in lung cancer

Genetic and epigenetic changes that lead to the formation of malignant cells cause alterations in the cell surface glyco- and lipoproteins that activate the complement cascade. Several reports support the capacity of complement to recognize malignant cells. For example lung cancer cell lines deposit C5 and generate C5a more efficiently than bronchial epithelial cells (11). Moreover, a significant increase in C5a was found in the plasma samples of patients with NSCLC, suggesting that the local generation of C5a within tumors may be followed by its systemic diffusion (11). Elevated levels of the complement activation product C4d in bronchial fluids allows for the diagnosis of lung cancer (12). In primary lung tumors, C3c can be detected by immunohistochemistry (13). C3c and C4 are elevated in patients with lung cancer (14) and complement levels correlate with tumor size (15). On the other hand, the capacity of lung cancer cell lines to produce C5a in the absence of an exogenous source of complement components (i.e., serum), suggests that, apart from the traditional pathways of complement activation, cancer cells may have the capacity to activate complement by an extrinsic activation mechanism (11). The production of anaphylatoxin C5a by cancer cells may be mediated by soluble and membrane-bound pro-teases, such as serine proteases of the coagulation and fibrinolysis systems or cell-bound proteases (16-18). The question that these observations raise is that if complement is activated in the TME, why does it not lead to tumor clearance? Studies have shown that malignant tumor cells are protected from such complement-mediated lysis because they over express complement regulators that limit complement activation and deposition *in situ* and, therefore, formation of the MAC (19,20). It has been postulated that the ability of the MAC to lyse foreign and host cells might enhance the efficacy of cancer immunotherapies involving monoclonal antibodies (mAbs) specific for particular tumor antigens, as complement proteins enhance antibody-dependent cytotoxicity (21,22). However it is now being realized that the peptide anaphylatoxin complement-activation product C5a is involved in promoting tumor growth.

C5a-mediated immune suppression in lung cancer

The TME consists of tumor cells, stroma, blood vessels,

immune infiltrates and the extracellular matrix. Genetic alterations in oncogenes and tumor suppressor genes or epigenetic changes in the tumor that modulate tumor growth and invasion into the surrounding tissue orchestrate the persistence of inflammatory infiltrates. These cellular infiltrates modulate tumor development and progression. The tumor infiltrates vary by size and composition in diverse tumor types and at different stages of tumor development. Contributing to the immune suppression in the TME are myeloid-derived suppressor cells (MDSC). Studies in murine lung cancer models have shown that the anaphylatoxin C5a in the TME recruits monocytes. The tumor then programs the monocytes to become MDSC, thus sustaining a dysregulated inflammation that promotes tumor progression (23). C5a may have a key role as a chemoattractant for a subpopulation of MDSC that is morphologically related to neutrophils polymorphonuclear MDSC and as an activator of the production of reactive oxygen and nitrogen species in the monocyte-like subpopulation (23) that inhibit T cell responses. The role of C5a in the immunosuppressive function of MDSC was confirmed *ex vivo* when isolated MDSCs from C5aR-deficient mice were unable to inhibit T-cell proliferation (23). Moreover, in the LLC lung cancer mouse model, the blockade of C5a signaling reduced MDSC and down regulated the expression of key immunosuppressive molecules ARG1, IL-10, IL-6, CTLA4, LAG3, and PDL1 within the tumors (11). MDSC negatively modulate immune responses and promote tumor progression and metastases (24). MDSC are a heterogeneous population of immature myeloid cells that consists of myeloid progenitors and precursors of macrophages, granulocytes and dendritic cells. In mice, MDSC are identified by antibodies that detect cell surface expression of Gr1 and CD11b. Increases in the number of MDSC evoke strong natural suppressive activity in cancer patients or tumor-bearing mice. Gr1⁺CD11b⁺ immune suppressive cells are capable of inhibiting the T cell proliferative response induced by alloantigens, CD3 ligation, or various mitogens, and can also inhibit IL-2 utilization as well as NK cell activity. These observations indicate that progressive tumor growth is associated with the down-regulation of T cell responses and that the MDSC are involved in negative immunoregulatory mechanisms. In murine tumor models, increases in MDSC in the tumors, spleens, bone marrow and blood down regulate T cell responses (24). The immune modulatory role of MDSC in lung cancer was shown in a recent study, demonstrating that MDSC depletion augments anti-tumor activity in lung cancer (25).

Combined inhibition of C5a/C5aR and PD-1 enhances anti-tumor responses in lung cancer

Ajona *et al.* in their publication “*A Combined PD-1-C5a Blockade Synergistically Protects Against Lung Cancer Growth and Metastasis*” hypothesized that a combined inhibition of C5a/C5aR1 and PD-1 signaling may have an anti-tumor synergistic effect. RMP1-14 antibody was used to block PD-1 and an L-aptamer to inhibit signaling of complement C5a with its receptor C5aR. The authors rationale to target C5a was to down regulate immune suppression mediated by MDSC as C5a contributes to lung cancer progression by promoting an immunosuppressive microenvironment in which MDSC are involved. Utilizing several syngeneic models of lung cancer the authors demonstrated that the combination of C5a and PD-1 blockade markedly reduces tumor growth and metastasis, and leads to prolonged survival. As depicted in *Figure 1*, the anti-tumor effect was accompanied by a reciprocal alteration in the frequency of CD8 T cells and MDSC within the tumors, which they suggested may result in a more complete reversal of CD8 T-cell exhaustion. Based on the experimental results the authors concluded that C5a blockade creates a more permissive environment for immune-mediated tumor rejection and, consequently, its combined administration with anti-PD-1 antibodies synergistically impairs lung cancer growth and metastasis.

