

Review

The Role of Complement in the Immunologic Microenvironment of Tumor Cells: Potential Therapeutic Targets

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The complement system comprises a set of essential molecules that bridge the innate and adaptive immune responses. Research has focused on how the complement system's destructive mechanism could potentially be harnessed for cancer treatment. However, cancer subverts the complement system to avoid immunosurveillance. In addition, a complement-triggered biological mechanism that contributes to cancer growth has been identified. Thus, drugs should be designed to homeostatically maintain a normal concentration of complement. This review explores three types of complement-related anti-cancer drugs: therapeutic antibodies, complement inhibitory drugs, and anti-complement regulatory drugs.

Keywords: Complement, immune system, tumour cells, immunosurveillance, inflammation, therapeutics

Introduction

Cancer is among the major causes of morbidity and mortality, causing approximately 8.2 million of deaths worldwide in 2012 [11]. Despite great advances in tumour therapies, more than half of all cancer patients do not recover after treatment. Moreover, some types of cancer therapy such as chemotherapy have severe side effects. Therefore, more personalised, highly tumour specific and less toxic therapies are the clinical goal. The complement system is a relatively novel target that is favoured due to its effectiveness.

The complement system is a main part of the innate immune system, so called because it is a first line of defence against microbes. Recent experiments suggest that cancer-induced high levels of complement augment

inflammatory and immunological responses to tumours. Such data extend the role of complement further than simple elimination of foreign intruders. Recent research also suggests a link between complement activated chronic inflammation and tumour growth [6]. Furthermore, scientists have discovered defence mechanisms that neoplastic cells use against complement attack [40]. Complement may kill cancer cells directly, and may also inhibit the invasiveness of tumour cells, yet the mechanisms involved cannot be fully explained due to the complexity of the interactions involved.

Cancer research has traditionally targeted tumour cells themselves, addressing less the tumour microenvironment that links to the initiation, development and progression of tumours. Only recently has intense research investigated the molecular events associated with the tumour microenvironment, and components of the immune system have become accepted as novel therapeutic targets [9]. In the past few decades, research on immunotherapy has proposed the interesting theory

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that the balance of complement levels is more important than overexpression of tumour-suppressive complement effectors. This theory has proposed that both inhibition and expression of complement may be effective anti-cancer therapies and that a specific mechanism is required for balancing the complement cascade to maximize the anti-tumour immune response. So far, cancer therapies have been developed to direct immune modulation but with only modest success. A better comprehension of the molecular interactions between tumours and the immune system may lead to better anticancer therapies.

Recent research has suggested numerous possible novel targets in the complement system. Drug development is preceded by the increasingly precise structural understanding of each specific target molecule. There are only two anti-complement drugs currently on the market that are linked to inflammatory and degenerative disease [31]. This review presents recent evidence on the relationship between complement and tumour cells and the recent controversy over complement homeostasis. In addition, the review will address possible therapeutic targets of complement-mediated action in cancer and potential use of current anti-complement drugs in cancer immunotherapy.

The complement system

The complement system was originally recognised as a key aspect of innate immunity, which protects the body against foreign cells. The defence depends on multiple steps leading to the activation of the inflammatory reaction, opsonisation of pathogen and direct cell lysis by the membrane-attacking complex [MAC], being a bridge between humoral and adaptive immunity [37]. Anti-infectious properties of complement cascade are amplified by other immune responses, including removal of unnecessary immune molecules, cell signalling pathways, angiogenesis, apoptosis, and tissue regeneration. Such diverse functions of complement reflect the high complexity of the system. It regulates numerous immunological processes devoted to homeostasis [38]. The complement system consists of more than 30 proteins circulating in the serum or bound to cell membranes [27]. The circulating complement molecules are managed in order to activate at the right moment. Cleavage occurs to enhance formation of the active complement effectors.

