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Subject Review

Cancer and the Complement Cascade

Martin J. Rutkowski, Michael E. Sughrue, Ari J. Kane, Steven A. Mills, and Andrew T. Parsa

Abstract

Despite significant research on the role of inflammation and immunosurveillance in the immunologic microenvironment of tumors, little attention has been given to the oncogenic capabilities of the complement cascade. The recent finding that complement may contribute to tumor growth suggests an insidious relationship between complement and cancer, especially in light of evidence that complement facilitates cellular proliferation and regeneration. We address the hypothesis that complement proteins promote carcinogenesis and suggest mechanisms by which complement can drive the fundamental features of cancer. Evidence shows that this diverse family of innate immune proteins facilitates dysregulation of mitogenic signaling pathways, sustained cellular proliferation, angiogenesis, insensitivity to apoptosis, invasion and migration, and escape from immunosurveillance. Given that the traditionally held functions for the complement system include innate immunity and cancer defense, our review suggests a new way of thinking about the role of complement proteins in neoplasia. *Mol Cancer Res*; 8(11); 1453–65. ©2010 AACR.

Introduction

A rich debate exists regarding the role of inflammation in neoplasia, one whose history began over a century ago with the competing theories of Rudolph Virchow and William Coley on whether inflammation promotes or impairs the development of cancer (1, 2). Clinical medicine offers a vivid picture of this association. Tissues chronically exposed to toxins such as tobacco, asbestos, and alcohol acquire secondary inflammation, conferring a greatly increased risk of bronchial, hepatocellular, gastric, and pancreatic cancer (3). Infectious agents such as *Helicobacter pylori*, *Shistosoma*, hepatitis C virus, and human papillomavirus strains can wreak neoplastic havoc on the tissues they target, resulting in mucosal-associated lymphoid tissue lymphoma, bladder and colon cancer, liver cancer, and cervical cancer, respectively (4). Endogenous disease processes marked by inflammation can also predispose to future cancer development, as evidenced by progression to colon cancer in inflammatory bowel disease and the development of esophageal metaplasia and adenocarcinoma in Barrett's esophagus (2).

Researchers have postulated that the chronicity of inflammation determines its cancer effect: Acute inflammation is believed to fight the development of neoplastic cells, whereas chronic inflammation encourages their genesis and spread (2, 5, 6). As a fundamental component of innate immunity,

the complement cascade (Fig. 1) contains some of the most powerful proinflammatory molecules in the body, including most notably the anaphylatoxins C3a and C5a. The contribution of the complement cascade to acute inflammation is well established, as is the continuous activation and consumption of complement proteins in chronic inflammatory states (7, 8). Nevertheless, emerging literature examining the mechanistic relationship between inflammation and cancer (4, 9, 10) has almost completely omitted the role of the complement cascade. Thus, the recent finding that complement proteins C3, C4, and C5a may aid tumor growth through immunosuppression (11) is unexpected and suggests an insidious and previously unrecognized relationship between the complement system and cancer.

Since its discovery, the complement system has been primarily considered an effector of innate immunity with the ability to “complement” antibody-mediated clearance of foreign pathogens, dispose of dead cells, and cause inflammatory states (12). This latter property is a recognized pathogenic factor in a wide spectrum of chronic inflammatory diseases, including rheumatoid arthritis (13), glomerulonephritis (14), atherosclerosis (15), asthma (16, 17), and multiple sclerosis (18). Thus, it is not surprising that evidence for complement-mediated disease pathogenesis has centered primarily on dysfunctional immunity caused by the absence, alteration, or overactivity of complement proteins (19–21). The relationship grows even more complex with the growing body of evidence suggesting that complement proteins mediate cellular turnover, growth, and regeneration, including bone marrow stem cell engraftment, bone and cartilage development, neurogenesis, synaptogenesis, white matter healing, and regeneration of the liver, limb, and lens (22–24).

Taken as a whole, evidence implicating the complement system in cellular proliferation and diseases of chronic inflammation suggests a potentially deleterious role in

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abnormal cellular growth. The following review addresses the novel hypothesis that complement proteins may promote the growth and spread of neoplastic tissues (Fig. 2), with a particular focus on research demonstrating mechanisms of complement-mediated oncogenesis.

Complement Promotes Oncogenesis

Complement proteins C3a and C5a increase activity of mitogenic signaling pathways

The proliferative abilities of the anaphylatoxins C3a and C5a have been documented repeatedly (22-24) and

reveal the participation of several signal transduction pathways with known links to neoplastic progression (Table 1). C3a receptor (C3aR) and C5a receptor (C5aR) are both coupled to G-proteins (25, 26). C3a/C3aR and C5a/C5aR binding activates members of the mitogen-activated protein kinase (MAPK) family including extracellular signal-regulated kinases (ERK; refs. 27, 28) and p38 (29). Furthermore, C3a and C5a increase the activation of phosphatidylinositol 3-kinase, Akt, and mammalian target of rapamycin (30, 31), three other proteins strongly associated with neoplasia when overexpressed (32, 33).

