Unwelcome Complement

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Abstract

For decades, the complement system has been recognized as an effector arm of the innate immunity system that contributes to the destruction of tumor cells. However, recent studies have challenged this paradigm by demonstrating that a complement component, the anaphylatoxin C5a, promotes the growth of malignant tumors in a mouse model of cervical carcinoma. The effect of C5a on tumor growth was associated with the recruitment of myeloid-derived suppressor cells to tumors, followed by the activation of these cells. These unexpected findings identify the complement system as a potential new target for anticancer immunotherapy. [Cancer Res 2009;69(16):6367–70]

Lessons from the Past

The complex biological process known as inflammation serves to eliminate pathogens and other factors that disrupt tissue integrity (1). Therefore, inflammation is considered an initial defense response by the host to the threats associated with both infectious and noninfectious factors. A well-coordinated inflammatory response rapidly eliminates invading pathogens or limits their spread, invokes the adaptive arm of the immune system, and facilitates the clearance and healing of damaged host tissues. This process is recognized as being essential to the survival and well-being of humans and animals. However, when the acute inflammatory process fails to eliminate the causative factor and becomes chronic, this initially defensive response can contribute to the pathogenesis of numerous diseases, including cancer (1).

An association between chronic inflammation and cancer was initially suspected on the basis of epidemiological data demonstrating an increased incidence of various malignancies in patients suffering from chronic inflammatory diseases (2). These preliminary observations have recently been confirmed in numerous experimental studies, which have shown that chronic and indolent inflammation increases the risk of malignant transformation, accelerates the progression of established tumors, contributes to the local invasion of normal tissue, and facilitates metastasis (3, 4). Despite these findings, and the critical role that complement effectors play in controlling various steps in the inflammatory response, a role for the complement system in promoting the development and progression of malignant tumors was not suspected for a very long time. In fact, the activation of complement in various malignancies was interpreted as evidence that this system can contribute to killing tumor cells (5–8), a conclusion that was based on an analogy to the well-characterized role of complement in eliminating microorganisms.

Activated complement proteins opsonize pathogens and facilitate their clearance by phagocytes, enhance antibody-dependent cellular cytotoxicity (ADCC), and can lead to the direct lysis of certain species of bacteria (9). However, although these complement activities are highly efficient in eliminating infection, they fail to reduce the growth of malignant tumors. The resistance of tumors to complement-mediated attack has been attributed to the high levels of complement-regulatory proteins that are expressed by cancer cells. These regulatory proteins can be found on the surface of tumor cells or can be secreted by these cells into the interstitial fluid (Fig. 1). Membrane-bound and secreted complement regulators are both capable of limiting the activation of the complement cascade and the subsequent coating of the tumor cells with complement fragments (10, 11).

Interest in the expression of complement-regulatory proteins by malignant cells has revived as a result of the successful use of monoclonal antibodies (mAbs) to target tumor-associated antigens, because the mechanisms by which these antibodies limit tumor growth include ADCC and complement-dependent cytotoxicity (CDC), both of which involve complement (12). Therefore, overcoming the inhibitory activity of complement regulators should increase the deposition of complement proteins onto the tumor cells as a result of mAb binding to the tumor cells. This enhanced binding of complement cleavage products to the tumor cells would be expected to enhance ADCC and CDC (Fig. 1), leading to an improvement in the therapeutic efficacy of the mAbs. In fact, several *in vitro* and animal studies have confirmed the appropriateness of this type of approach (13). Thus, it would seem reasonable to conclude that enhancing complement activation should be beneficial for cancer patients, at least for those patients who are treated with mAbs targeting tumor antigens. In contrast, the functional implications of complement activation in the absence of exogenous antibodies (such as tumor-targeting mAbs) have until recently been unclear.