There are three distinct complement pathways, which have different mechanisms of target recognition and sources of activation. The pathways are called the classical, lectin and alternative pathways. First, the classical pathway is triggered when C1q binds to antibody-antigen complexes and membrane fragments caused by tissue damage: damage associated molecular patterns (DAMPs). The complexes are bound to pathogen-associated molecular patterns (PAMPs) such as serum amyloid or C-reactive proteins. C1q then activates C1r and C1s and the complex then cleaves C2 and C4. Second, the lectin pathway starts with the mannose-binding lectin (MBL) or ficolins bound to PAMPs or host cells. This bound complex activates MBL-associated serine protease which cleaves C4 and generates C4a and C4b fragments. As result of C2 and C4 cleavage, C4b and C2a come together as C3 convertase, which cleaves C3 into C3a and C3b. Finally, the alternative pathway is stimulated by continuous, random hydrolysis of C3 to C3 + H₂O. This process is called 'tick over'. Hydrolysed C3 then binds with factor B. The complex is cleaved by factor D which divides it into Ba and Bb. The cleavage forms the initial C3 convertase. The enzyme cleaves another C3 into C3a and C3b. C3a leaves the site but C3b binds to membrane surfaces. Then C3b binds to additional factor B, which is cleaved by factor D directly to generate final C3 convertase that is stabilised by properdin. The C3 convertase from the alternative pathway has the ability to amplify complement activation by the other two pathways. Altogether, C3b can bind to C4b2a or C3bBb to form the C5 convertase which has the ability to cleave C5 into C5a and C5b. Like C3a, C5a leaves the site and C5b activates, with C6- C9, formation of the membrane-attacking complex (MAC) which is a key of complement-mediated lysis. Fig. 1 illustrates schematic diagrams of three complement pathways.

Although the differences between the three pathways can be seen, at the centre of each there is component C3. In addition, C5 is cleaved after C3 cleavage and activation, initiating formation of the pore-inducing MAC. C3 and C5 cleavage leads to secretion of anaphylotoxins C3a and C5a, which are the two main inflammatory mediators that activate leukocytes and chemoattractant [28]. Like other immune responses, the complement system is very tightly controlled by several different defence mechanisms because inappropriate complement activation

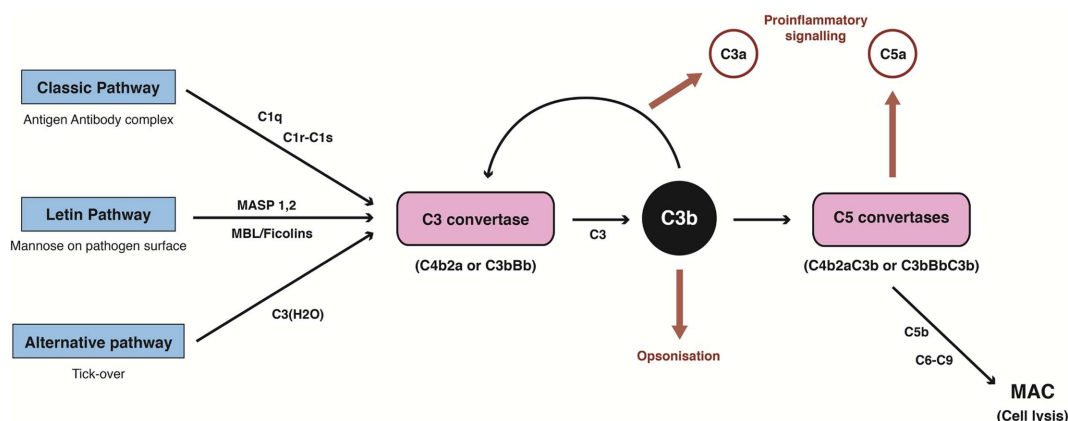


Fig. 1. Simplified scheme of complement activation pathways.

can severely harm normal cells. To prevent such misdirected activation, complement regulators generally protect normal cells and tissues. This kind of regulatory system is also used by cancer cells to protect them from complement-mediated attack [52].

The immune hypothesis

The cancer immunosurveillance theory was first formulated in 1957 [28]. Burnet and Thomas proposed the theory that lymphocytes act as sentinels in recognising and eliminating continuously arising, nascent transformed cells. It appears that cancer immunosurveillance plays an important role in the host protection process.

Generally, the immune system has three key roles in tumour suppression. Firstly, the system protects the host from virus-induced tumours by repressing viral infection. Secondly, continuous elimination of pathogens and homeostasis of inflammatory action can prevent inauguration of inflammatory triggered tumorigenesis. Third, the immune system can find and destroy tumour cells through their expression of tumour-specific antigens or molecules indicating cellular stress. The third process is referred to as tumour immunosurveillance where the immune cells identify and eliminate cancerous cells.