Commentary

With the success of PD-1 immune checkpoint blockade therapy for cancer, it is important to understand the role of complement activation in response to therapy. Although activated complement proteins have the potential to inhibit tumor growth, it is now being realized that the complement-activation peptide C5a is involved in promoting tumor growth in the context of recruitment/activation of MDSC. MDSC are key immune suppressive cells that down regulate T cell activity in the TME. In addition to mAb therapy induced complement activation, neoplastic transformation is accompanied by an increased capacity of the malignant cells to activate complement. However inhibitory mechanisms of complement activation allow cancer cells to escape from complement-mediated elimination and impede the clinical efficacy of mAb-based cancer immune therapies. PD-1/PD-L1 blockade is not able to reduce the T cell suppressive activity of the TME, which is induced by the accumulation of MDSC. Blocking C5a signaling reduces MDSC in the TME and synergizes

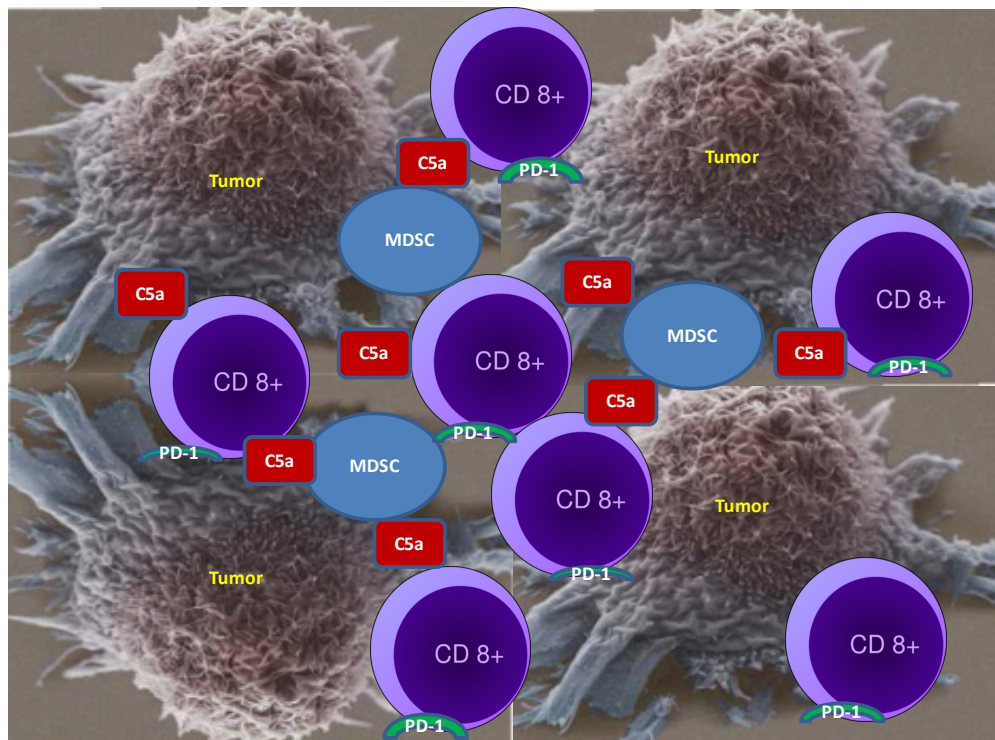


Figure 1 C5a and PD-1 inhibition reduces the frequency of MDSC and increases CD8 T cell effectors in the tumor. Combined C5a and PD-1 inhibition modulates the balance of CD8+ T cell immune effectors and MDSC in the lung tumor microenvironment that enhanced anti-tumor activity leading to potent tumor regression. MDSC, myeloid-derived suppressor cells.

with PD-1 blockade therapy in lung cancer. The study by Ajona *et al.* provides support for further evaluation of anti-PD-1 and anti-C5a agents as a novel combination therapeutic strategy for lung cancer. Studies are warranted to determine the generality of Ajona *et al.* observations for the broad applicability of the combined approach. Challenges that need to be overcome for the overall clinical success of this combinatorial approach, include appropriate scheduling, dosage, sequencing of the therapeutic agents and manageable toxicity profiles. In addition further analysis of the pathways by which cancer cells activate complement and induce immune suppression will provide insights on the interplay between complement and cancer and identify new diagnostic biomarkers and molecular targets for combined immune based anti-cancer therapies.

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Footnote

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