Shifting a Paradigm

A recent study involving a mouse model of cervical carcinoma has shown that proteins of the complement system can promote the growth of tumors (14). Because complement proteins require various types of cleavages to be activated, and their activation is often associated with the deposition of the cleavage products onto microbial or cellular surfaces (9), initial experiments in this study have been designed to confirm the deposition of complement in tumor tissue. These studies have focused on complement component C3, because this protein has a central position in the complement cascade, and all three pathways of complement activation lead to its cleavage (Fig. 1). These experiments have shown an association of C3 cleavage product deposition with the tumor vasculature in mice (Fig. 1). At the same time, they have

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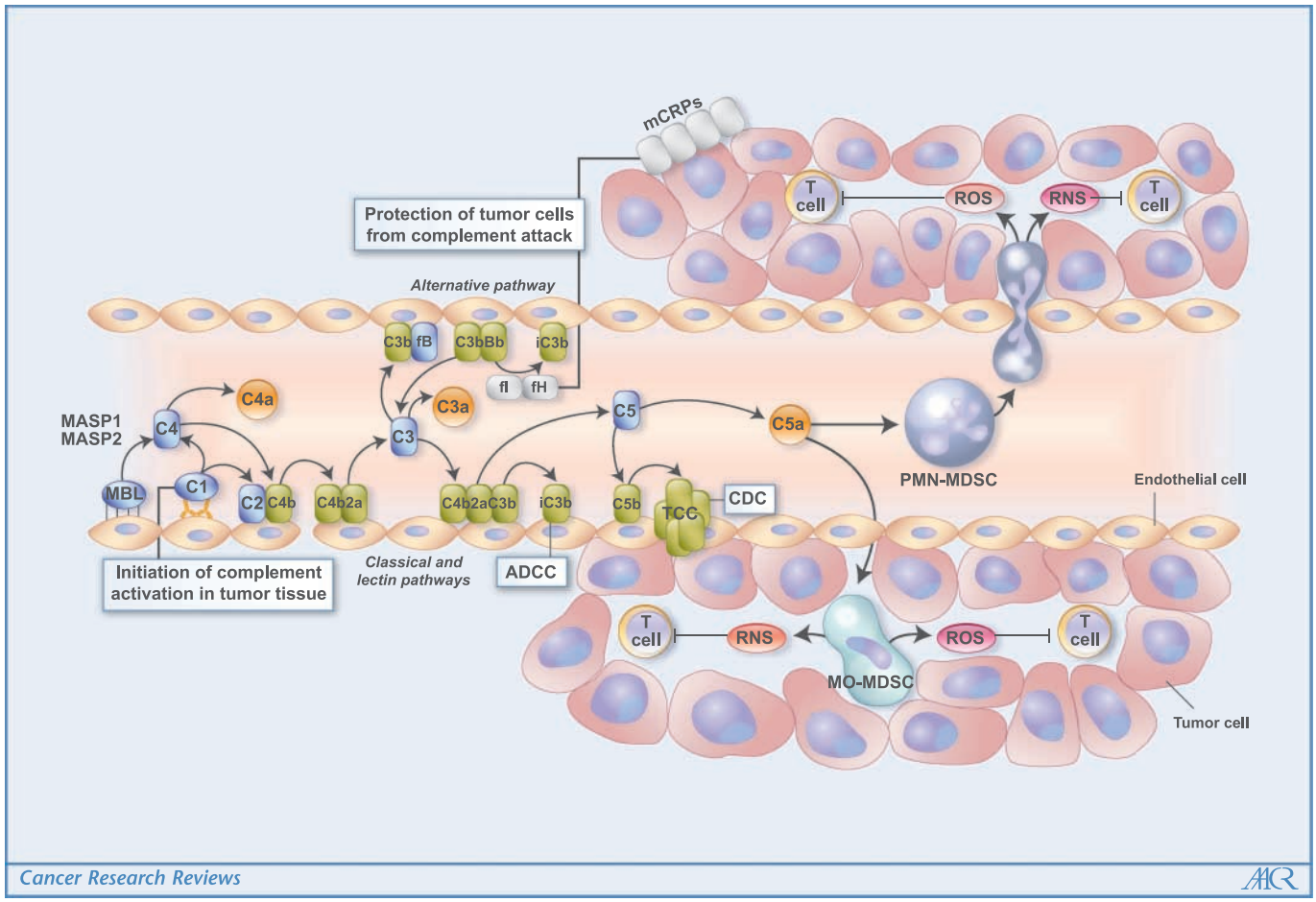