Although tumour immunosurveillance continuously protects the host, tumour cells are able to grow in the presence of a fully functioning immune system. Tumour immunoeediting refers to the concept that the immune system both protects the body from cancer growth and promotes tumour progression. Tumour immunoeediting is divided into three phases; elimination, equilibrium

and escape [5]. The elimination phase relates to the initial theory of tumour immunosurveillance where the system detects and removes cancer cells that have developed as a result of failed tumour suppression. After the elimination processes, according to immunoeediting theory, equilibrium arises between the immune system and the growing cancer. In this period, tumour cells may continuously evolve by modulation of their antigens. During this process, the immune system exerts selective pressure to control tumour growth. However, if the immune system fails to absolutely kill all the tumour cells, the process eventually results in selection of tumour cells that have better resistance, avoidance and suppression of the anti-tumour response, which leads to the escape phase. After the tumour enters the escape phase, the immune system can no longer control tumour suppression due to the high level of resistance. Various experimental and clinical data support the concept of cancer immunoeediting [45].

Clinical data on relationships between complement and cancer cells

Clinical research lacks direct results to support the argument that complement proteins can have an effect on nascent tumour cells. Yet, considering the function of complement in recognition of foreign antigens, it is assumed that membrane proteins of tumour cells could be targets of complement recognition. Activation of the complement system could be a key component of immunosurveillance and reduce cancer growth in various ways. Despite the lack of direct evidence, many reports

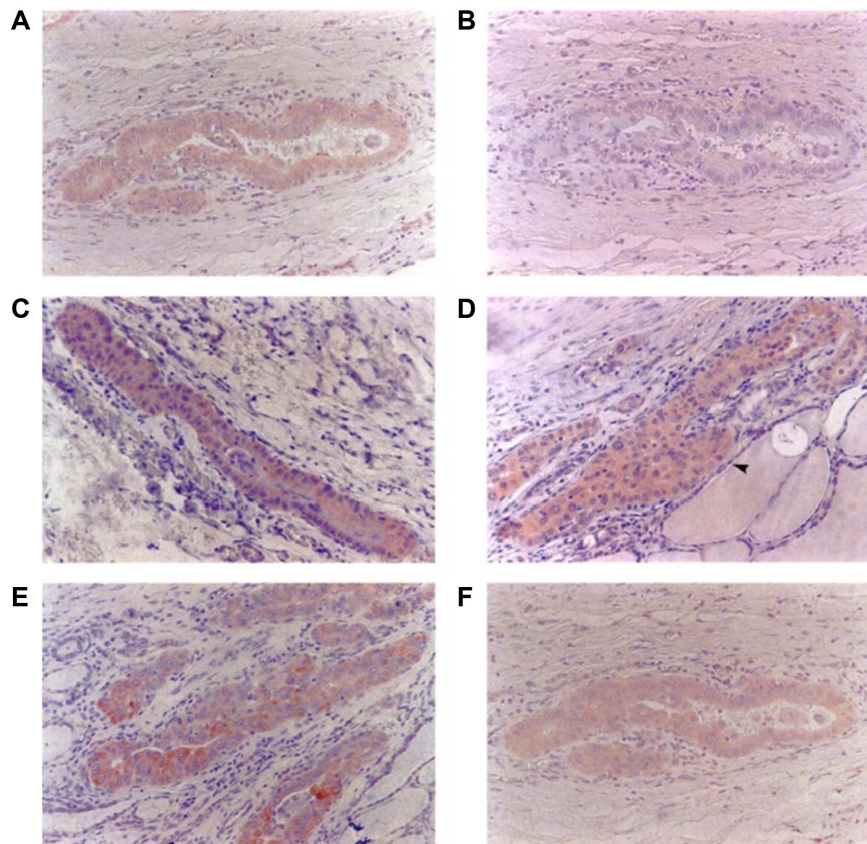


Fig. 2. IgG and complement staining patterns in PTC (papillary thyroid carcinoma). Photomicrographs of typical staining results in parallel sections of a representative PTC specimen. Panel A shows unequivocally positive (red) tumor cell staining with the IgG1 subclass antibody and panel B an IgG3 staining, assessed as negative. IgG4 (C), C3d (D), C4d (F), and C5 (F) all gave positive staining. Note the lack of staining in adjacent normal thyroid cells (arrow, panel D). (Original magnification $\times 400$) [25].

have mentioned that activation of complement protein is seen in cancer patients [26, 35].