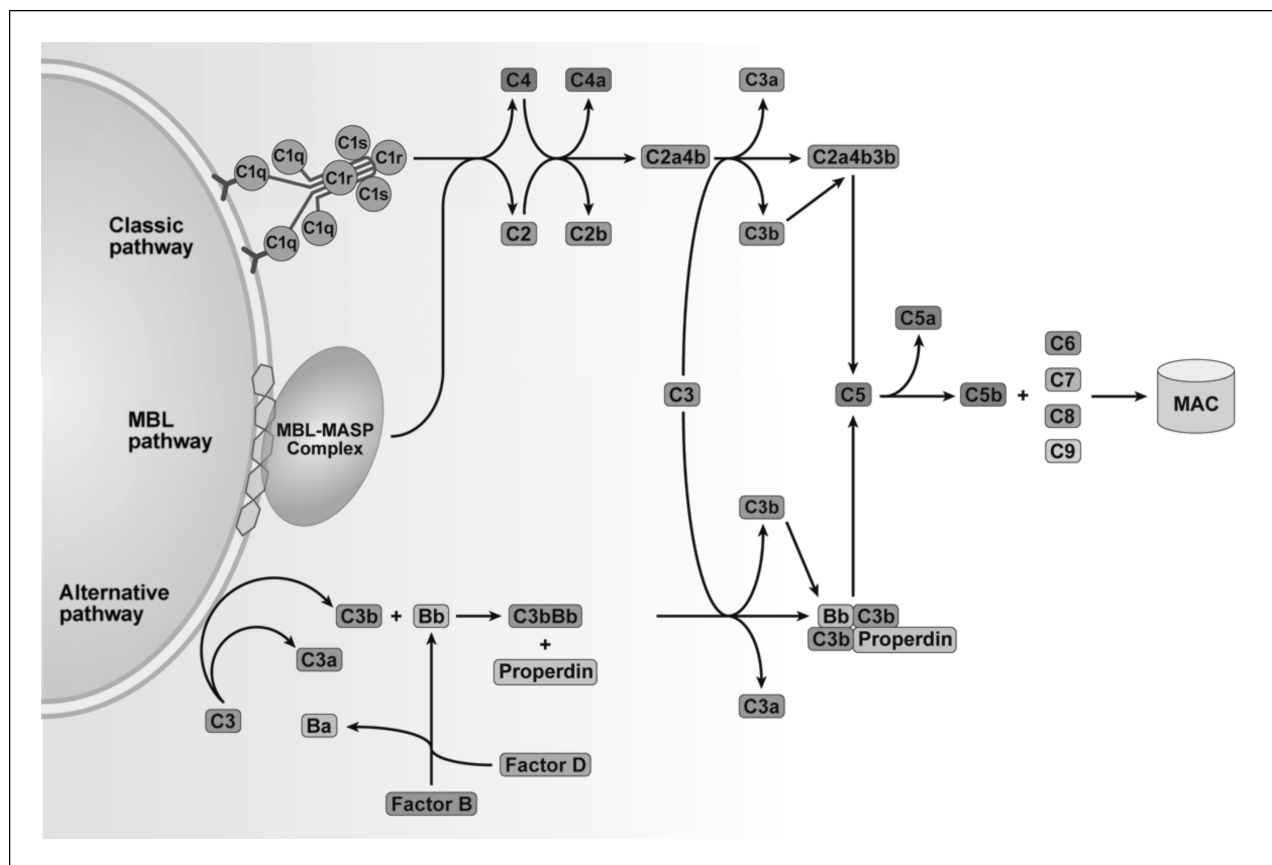


FIGURE 1. The complement cascade. The complement cascade comprises the classic, alternative, and MBL pathways. The classic pathway is activated by the Fc portion of immunoglobulins bound to antigen, apoptotic cells, Gram-negative bacteria, and viruses. The C1 complex, made up of C1q, C1r, and C1s subunits, initiates the downstream classic cascade. Upon binding of C1q to an inciting stimulus, C1r catalyzes breakage of a C1s ester bond, resulting in its activation and subsequent cleavage of C2 and C4 into their respective "a" and "b" fragments. The formation of C2a4b creates C3 convertase, which cleaves C3 into C3a and C3b. C3b similar to other C3 convertases, with subsequent production of C2a4b3b, also known as C5 convertase. It facilitates the final steps of the cascade by splitting C5 into C5a and C5b. The latter fragment is the critical first protein that combines with C6, C7, C8, and multiple C9 proteins to form the MAC, the terminal, pore-forming complement protein complex responsible for lysis of cells and pathogens. The MBL pathway is activated by surfaces bearing mannose groups or other pathogen-associated molecular patterns. MBL or ficolin activation of mannose-associated serine proteases (MASP) results in cleavage of C2 and C4 similar to the C1 complex, with subsequent production of C3 convertase and complement cascade activation resembling the classic pathway. Lastly, the alternative pathway is activated by a multitude of infectious agents including various bacteria, viruses, and fungi, as well as neoplastic cells. This pathway exhibits a unique "tickover" effect whereby low-level C3 cleavage occurs continuously. Generated C3b binds Bb, a cleavage fragment of factor B, and properdin, resulting in the formation of the alternative pathway C3 convertase. Binding of additional C3b to the alternative pathway C3 convertase renders it capable of C5 cleavage, and forms the basis for the amplification loop of the alternative pathway. Additionally, C3b generated by alternative pathway C3 convertase can attach to target surfaces and bind Bb, forming a C3 convertase that amplifies downstream complement proteins locally at the target surface. Although the activation and amplification of the three pathways differ initially, they commonly cleave C3 into C3a and C3b, resulting in terminal formation of the MAC.