Figure 1. Functions of complement in tumor growth. The complement system can be activated through three distinctive pathways: The classical pathway (CP) is initiated when C1q binds to its various targets. Bound C1q activates C1r and C1s within the C1 complex, and C1s then cleaves C2 and C4. The lectin pathway (LP) begins with the binding of mannose-binding lectin (MBL) or ficolins to pathogen-associated molecular patterns (PAMPs) or apoptotic host cells. This binding activates MBL-associated serine proteases (MASPs), which cleave C4, generating C4a (which is released) and C4b. As a result of the cleavage of C2 and C4 by the CP or LP, C4b combines with C2a to form the CP C3 convertase, which cleaves C3 into C3a and C3b. The alternative pathway (AP) is initiated by low-grade cleavage of C3 in plasma and the generation of a small amount of C3b. This C3b binds factor B (fB), which is then cleaved into Ba and Bb. This cleavage leads to the formation of the "initial" AP C3 convertase, which, like the CP convertase, cleaves C3 into C3a and C3b. C3a is released, and C3b binds to activating surfaces. Surface-bound C3b binds additional fB, which is cleaved within this complex, leading to the formation of the AP C3 convertase. The C3 convertase can amplify complement activation induced by the CP or LP. C3b can bind to C4b2a or C3bBb to form the CP or AP (not shown) C5 convertase, respectively, which cleave C5 into C5a and C5b. The C5a is released, and C5b contributes, together with C6-C9, to the formation of the terminal complement complex (TCC). Complement effectors generated during activation can limit tumor growth. The C3b cleavage product iC3b binds to tumor cells, and through its interaction with complement receptor (CR) 3 on mononuclear phagocytes and natural killer (NK) cells enhances ADCC. The TCC is thought to contribute to CDC, which is one of the mechanisms for eliminating tumor cells by means of therapeutic antibodies. Some complement proteins accelerate tumor growth. C5a probably generated through the CP, recruits PMN-MDSCs to tumors. PMN-MDSCs migrate to tumors and produce highly immunosuppressive ROS and RNS that inhibit the antitumor T-cell response. In addition, C5a enhances the production of ROS and RNS by MO-MDSCs in the tumor microenvironment. Tumor cells are protected from complement attack by membrane complement regulatory proteins (mCRPs) and soluble complement inhibitors, secreted in the tumor microenvironment, such as factor H (fH) and factor I (fI). Factor H is a cofactor for fI-mediated cleavage of C3b to iC3b and accelerates the decay of the alternative pathway C3 convertase.

indicated an absence of these cleavage products from the plasma itself, suggesting that the activation of complement had been limited to the tumor tissue and had likely been triggered by factors produced locally in the tumor microenvironment.

The reduced tumor growth that is seen in mice lacking C3 is a first hint that complement proteins can promote the progression of malignant disease (14). Furthermore, given the essential role of C3 in activating the complement cascade and the presence of C3 cleavage products in tumor tissue, it seems that complement activation itself contributes to this process. Because complement can be activated through various mechanisms, the next round of experiments was aimed at establishing which pathway of activation is triggered in tumors. For this purpose, tumor growth was evaluated in mice lacking complement fragment C4 and factor B. The limited tumor growth that was seen in C4-deficient mice has pointed to the classical or lectin pathways as the mechanism(s) of complement activation in cancer, because C4 is required for the formation of the C3-cleaving enzymes (the C3 convertases) in both of these pathways (Fig. 1). The lack of effect of factor B deficiency on tumor growth has excluded the contribution of the alternative pathway to this model (Fig. 1).

Finally, to determine which of the two pathways, the classical or lectin, plays an essential role in this activation, tumor tissue has been assayed for the presence of molecules that initiate these pathways. Because the activation of complement is tightly regulated and limited to sites in which the causative factor is

present, the pattern of C1q distribution in the tumor tissue (which parallels that of C3) has suggested that the complement system is mainly activated through the classical pathway, because C1q triggers this pathway (Fig. 1; ref. 14). However, because C1q can bind to numerous targets (15), the factors that initiate the activation remain to be determined.