In the case of primary tumour cells, there are many studies suggesting that different type of cancers are linked mainly with the classical complement pathway. The research identified deposition of complement protein C5b-9 complexes among the tumour cell membrane and necrotic areas of breast carcinoma [34]. The presence of IgG, C3 and C4 with the complement complex C5b-9 in carcinoma samples means that activation of the classic complement pathway has occurred. The absence of C5b-9 on macrophage membranes suggests that complement activation is only achieved in the presence of tumour cells. C5b-9 deposits were not found on the cells of benign breast tumours. Fig. 2 demonstrated the presence of complement factors C3d, C4d and C5 in papillary thyroid carcinoma tumour parenchyma [25]. This indicates that the complete complement system could poten-

tially be activated, despite the presence of complement regulatory proteins, CD55 and CD59, in nearly every thyroid tissue. Both primary papillary thyroid carcinoma tumours and their metastases were positive when tissues were stained for C3d, C4d and C5.

The researchers found from single and double immunostaining that C4d was located around dendritic cells in neoplastic follicles [2]. Their results show that follicle dendritic cells have Fc receptors which could eventually activate the complement cascade. The deposition of C4d was established in capillaries, arterioles and veins in lymphoma tissues which indicate that high levels of complement activation could be turned on and off.

Additionally, several *in vitro* studies have observed complement activation in tumour cell lines. Spontaneous activation of the classical complement pathway has been demonstrated in two neuroblastoma cell lines [13]. In contrast, other studies have suggested that alter-

native pathways are activated by lymphoma and myeloma cells [23, 30]. An increased activity of the lectin pathway of complement activation has been observed in colorectal cancer patients [51].

A research suggests that complement activity is depressed after primary Epstein-Barr virus (EBV) infection [30]. A failure of defensive immune system against EBV-transformed B lymphocytes could lead to malignancy. Typically, serum complement abnormalities have been seen in patients with Burkitt lymphoma (malignant cancer of the lymphatic system caused by EBV-infection).

Thus clinical data from several decades suggest a role of complement proteins within the tumour microenvironment.

Complement activation in cancer cells

Other evidence for the role of complement in immunosurveillance is the observation that cancer cells have a variety of mechanisms to protect themselves from complement-directed attack. There are two main types of

resistance by which cancer cells protect themselves from complement-mediated lysis: extracellular and intracellular regulators.

Extracellular regulators inhibit complement deposition on the membrane surface of targeted cells. The inhibitors interact with the complement binding system at specific points and counterattack the activated complement proteins. There are also many different subtypes of extracellular protectors. The most well-known inhibitors are membrane complement regulatory proteins, mCRPs, which are reviewed in many papers [12]. Other types are soluble complement inhibitors. For example ecto-protease, ecto-protein kinase and sialic acid residues are molecules that block complement binding extracellularly.

Intracellular protectors which are produced within the intracellular environment have the function to reduce damage caused by the MAC, increase the rate of repair cascades and clear MAC compartments from cell surfaces. Their subtypes are intracellular calcium ions, cAMP, PKC, MAPK ERK, heat-shock protein 70 and