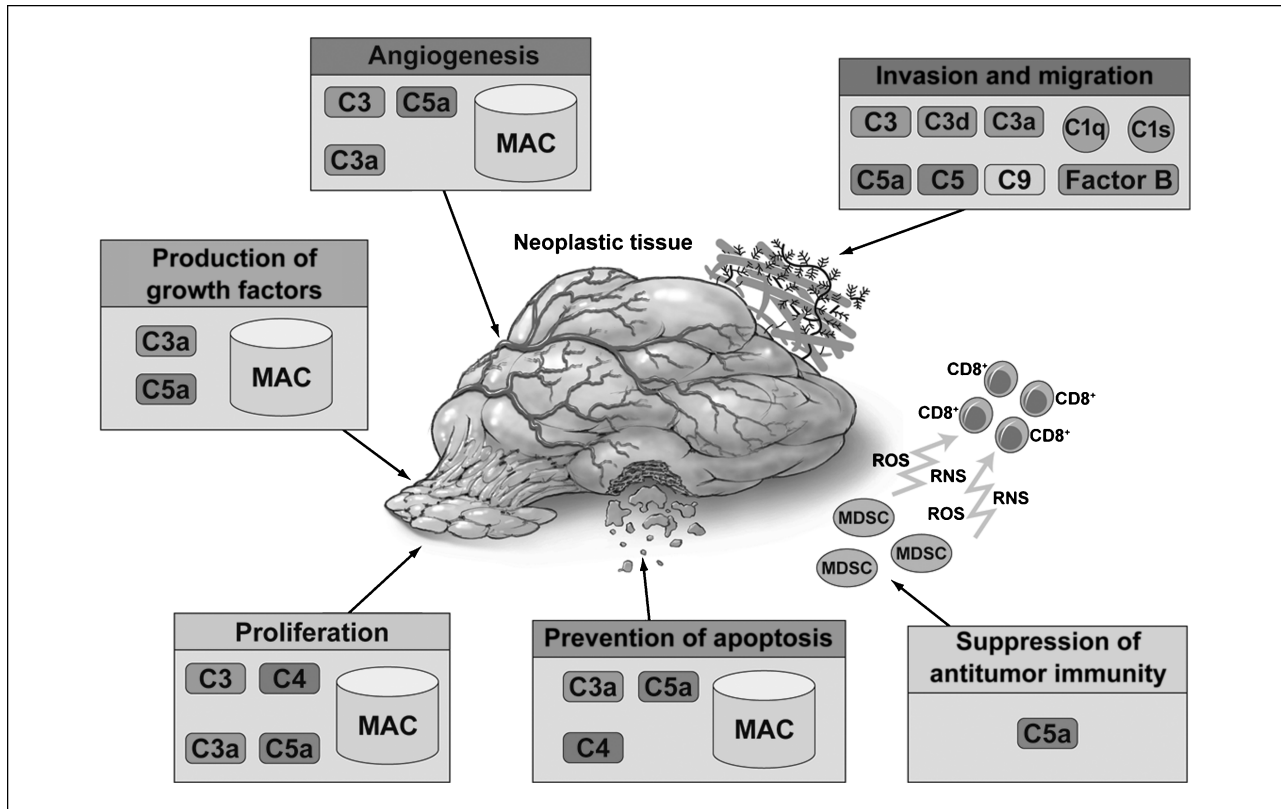


FIGURE 2. Potential oncogenic roles for complement proteins. Complement proteins contribute to each major hallmark of cancer, including mitogenic signaling cascades and growth factor production, angiogenesis, protection from antigrowth signals and apoptosis, cellular invasion and migration through the extracellular matrix, and suppression of antitumor immunity.

A newly discovered receptor for C5a, the C5L2 receptor, remains poorly understood. Although it does not seem to be G-protein coupled, it has a role in enhancing the downstream effects of C3a and C5a. In neutrophils, macrophages, and fibroblasts, C5L2 modulates downstream mitogenic signaling through ERK 1/2, c-Jun amino-terminal kinase (JNK), MAPK p38, and β -arrestin pathways (34, 35).

Both immunologic and explicitly nonimmunologic cells respond to C3a and C5a with activation of mitogenic pathways. Endothelial cells constitutively express C3aR and activate ERK1/2 following C3a/C3aR binding (27, 28). Leukocytes show sustained ERK1/2 and Akt pathway activation in response to C3a (30), whereas macrophages activate MAPK p38 pathways in response to C5a (29). C5a causes potent activation of ERK1/2, Akt, and JNK in monocytes (36), neutrophils (37), and neurons (38), and induces transactivation of epidermal growth factor receptor in endothelial cells, enhancing their migration (27). This finding is especially interesting in light of the mitogenic properties of epidermal growth factor receptor activation (39). Finally, C5a/C5aR binding is known to induce cyclin E and D1 mRNA levels (40), phospholipase C β 2 (41), phospholipase D (42), and Raf-1/B-Raf-mediated activation of MAPK/ERK kinase-1 (43), all demonstrable mediators of oncogenic transformation and progression.

Intriguingly, two studies have shown direct complement-mediated neoplastic proliferation. In the search for evidence of C5aR in neurons, researchers discovered that C5a promotes proliferation of undifferentiated human neuroblastoma cells (44). The proliferative effects of C5a/C5aR binding seem to be partially mediated by protein kinase C and NF- κ B activation (44). Complement-mediated tumor proliferation has also been observed in the TC-1 syngeneic model of murine cervical cancer (11). Investigators injected mice with subcutaneous TC-1 tumor cells and discovered that mice deficient in C3 or C4 show significantly decreased tumor proliferation compared with wild-type C3 and C4-sufficient mice (11). Wild-type mice exhibit widespread deposition of C3 split products across tumor vasculature without a concomitant increase in plasma C3 split products, suggesting a local and tumor-specific proliferative effect for C3.

Interestingly, tumor growth was no different in factor B-deficient mice compared with wild-type mice (11), indicating the importance of complement protein amplification outside of the alternative pathway. Factor B is a critical component of the alternative pathway amplification loop. Spontaneously activated C3, designated C3(H₂O) due to hydrolysis of a thioester bond, resembles C3b and binds factor B. Factor D then cleaves factor B, creating the C3(H₂O)Bb C3 convertase of the alternative pathway that is

responsible for C3b production. C3b has diverse effects that include opsonization of target surfaces and promotion of the alternative pathway amplification loop. In this loop, C3b generated by the alternative pathway C3 convertase or

through the classic or mannose-binding lectin (MBL) pathways binds factor B to form the C3bBb convertase, propagating further C3b production and self-amplification (45, 46). Because factor B is central to this amplification

Table 1. The specific contributions of various complement proteins to neoplastic phenomena