The activation of complement leads to the generation of numerous effectors, including the potent inflammatory mediator C5a (Fig. 1; ref. 9). Because chronic inflammation is known to promote cancer, it has been hypothesized that this mediator can enhance tumor growth. This hypothesis has been confirmed by the reduced tumor growth that is seen in C5a receptor (C5aR)-deficient and in wild-type mice treated with a C5a-blocking peptide. The increased infiltration of tumors by $CD8^+$ T cells in mice treated with a C5aR inhibitor suggests that C5a promotes tumor growth by suppressing the antitumor T-cell response. Furthermore, depletion of $CD8^+$ T cells from C5aR-deficient mice completely eliminates the tumor growth-reducing effect of this deficiency, confirming a role for C5a in the suppression of T-cell-mediated antitumor responses (14).

Although various mechanisms contribute to the inhibition of the immune response to tumor antigens, recent studies have established a pivotal role for myeloid-derived suppressor cells (MDSCs) in this process (16, 17). Given that these cells are immature counterparts of monocytes and neutrophils, and the functions of monocytes and neutrophils are regulated by C5a during an inflammatory response (9), further research has focused on determining whether the immune suppression exerted by C5a in tumor-bearing mice occurs through the regulation of MDSCs. The results of initial studies have indicated that MDSCs from both normal and tumor-bearing mice express C5aRs. Importantly, these receptors are partially internalized in tumor-associated MDSCs, suggesting that these cells are constantly exposed to ligand-C5a generated in the tumor microenvironment. Therefore, the internalization of C5aRs is consistent with the previous observation that complement has been found to be locally activated in tumor tissue.

Because C5a is a strong chemoattractant, finding that C5a contributes to the recruitment of MDSCs to tumors has not been surprising. This C5a activity has been associated, at least in part, with the upregulation of adhesion molecules on MSDCs that contribute to the process of extravasation. It is particularly interesting that the capacity of C5a to attract suppressor cells to a tumor has been limited to the subtype of MDSCs corresponding to neutrophils (PMN-MDSCs) (Fig. 1). In contrast, C5a does not influence the migration of another subpopulation of MDSCs that morphologically resemble monocytes (MO-MDSCs); however, it enhances the production of reactive oxygen (ROS) and reactive nitrogen (RNS) species by this monocyte-like subtype of MDSCs (Fig. 1; ref. 14). Although MDSCs influence T-cell function through a variety of mechanisms, the production and release of ROS and RNS seem to be critical to their suppressive capabilities (Fig. 1; ref. 16). Therefore, C5a-mediated enhancement of ROS and RNS production by MO-MDSCs apparently contributes to the suppression of the antitumor immune response (Fig. 1). The influence of C5a on the function of MDSCs has been finally confirmed by experiments demonstrating that MDSCs isolated from C5aR-deficient mice are unable to inhibit T-cell proliferation *ex vivo*. Thus, C5a suppresses T-cell-mediated antitumor response by recruiting PMN-MDSCs to the tumor and activating ROS and RNS production in tumor-associated MO-MDSCs (Fig. 1; ref. 14).

Back to the Future

Although the discovery that complement promotes the growth of malignant tumors has been viewed as a rather unexpected finding (18, 19), these tumor-promoting properties of complement are consistent with a growing appreciation for the role of inflammation in cancer initiation and progression. These recent observations illustrate yet again the irony that is becoming apparent when we look more closely at the response of the immune system to tumors: Immune mechanisms that cooperate to defend us from invading pathogens suddenly become an opposing force in the presence of a malignant tumor. The inflammatory response is critical for our survival in an environment full of pathogenic microorganisms, supports the healing of damaged tissue, and boosts adaptive immunity to foreign antigens; however, in the presence of a malignant tumor, its defensive activity is subverted, making it a major obstacle to the elimination of cancer cells by the adaptive immune system.

Several attempts are currently being made to reverse this situation. In light of the recent studies described here, inhibiting the complement system seems to be a promising new approach to achieving this goal, especially because the complement-inhibiting peptide that has been used for this research (14) has already been tested in clinical trials involving patients with inflammatory diseases (20). Also, because MDSCs in cancer patients are thought to be one of the main reasons for the failure of antitumor immunization to reduce or eliminate the growth of advanced tumors, targeting the complement system should, in theory, improve the efficacy of currently existing antitumor vaccines. These promising approaches should be viewed, however, with cautious optimism, because the results discussed here have been obtained in a single experimental model. Therefore, it is critical that their potential clinical applicability be verified through more extensive research in a variety of model systems.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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