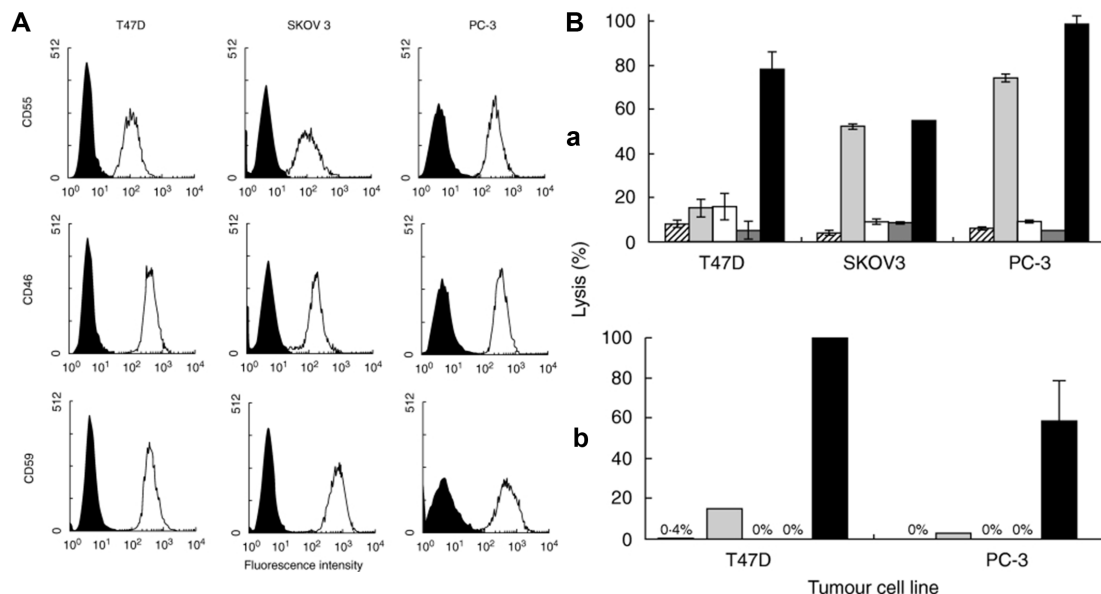


Fig. 3. (A) Expression of CD55, CD46 and CD59 on T47D, SKOV3 and PC-3 cells. T47D, SKOV3 and PC-3 cells (0.5×10^6) were treated for 30 min on ice with mAb anti-human CD59, CD55 or CD46 (\square) or without antibody (\blacksquare) and washed. Then, the cells were treated for 30 min on ice with FITC-conjugated goat anti-mouse IgG, washed and analysed by flow cytometry [8]. **(B) Neutralization of mCRP augments susceptibility of carcinoma cells to complement-mediated lysis.** Cells were incubated with neutralizing mAb directed to CD59, CD55 or CD46 or a mixture of these antibodies or with buffer alone. (a) 10 µg/ml mAb (an optimal concentration) (b) 2.5 µg/ml mAb (a suboptimal concentration). Next, the cells were subjected to lysis by rabbit anti-CA antibodies and NHS. Cells in Fig. 3B(b) also received a lower concentration of anti-CA antibodies (1 : 500 antiserum dilution) than in Fig. 3B(a) (1 : 300 dilution). Percentage lysis was determined by released-TDA fluorometry (see calculation on Methods). Ab; anti-CD59 Ab; \square anti-CD55 Ab; \blacksquare anti-CD46 Ab; \blacksquare Abs mixture [8].

Bcl-2. In addition, protein synthesis and linked membrane lipid metabolism also produce molecules that counteract MAC attack. Although attracting less attention than other resistive mechanisms, their importance for complement resistance of tumour cells matches that of the mCRPs [46].

The experiments researched the effect of biological regulators on complement resistance in human carcinoma cell lines T47D, SKOV3 and PC-3, each representing different organs [8]. They studied the level of expression of the mCRPs CD59, CD55 and CD46, showing overexpression on the membrane surface of carcinoma cell lines. In addition, experiments tested the impact of mCRPs on complement resistance by blocking mCRP activity with specific antibodies, demonstrating significant sensitization of cancer cells to complement-mediated lysis. Fig. 3A visualises the expression of regulatory proteins; CD44, CD46 and CD58 those can neutralise complement-mediated lysis. Fig. 3B shows how the neutralisation of complement regulatory proteins leads to susceptibility of carcinoma cells to complement-mediated lysis. The results confirm the presence of regulatory proteins increase number of carcinoma cells in Fig. 3A. Also the results confirm the neutralisation of complement regulatory protein leads to decrease percentage of complement-mediated lysis in Fig. 3B.

Previous research by this team also presented evidence that protein kinase C (PKC) aids resistance of K562 human erythroleukaemia cells to complement-mediated lysis [21]. They analysed carcinoma cell lines treated with PKC inhibitor GF109203X. Pre-incubation of cancer cells with the inhibitor raised their sensitivity to complement compared to controls. In addition, pre-incubation of tumour cells with the protein kinase A (PKA) inhibitor, H89, also enhanced complement-mediated lysis relative to the control. Another protein kinase which has a vital role in cell protection from complement is the extracellular-regulated protein kinase (ERK). Cells treated with the ERK inhibitor, PD98059, showed greater sensitivity to the lysis compared to untreated cells. This research team also found that removal of sialic acid from carcinoma cell surfaces with neuraminidase conferred increased sensitivity to complement-mediated lysis in certain cell types [8].

Relating to the immunoediting hypothesis, the high expression of regulatory molecules is recognised as selec-

tive pressure created by complement activation in the tumour microenvironment. This enables cancer cells to evade the damage from effect of complement.