Complement protein	Tumorigenic effect	Tumorigenic hallmark
C1q	Extracellular matrix anchoring	Invasion and migration
C1s	Extracellular matrix disintegration	Invasion and migration
C3	Activation of MMP-9	Invasion and migration
	Production of VEGF	Angiogenesis
C3a	Extracellular matrix reorganization	Invasion and migration
	Extracellular matrix disintegration	Invasion and migration
	Activation of ERK1/2, Akt	Mitogenesis
	Induction of IL-6	Growth factor/cytokine production
C3d	Production of VEGF	Angiogenesis
	Extracellular matrix reorganization	Invasion and migration
	Cellular chemotaxis	Invasion and migration
Factor B	Extracellular matrix anchoring	Invasion and migration
C5	Extracellular matrix disintegration	Invasion and migration
C5a	Induction of TGF- β , IGFs, IGFBPs	Growth factor/cytokine production
	Extracellular matrix disintegration	Invasion and migration
	Activation of ERK1/2, Akt, JNK, p38 MAPK, phospholipase C β -2, phospholipase D, MEK-1, PKC, NF- κ B	Mitogenesis
	Stimulation of HGF, c-MET receptor	Growth factor/cytokine production
	Transactivation of EGFR	Mitogenesis, cellular migration
	Induction of cyclin E, D1	Mitogenesis
	Induction of IL-6	Growth factor/cytokine production
	Inhibition of caspase-3	Prevention of apoptosis
	Production of VEGF	Angiogenesis
	Cellular chemotaxis	Invasion and migration
	Inhibition of CD8 ⁺ T cells	Antitumor Immunosuppression
	MDSC migration and production of ROS/RNS	Antitumor immunosuppression
	C9	Extracellular matrix disintegration
MAC	Induction of CDK 2 and 4, p21, RGC-32	Mitogenesis
	Activation of ERK, p38 MAPK, JNK, PI3K, Ras, p70 S6 kinase, JAK-STAT	Mitogenesis
	Activation of c-jun, junD, c-fos	Mitogenesis
	Induction of bFGF, PDGF, TGF- β	Growth factor/cytokine production, angiogenesis
	Inhibition of caspase-3, caspase-8, BAD, BID, TNF- α , FasL	Prevention of apoptosis
	Induction of bcl-2, IGF-I	Prevention of apoptosis
	Production of VEGF	Angiogenesis

Abbreviations: BAD, bcl-xL/bcl-2-associated death promoter; Bcl-2, B-cell lymphoma-2; bFGF, basic fibroblast growth factor; BID, bcl-2 interacting domain; CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FasL, Fas ligand; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; IL-6, interleukin-6; JAK-STAT, Janus activated kinase-signal transducer and activated transcription; JNK, c-jun amino terminal kinase; MAPK, mitogen-activated protein kinase; MDSC, myeloid-derived suppressor cells; MEK-1, mitogen-activated protein kinase or ERK kinase-1; METr, mesenchymal-epithelial transition receptor; MMP-9, matrix metalloproteinase 9; PI3K, phosphatidylinositol 3-kinase; PDGF, platelet-derived growth factor; PKC, protein kinase C; RGC, response gene to complement; ROS/RNS, reactive oxygen species/reactive nitrogen species; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor- α ; VEGF, vascular endothelial growth factor.

loop, the classic pathway is likely responsible for the production of C3, its split products, and the downstream proliferative effects observed in mice injected with TC-1 tumor cells (11). Importantly, classic pathway cleavage of C3 is preceded by C4 cleavage, making it difficult to ascertain whether the protumorigenic effects of C4 are specific to this protein or due to its downstream effects on C3. Nevertheless, these results offer remarkable evidence that complement can mediate cancer growth.

The membrane attack complex activates the cell cycle and oncogenic pathways

In the classic view of complement's role in innate immunity, formation of the membrane attack complex (MAC) represents the common terminal event in the activation and amplification of each complement pathway. Composed of C5b, C6, C7, C8, and multiple C9 molecules, the MAC forms structural pores within cell membranes, allowing for osmotic fluid shifts that rupture metabolically inactive cells such as erythrocytes. In metabolically active cells, shifts in calcium are critical to MAC-mediated destruction (47), as this cation influx disrupts mitochondrial transmembrane potential and ATP production, resulting in ensuing cell death (48, 49).

Lytic efficiency is driven in part by the number of MACs present within a cell membrane and the ability of a cellular target to defend and repair itself. Many cells use a defensive armamentarium against MAC-mediated destruction that includes ion pumps to reverse solute shifts, endocytosis and exocytosis, proteases and kinases that inactivate the MAC, and membrane-bound inhibitors such as CD59 (50). In these settings, the so-called sublytic doses of the MAC induce dramatically different effects, including cell cycle activation and proliferation (51, 52).

The observation that insertion of the MAC into the cell membrane causes ion shifts mimicking those in signal transduction pathways led to one of the first studies on the phenomenon of MAC-induced cellular proliferation (53). The authors studied Swiss 3T3 cells, an immortalized fibroblast cell line. MAC insertion into their membranes was found to increase DNA synthesis and cell proliferation in a dose-dependent manner (53). MAC-induced increases in cytosolic calcium activate proteins such as protein kinase C, diacylglycerol, and ceramide, important mediators of the cell cycle through mitogenic signaling cascades (54). The MAC can also directly stimulate the cell cycle. In one study, MAC insertion into the membrane of smooth muscle cells caused activation of cyclin-dependent kinases 2 and 4 and increased mRNA and protein levels of p21, a molecule known to regulate G₁ progression (55). This resulted in transition from the G₁ to S phase, with consequent increases in cell proliferation (55). Similar MAC-driven proliferation occurs in endothelial cells (56) and Schwann cells (57).

Response gene to complement (RGC)-32 also seems to act as a mediator of MAC-induced cell cycle progression. In response to the MAC, RGC-32 protein shows complex formation with cyclin B1/CDC2, stimulating the cell cycle in rat oligodendrocytes (58) and human aortic smooth mus-

cle cells (59). Upregulation of RGC-32 mRNA and protein is an especially noteworthy effect given the widespread presence of RGC-32 in cancer. Investigators have shown that RGC-2 mRNA expression is 2-fold higher in cancers of the kidney, colon, stomach, rectum, ovary, and small intestine compared with their normal tissues (60). RGC-32 protein was present in more than 80% of the tumors they stained, including those of the gastrointestinal tract, lung, liver, breast, ovaries, and brain (60). Given its putative role in stimulation of the cell cycle and proliferation, the finding of upregulated RGC-32 in malignant tissues is not necessarily unexpected. However, until the association between RGC-32 and malignant tissue is explored further, a causal role for RGC-32 in neoplasia remains speculation.

Potentially oncogenic signal transduction mechanisms also play a large role in the transmission of downstream cellular effects by the MAC. Each of the MAPK family of proteins, including ERKs, p38 MAPKs, and JNKs, has a demonstrable importance in the pathogenesis of cancer, and each is significantly activated by the MAC with resulting cellular proliferation (51, 54, 55, 61, 62). Other oncogenic targets activated by the MAC include the phosphoinositide 3-kinase pathway (55), Ras (63), p70 S6 kinase (51), and the Janus-activated kinase-signal transducer and activated transcription pathway (56). These pathways have powerful effects on transcription of proto-oncogenes (51), providing another mechanism by which the MAC can influence cell cycle progression and proliferation. As an example, exposing quiescent oligodendrocytes to the MAC facilitated membrane insertion and consequent activation of the *c-jun*, *junD*, and *c-fos* proto-oncogenes, causing these cells to enter the S phase (61). Similar transcriptional activation of *c-fos* due to MAC stimulation occurred in myotubes (64).

Complement Sustains Tumorigenesis

Complement proteins upregulate oncogenic growth factors and cytokines

A fundamental characteristic distinguishing neoplastic cells from their normal counterparts is their differential ability to respond to growth factors and cytokines. Although normal cell survival depends heavily on exogenous growth factor stimulation and intercellular interaction with their neighboring cells, cancer cells possess two critical mechanisms of autonomy that free them from similar dependence. First, they develop in the absence of growth signals from other cells, and second, they are unresponsive to the array of antimitogenic signals that keep normal cells in check (65). In fact, many cancers such as gliomas possess the ability to create their own growth factors, resulting in continuous self-stimulation (65). The network of growth factors that support neoplastic transformation and growth is extremely complex, and numerous complement proteins have been shown to stimulate their production.

Research on complement-mediated liver regeneration has proven especially illustrative of this idea. In rats undergoing partial hepatectomy, C5a stimulation of injured

liver cells results in increased mRNA expression of hepatocyte growth factor and its corresponding c-MET receptor (40). Both are known to possess potent mitogenic and anti-apoptotic effects in liver regeneration (66, 67), and dysregulation of this pathway is strongly exhibited by many cancers (68). Additionally, C3a and C5a regulation of the cytokine milieu critical for liver cell regeneration and survival following injury (31) further indicates a potential role for complement in neoplasia. Investigators identified lowered levels of tumor necrosis factor- α (TNF- α) and IL-6 in C3-/- mice after partial hepatectomy, consequently interfering with liver regeneration and hepatoprotection (31). C3a and C5a induction of IL-6-mediated downstream control over the Janus-activated kinase-signal transducer and activated transcription, phosphatidylinositol 3-kinase/Akt, and mammalian target of rapamycin proliferative pathways (31). IL-6 is known to induce transcriptional changes implicated in cell cycle progression and prevention of apoptosis (3, 69). Astrocytes exhibit a similar phenomenon in response to complement. Binding of C3a and C5a to their receptors has been shown to increase IL-6 mRNA expression (70).

Further investigation into complement-mediated growth factor production has shown that endothelial cells release basic fibroblast growth factor and platelet-derived growth factor in response to MAC stimulation (71). These two mitogens are known to stimulate the cell cycle (51) and angiogenesis (72). Other evidence indicates that complement proteins induce production of transforming growth factor- β (TGF- β ; refs. 73-76), an especially compelling finding given its complex relationship to neoplastic progression (77). TGF- β has the potential to both inhibit and enhance neoplasia (74, 75, 77), suggesting that its effects may depend on more specific tumor characteristics such as stage and type (3). Notably, certain cancers secrete TGF- β (78, 79) and likely respond to it in an autocrine fashion, facilitating many of its neoplastic effects (77). These include angiogenesis, invasion and metastasis, suppression of anti-tumor CD8⁺ T-cells (77), and epithelial-to-mesenchymal transition (EMT) in numerous tumor types (77). Intriguingly, one of the downstream targets of TGF- β seems to be RGC-32, which has also been shown to regulate EMT (80, 81). Not only has RGC-32 been implicated in control of the cell cycle and cancer (58-60) but it also shows upstream control by complement proteins, similar to TGF- β . Dual control of the complement system over RGC-32 through TGF- β modulation and the MAC indicates a potentially powerful and synergistic impetus for tumorigenesis and development, although this remains conjecture as the relationships between complement proteins, TGF- β , and RGC-32 have not been explicitly studied.

Complement Prevents Cell Death

Complement proteins exert prosurvival and antiapoptotic effects

The insensitivity of neoplastic cells to exogenous growth signaling allows them to escape from apoptotic stimuli. This

prosurvival capability stands in stark contrast to the homeostatic growth control maintained in normal cells, almost all of which contain the ability to activate programmed cell death in response to appropriate signals (65).

C5a mediates diverse prosurvival and antiapoptotic functions in a variety of cells. Animal models of central nervous system pathology have especially highlighted its neuroprotective, antiapoptotic abilities. One study examining C5-deficient mice found greater levels of neurodegeneration among astrocytes and neurons subjected to kainic acid injury compared with C5-sufficient mice, suggesting its importance in both neural and glial response to injury (82). Co-infusion of C5a with kainic acid mitigated the extent of injury by significantly decreasing the number of apoptotic neurons (83). C5a likely decreases apoptosis through ERK1/2 inhibition (38) of caspase-3 (83) and downregulation of glutamate receptor subunit 2-mediated apoptosis (84).