Further immunosurveillance research

Results to date have proven that complement components are associated with cancer cells. Experimental data provided evidence of complement activity in cancer patients and tumour cells, and corresponding defence mechanism of tumour cells against complement-mediated attack. However, the information and results so far are scattered and most are several years old. The aim of the studies focuses on the pathology of cancer cells rather than the general complement activity of related immune responses. Most importantly, studies dealing with different types of tumour cells have not created a focussed research stream on immunosurveillance of complement proteins.

Each unique tumour type has its own specific antigenic identity and characteristics with respect to complement regulators and receptor functions. The various different complement recognition molecules and their regulators provide diverse control of activation pathways. Each tumour cell has a unique antigenic recognition and associated complement regulators. For example of the complement regulators, there are soluble and membrane bound proteases such as serine proteases which are categorised as coagulation and fibrinolysis systems mediate extrinsic complement activation pathways. Other mechanisms such as membrane lipid regulation and protein synthesis control complement regulatory proteins. The complement system is more complex than previously thought. Therefore, a more systematic analysis of pathways and mediators of complement activation in cancer cells is required. Such studies may lead to greater understanding of the dynamics between complement and cancer and could bring the opportunity to recognise new molecular biomarkers and therapeutic targets.

Other possible uses of complement

Besides their role in immunosurveillance of foreign intruders and tumour tissues, complement molecules and their activation products have been recommended as markers to visualise tumour pathologies. Lung cancer patients generally show a significantly higher level of

complement proteins and activation fragments than control donors [14]. Interestingly, the raised complement protein levels are correlated with lung tumour size [35]. Fascinatingly, complement activity can be linked with clinical perspective. A correlation has been seen between survival time and initial activity of the classical complement pathway in chronic lymphocytic leukaemia [49]. Thus, clinical data on complement levels can be associated with the size of the tumour and gives the ability to estimate survival time. In addition, high levels of complement regulatory proteins have been correlated with poor prognosis in different malignancies [10]. Greater understanding of differences in the levels of regulatory proteins between cancer and normal cells may lead to useful early predictive markers for tumour treatment such as chemotherapy.

Controversy over complement

Traditionally, researchers have viewed the complement system as the recognition and effector activity against the growth of tumour cells. Therefore, many scientists have designed strategies to raise levels of complement activation, hoping to see improvements in immunosurveillance effects against tumours, leading to development of possible immunotherapy. However, more recent research has demonstrated the complement system acting as a tumour-promoting system in mouse models [28]. Despite the unexpected discovery of complement acting as a tumour promoter; the idea is consistent with the immunoeediting theory.

This new concept of the role of complement in cancer was first suggested in a complement deficient mouse model of cervical cancer [29]. The results supported the complement mediator C5a, which can act as a chemoattractant and proinflammatory molecule, as having a key role in the promotion of tumour growth. The promotion of tumour cells was observed to be due to suppression of the anti-tumour CD8⁺ T cell response, which was due to expression of myeloid derived suppressor cells (MDSC). MDSC augmentation by C5a regulates regulation of T-cells and blockade of the complement receptor C5aR significantly suppressed tumour growth [29]. Onward study confirmed the contribution of C5a to lung cancer development [6].

Further research proceeded, to gain more understanding of C5a as tumour promoter because such knowledge

could lead to a novel therapeutic target for cancer suppression. Surprisingly, contrasting data were obtained from experiments using immunodeficient mice with injected cancer cells transfected with mouse C5a [15]. Human SKOV-3 ovarian adenocarcinoma cells were injected, and showed shown reduced progression through overexpression of C5a. Due to these conflicting results, it is suggested that the level of C5a may control suppression and activation of tumour progression. The researchers designed experiments in a lymphoma model which suggest that C5a has a concentration dependent effect [15]. They proposed that over-activation of C5a may lead to enhancement of alternative functions of complement such as inflammation and angiogenesis which perpetuate tumour progression. However, low levels of the C5a enhance the anti-tumour immune response. These experiments support the idea that complement protein level has a strong influence over the control of tumours.

Complement-mediated inflammation associated with tumour growth

It is believed that complement has a variety of roles in cancer-related biological processes. Experiments on C5a have given insight into the relationship between complement-mediated inflammation and cancer promotion. It is known that during common complement pathways, proinflammatory mediators are formed and cause inflammation. Both acute and chronic inflammation increase the risk of neoplastic transformation and increase tumour-promoting effects. During the events of inflammation, complement activation stimulates the release of cytokines and reactive oxygen species, which creates a tumour-supportive microenvironment [16]. The presence of a microenvironment favourable towards neoplastic cells is able to protect cells from complement-mediated damage. This resistance provides permanent complement activation which promotes proinflammatory complement mediators. Complement proteins such as C3-, C4-, C5- fragments, MAC and C1q are key elements of inflammatory conditions. Researchers have shown that complement-mediated cytokines have major tumour-promoting activity, e.g. IL6 which promotes angiogenesis and increased drug resistance [17]. Additionally toll-like receptors [TLRs] provide upregulation of proinflammatory cytokines once activated by comple-

ment fragments such as C3a and C5a. Overall, complement activation consistently contributes to upregulation of cytokines and growth factors.

Other possible complement-mediated biological processes affecting cancer growth

The biological process of angiogenesis leads to creation of new vessels in order to provide oxygen and nutrients for neoplastic tissues. As related to cytokine and complement activity, angiogenesis is widely studied and related to cancer growth. For example, C3 and C5aR deficiencies are associated with reduction of vascularisation in an in vivo mice model of ovarian cancer [36]. In addition, experimental data suggests that deposition of MAC prompts ion shifts that activates proliferation, differentiation and apoptotic resistance [48]. Several researchers have shown that cancer-associated signalling pathways involving mitogen-activated protein kinases, phosphatidylinositol 3-kinase and Ras are activated by the presence of sublytic MAC molecules such as C9 [22, 32, 33]. This evidence suggests the influence of the complement system over tumour signalling pathways. Further

understanding could lead to the discovery of novel mechanisms of tumour suppression.

Novel approaches to cancer therapy via the complement system

Since both activation and inhibition of complement provides anti-tumour responses this suggests a wide choice of therapeutic targets. Various strategies are already in place to provide therapeutic effects on cancer via the complement system. For example, researchers have improved the efficacy of monoclonal antibody based anticancer therapies using complement [20]. Many therapeutic strategies designed to overcome complement inhibitors have been tested in vitro and in mouse models [12]. The beauty of therapeutic complement is its ubiquitous presence in serum, hence complement-derived therapies show relatively few side effects, in contrast to the high toxicity of common anticancer chemotherapies. In 2007, the first complement-specific drug, eculizumab, an antibody against complement C5, was approved by the US Food and Drug Administration. Since this first vali-