C5 exhibits additional antiapoptotic functions in experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis. C5-sufficient mice subjected to experimental autoimmune encephalomyelitis had far fewer apoptotic cells during recovery compared with C5-deficient mice (85), raising the possibility that the chronic regenerative effects of C5 outweigh its acute proinflammatory damages. C5 seems to partially mediate its effects through gene regulation of insulin-like growth factors (IGF), their binding proteins (IGFBP), and TGF- β 3 (75). In particular, IGFs and IGFBPs are known to facilitate myelination by enhancing oligodendrocyte progenitor cell survival and differentiation and by inhibiting mature oligodendrocyte death (75, 86, 87).

Although the evidence is more limited, C3a also seems to hold some neuroprotective, antiapoptotic properties. *In vitro* experiments have shown that purified human C3a protects neurons against *N*-methyl-D-aspartate induced injury in a dose-dependent manner (88).

The MAC also uses a large repertoire of prosurvival and antiapoptotic mechanisms. Apoptosis of both Schwann cells (89) and oligodendrocytes (90) is inhibited by the MAC. Sublytic doses inhibit numerous proapoptotic proteins, including caspase-3 and caspase-8 (91), BAD and BID (92), and TNF- α and FasL (93), while they provide protection through increased synthesis of antiapoptotic bcl-2 (92) and IGF-I (94). Interestingly, IGF-I is released by *in vitro* smooth muscle cells following experimental exposure to the MAC, enabling protection from apoptosis in an autocrine manner (94).

Complement Promotes Angiogenesis

Complement proteins induce and sustain angiogenesis

Decades of cancer research have firmly established the principle that progression from a small population of neoplastic cells to a clinically significant mass requires creation of new vessels to perfuse the newly formed malignant tissue. Furthermore, the growth rate and aggressiveness of some tumors can be predicted by the density of tumor vessels,

suggesting a connection between increased angiogenesis and tumor aggressiveness (95). Notably, complement proteins are direct and indirect participants in angiogenesis.

Identification of the murine stem cell antigen AA4 has hinted at the potential for complement-mediated angiogenesis. AA4 is believed to be homologous to the human receptor C1qRP, a C1q receptor expressed in myeloid progenitor cells, endothelial cells, and platelets (96). AA4 is a transmembrane protein present throughout fetal and adult murine cells of the hematopoietic and cardiovascular systems (97). Between fetal days 9 to 14, it is particularly expressed in preexisting vessels and in endothelial cells during vascular remodeling, suggesting roles in angiogenesis and cell adhesion (96). Furthermore, its expression occurs concomitant to capillary formation in early organogenesis (96). AA4 also shows expression in hematopoietic cells associated with the fetal aorta and liver, whereas adult mice show abundant expression in the lungs, heart, and bone marrow (96). Because it shares 68% homology with mouse AA4, human C1aRP has been purported to play a similar developmental role, including angiogenic properties and involvement in endothelial cell migration and intercellular adhesion (96).

The potential role for complement activation in angiogenesis has also been shown in age-related macular degeneration (AMD; ref. 98). The pathogenesis of AMD involves a process known as choroidal neovascularization whereby inappropriate angiogenesis in the choroid causes vascular invasion into the adjacent retina. Lipoproteinaceous deposits known as drusen concurrently arise between the choroid and retinal pigmented epithelium, and offer one of the earliest markers of AMD (98). The anaphylatoxins C3a and C5a are known components of drusen and seem to be confined to pathologic tissues of eyes with AMD (98). C3a and C5a stimulate the secretion of vascular endothelial growth factor (VEGF) in adjacent retinal pigmented epithelium and choroid cells in a dose-dependent manner (98). Choroidal neovascularization and VEGF levels are decreased in C3aR^{-/-} and C5aR^{-/-} mice, wild-type mice treated with C3aR and C5aR antagonists, and wild-type mice treated with neutralizing antibodies against C3a and C5a (98). Additionally, C3 and the MAC are deposited in laser-induced choroidal neovascularization, causing subsequent increases in VEGF and other angiogenic growth factors, including TGF- β 2 and β -fibroblast growth factor (74), further supporting a role for complement proteins in angiogenesis.

Complement Promotes Cellular Invasion and Migration

Complement proteins reorganize intercellular and extracellular matrix connections

Following the establishment of a neoplastic colony of cells, cancers have a remarkable ability to reorganize their surrounding microenvironment in a manner that facilitates invasion through the extracellular matrix and into distant tissues. The migration and establishment of

neoplastic colonies distant from their origin, the process of metastasis, is particularly pernicious due to the overwhelming morbidity and mortality it unleashes on the cancer host (65, 99). It has been estimated that ~90% of cancer deaths arise from metastatic disease (100). Research has revealed that complement proteins stimulate both invasion and migration, two highly intertwined mechanisms that involve reorganization of intercellular connections and disintegration of the extracellular matrix (65). As major participants in the inflammatory milieu surrounding neoplastic tissue (101-107), activated complement proteins are abundantly dispersed throughout the extracellular matrix surrounding tumors.

Broadly speaking, the intercellular connections reorganized during neoplastic invasion involve the cadherin family of cell-cell adhesion molecules (65). As an omnipresent member of epithelial intercellular junctions, E-cadherin is a well-studied member of the cell-cell adhesion molecule family that seems to be disrupted in most cancers arising from the epithelium (108). Beyond its structural role, E-cadherin also has a role in the transmission of extracellular stimuli to the intracellular environment. E-cadherins create a widespread signaling network capable of relaying antiproliferative messages, thus serving an important tumor-suppressor role (100). Tubulointerstitial damage in proteinuric nephropathy provides evidence of complement-mediated alterations in E-cadherin; more specifically, researchers discovered that C3a/C3aR binding partially induces EMT through decreased expression of E-cadherin protein (109). Indirect evidence of complement-directed loss of E-cadherin function is provided by evidence that C5 induces IGF expression (75). IGFs have shown the ability to downregulate E-cadherins, leading to pancreatic cancer invasion and metastasis (110) and EMT (111). The downregulation of E-cadherin accompanying EMT may provide a useful paragon for the intercellular changes that facilitate cancer invasion.

Cellular connections to the surrounding matrix are primarily mediated by the large family of integrin proteins (108). Similar to E-cadherins, integrins facilitate extracellular transmission of antimetastatic, anti-invasive, and antimigratory signals (100). In a fascinating early study of complement-mediated cellular proliferation, complement proteins C3 and C5 were discovered to mediate limb and lens regeneration in amphibians (112, 113), processes that heavily depend on reorganization of the extracellular matrix surrounding injured tissues. C3 primarily localizes to the blastema, a stem-cell like structure that forms from injured tissues that subsequently dedifferentiate through a combination of changes involving cellular adhesion and integrin regulation (114). The authors speculate that the homology of C2 and factor B to certain proteins of the extracellular matrix indicates additional mechanisms of control over cellular adhesion and communication (112). Of note, both complement receptors 3 and 4 belong to the β_2 integrin subgroup (115), further suggesting that complement proteins such as iC3b may modulate their functions and downstream effects.

Conversely, the extracellular matrix itself can regulate complement activation and amplification, with occasionally detrimental effects when this balance is lost. In inflammatory states that involve tissue damage and extracellular matrix exposure to activated complement proteins, these interactions offer a potent source for continuous inflammation and disease progression. Diseases including Alzheimer's, AMD, systemic lupus erythematosus, and inflammatory arthritides can be partially attributed to dysregulation of complement proteins by ECM components such as pentraxins, small leucine-rich repeat proteins, fibronectin, and laminin (116). The small leucine-rich repeat protein fibromodulin, a component of cartilage, can activate both the classic and alternative pathways, resulting in the production of C3b and C4b; furthermore, it can directly bind to C1q and promote inflammation due to downstream production of activated complement proteins such as C5a (117). Osteoadherin similarly activates complement through C1q (118). Histidine-rich glycoprotein binds to C1q, C3, C4, C8, factor H, C4 binding protein, and MBL, although the consequences of these interactions remain unclear as histidine-rich glycoprotein neither activates nor inhibits complement protein functionality (119).

Extracellular matrix components also possess the ability to dampen complement cascade amplification by binding to inhibitors such as factor H and C4 binding protein that attenuate inflammation (118, 120). The proteoglycans decorin and biglycan can inhibit the classic pathway directly through binding and inhibition of C1q, whereas biglycan inhibits the MBL pathway by inactivating MBL (121). Decorin and biglycan show further inhibition of complement by preventing C1q binding to human umbilical vein endothelial cells and U937 cells, with biglycan further able to inhibit C1q-mediated release of inflammatory monocyte chemoattractant peptide-1 and IL-8 from human umbilical vein endothelial cells (121).

Complement proteins facilitate invasion and migration

Disintegration of the extracellular matrix is the next key step for neoplastic cells to invade and migrate to distant tissue sites (65). The proteases responsible for this process most often arise from the surrounding stroma and reactive inflammatory cells, demonstrating the ability of neoplastic cells to subvert the normal physiology of their microenvironment toward their own invasive aims (122).

The ability of complement proteins to degrade the extracellular matrix has been recognized for some time. Factor B, C3, C5, and C9 are associated with endochondral bone formation and cartilage degradation (123). C1s can degrade collagens and gelatin present in human cartilage (124) and can activate matrix metalloproteinase-9, a protease with established roles in the metastasis of cancers (125-127). Interestingly, matrix metalloproteinase-9 mediates other oncogenic mechanisms, including cellular growth (125), angiogenesis (128), and induction of myeloid-derived suppressor cells (MDSC; ref. 129), cells believed to interfere with antitumor immunity.

C3a and C5a are known for their ability to effect inflammatory states, in part through the promotion of leukocyte chemotaxis. Because mesenchymal stem cells also migrate to sites of injury as part of the tissue regenerative response, one group of researchers investigated the ability of C3a and C5a to induce similar chemotaxis in mesenchymal stem cells. C3a and C5a promote migration of mesenchymal stem cells, inducing downstream signaling cascades including ERK1/2 and Akt upon binding their receptors (130), results that parallel the finding that C3a exhibits chemotactic influence over hematopoietic progenitor cells (131). In this latter population of stem cells, C3a provides a critical migratory signal leading to their engraftment into bone marrow (131). C3a also provides a chemotactic stimulus for the migration of neural stem cells in several models of murine neurogenesis (132, 133).

These results raise the intriguing possibility that the manifold consequences of complement-stromal interactions include alteration of the tumor microenvironment into a prometastatic niche. Complement proteins enhance EMT, disrupt the ECM through proteases such as MMP-9, provide chemotactic stimuli, and induce production of growth factors including hepatocyte growth factor-1 and TGF- β , complex events that can prime and encourage tumor invasion and migration (134, 135).

Neoplastic cells also possess the ability to co-opt stromal cells such as fibroblasts into contributing to carcinogenesis. These tumor-associated fibroblasts and myofibroblasts have unique genotypic and phenotypic profiles that further promote tumorigenesis, proliferation, invasion, and angiogenesis (136-138). Both fibroblast and myofibroblast cells respond to complement through specific (139-142) and nonspecific receptors (143), raising the possibility that complement proteins present in the tumor microenvironment may similarly activate their tumorigenic functions.

Complement and Immunosurveillance

Complement proteins play a dual role in the tumor microenvironment

Complement split products such as C1q, C3, C3a, C4, C5, and the MAC are prominent features of the inflammatory tumor microenvironment (101-107). Classically, the assumption has been that these activated complement proteins play a role in tumor defense directly through complement-dependent cytotoxicity (144) and indirectly through antibody-dependent cell-mediated cytotoxicity (145). Unfortunately, neoplastic cells are known to express a wide variety of defenses against complement-mediated attack. Membrane-bound regulatory proteins and soluble complement inhibitors, including CD21, CD35, CD46, CD55, CD59, and factor H, hinder complement cytotoxicity (146-148). The antagonistic interaction between complement and tumor cells, in which tumor escape is partly facilitated by neutralization of complement attack, underscores the opposing roles of complement in carcinogenesis.

Evidence suggests that enhancement of the lytic properties of complement proteins such as the MAC is an effective cancer therapy (145, 149). At the same time, complement proteins such as C3a have shown anti-inflammatory properties that might preclude the further amplification of complement activation products necessary for complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity. In a murine model of sepsis, C3aR^{-/-} mice showed greater mortality in response to lipopolysaccharide shock when compared with wild-type mice, with concomitant increases in the proinflammatory cytokine IL-1 β (150). Furthermore, C3a/GFAP mice, which express biologically active C3a exclusively in the central nervous system, were more resistant to lipopolysaccharide shock than their wild-type and C3aR^{-/-} counterparts (151). The anti-inflammatory effects of C3a may arise from downregulation of inflammatory cytokines such as IL-1 β , IL-6, and TNF- α (152, 153).

The degree of inflammation and complement activation surrounding a tumor may also have an effect on the migratory properties of tumor cells. ME180 cervical cancer cells subjected to shRNA knockout of decay-accelerating factor and membrane cofactor protein, inhibitors of C3 convertases and C3b and C4b, showed significantly decreased migration compared with those expressing decay-accelerating factor and membrane cofactor protein (154). Although these results associate uninhibited complement amplification with increased migration of ME180 cervical cancer cells, a direct influence of these complement proteins on tumor chemotaxis remains unverified. In combination with data demonstrating that excessive C5a disrupts neutrophil chemotaxis (155), it would seem that complement-mediated cellular migration is highly dependent on a variety of local immunologic cues.

Finally, angiogenesis represents another phenomenon dually influenced by complement: In contrast to complement-mediated promotion of angiogenesis in AMD, proteins such as C3 and C5 seem to have antiangiogenic functions as well. Studies of retinopathy of prematurity and placental dysfunction have shown that complement proteins can inhibit angiogenesis (156, 157). For example, C3^{-/-} and C5aR^{-/-} mice show increased pathologic retinal angiogenesis, an effect recapitulated in mice undergoing C5 antibody blockade or C5aR antagonism. Macrophages seem to mediate the effect through increased expression of antiangiogenic signaling molecules such as IL-6 and TNF- α , decreased IL-10, and increased monocyte/macrophage secretion of sVEGFR1, a soluble VEGF inhibitor (157). In studies of placental dysfunction, C5a similarly causes sVEGFR1 production from monocytes, resulting in decreased VEGF and consequent recurrent miscarriage and intrauterine growth restriction (156).

Complement proteins promote immunosuppression and cancer growth

Given the complicated role of complement in the tumor microenvironment, the ability of tumor cells to neu-

tralize activated complement proteins raises questions regarding their putative role in cancer immunosurveillance. If cancer activates complement but is defended against its immune attack, is complement doing something entirely different?

The recent work of Markiewski and colleagues shows a complicated immunologic role for complement proteins and their contribution to tumor growth. Using the murine TC-1 syngeneic model of cervical cancer, the authors discovered that mice deficient in C5aR and wild-type mice exposed to pharmacologic blockade of C5aR showed decreased tumor growth (11). Tumors showed enhanced infiltration of cytotoxic CD8⁺ T cells, resulting in an inverse relationship of tumor growth to the number of cytotoxic CD8⁺ T cells. This augmented antitumor response was further associated with inhibition of MDSC recruitment into tumor tissue (11). MDSCs are a population of intermediately differentiated myeloid cells known to suppress cancer immunosurveillance and consequently potentiate neoplastic proliferation (144). MDSCs accumulate in the blood, lymph nodes, and tumor tissue of patients with cancer, where they can directly interfere with antitumor immune responses (144). Notably, MDSCs express high levels of membrane C5aR. C5a/C5aR binding on MDSCs promotes their migration and augments their production of immunosuppressive molecules such as reactive oxygen species and reactive nitrogen species, consequently decreasing cytotoxic CD8⁺ T cells (11). Intriguingly, depletion of CD8⁺ T cells in C5aR^{-/-} mice leads to restoration of the wild-type phenotype, suggesting that the proneoplastic effects of C5a are specifically mediated through CD8⁺ T-cell suppression.

Conclusion

The assumption that the complement system facilitates innate immune attack against cancer cells through cytotoxic and lytic effects may be in need of revision. Exciting research is revealing that the complement cascade enables a remarkable array of proliferative events. We have highlighted the body of evidence demonstrating diverse means by which complement proteins may facilitate the major features of carcinogenesis, including dysregulation of mitogenic signaling pathways, sustained cellular proliferation, angiogenesis, insensitivity to apoptosis, invasion and metastasis, and escape from immunosurveillance.

At present, a number of studies are exploring the potential role for complement-mediated therapeutics in the fight against cancer. There is particular interest in the use of monoclonal antibodies against the soluble and membrane-bound complement inhibitors displayed by various tumors (2), a strategy believed to enhance antitumor complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity. Despite some promising early results (158-160), we believe that such strategies ignore the possibility that the complement system promotes neoplastic development and progression rather than exclusively retarding it. Ultimately, the increasingly

complex picture of complement interaction with cancer demands further study, but does offer the intriguing possibility that anticomplement strategies may offer an entirely new means of fighting cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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