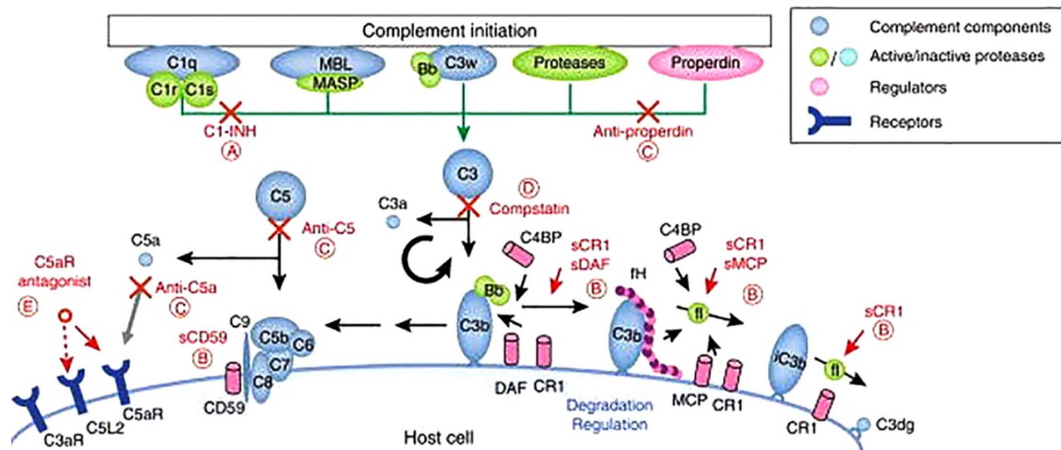


Fig. 4. Regulation, deactivation and inhibition of the complement cascade on host cells by natural regulators and complement-specific therapeutics. Several pathogenic processes and diseases are the result of an erroneous activation or insufficient downregulation of the complement cascade. Under normal conditions, any host-associated C3 convertase (C3bBb) undergoes an accelerated decay mediated by complement receptor 1 (CR1), decay accelerating factor (DAF), C4b-binding protein (C4BP) or factor H (fH). C3b is degraded to inactive iC3b by factor I in a reaction that requires as cofactor CR1, fH, C4BP or membrane cofactor protein (MCP). In addition, CD59 prevents the formation of the MAC. Some of the therapeutic interventions focus on increasing this downregulation by using soluble forms of these regulators (that is, sCR1, sDAF, sMCP, sCD59). Other approaches involve the substitution of the natural C1 inhibitor (C1-INH), the inhibition of the central conversion of C3 to C3b and C3a (compstatin), blockage of C5 or C5a by antibodies, and the suppression of anaphylatoxic signaling by C5a receptor antagonists. For clarity, only the regulation of the alternative pathway, which may contribute up to ~80% of all complement activity, is shown here. The C3 convertase of the classical pathway (C4bC2a), as well as the C5 convertases (C4bC2aC3b and C3bC3bBb), can be modulated by the same regulators and drug compounds [39].

dated approval, multiple arrays of diverse therapeutic options have been proposed. The complexity of complement provides many potential targets yet there is also a downside. One of the critical issues being considered is the maximal point of drug interactions. Inhibition or activation of complement protein acting between the cascades could potentially trigger unwanted effects. For example, protease inhibitors of C1q approved for hereditary angioedema treatment could effectively lead to shut down of multiple common complement pathways [7]. This blockade could further down regulate biological processes such as inflammation and lead to potential immunodeficiency. Numerous studies have been designed to test the effects of potential complement-mediated tumour suppressors. However, several attempts to devise successful therapeutics have failed [3, 41].

Protein to protein interaction is required at each step of the complement cascades. High numbers of biopharmaceuticals such as proteins, antibodies, peptides and nucleotides are involved in current drug development. A number of different approaches, illustrated in Fig. 4, have been tried for the clinical substitution, inhibition, promotion and modulation of complement-related molecules.

Therapeutic antibodies

Antibody-mediated therapeutics are in development for many different classes of disorder. Complement synergises with antibody-mediated mechanisms of action. Experience with monoclonal antibodies in cancer treatment suggests that initiation of apoptotic activation through antibody-dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC) constitute the therapeutic mechanisms. However, to initiate the classical complement pathway via recruitment of C1q, high levels of antigen densities are required. Recently, Genmab from Copenhagen have described anti-CD20 antibodies those are highly effective at recruiting C1q and hence activating CDC [47]. The CDC inducing activity of therapeutic antibodies could become an essential parameter in improving them for future drug development.

Complement inhibition for cancer treatment

For a long time, only activation of complement was believed to be beneficial for cancer treatment. With the

recent discovery of a new role for complement, the novel therapeutic option of complement inhibition has been proposed. C5a has been shown to mediate cancer growth. Selective inhibition of the C5a interaction with its receptor could stop the protumoural state without reducing other complement activity that could be vital for defensive mechanisms. Eculizumab, mentioned above as a drug that inhibits development of C5a, and PMX-53, a drug that neutralises the C5a-C5aR binding interaction are in different stages of clinical or preclinical development [50].

In addition, other complement effector molecules, C3a and C3aR, are maybe potential targets. Cleavage of C3 is the one of major events taking place in all three complement pathways. It is the intermediary step leading to cleavage of C5. Compstatin and its analogs are cyclic tri-decapeptides that prevent the cleavage of C3 into active fragments [18]. Despite unknown mechanisms, compstatin successfully inhibits the central steps of the complement cascade. This suggests that C3 blockade could be developed for therapeutic applications. The problem arose that compstatin did not show effectiveness against C3 in a mouse model [42]. This means that evaluation in standard preclinical model is restricted. Thus, for its validation, an alternate animal model needs to be designed for further development.

Complement inhibiting drugs in various stages of research provide a new type of cancer therapeutic. In the future, the use of complement-directed drugs should be considered as part of combinational therapies. Complement inhibition can override tumour immunosuppression and could be developed to supplement antitumor vaccines. Complement inhibition may also increase the efficacy of cell-based tumour immunotherapies [19].

Anti-complement regulatory drugs

The effectiveness of anticancer antibodies is likely to be reduced due to presence of complement regulatory proteins. There are several ways to overcome this: blockade of regulators, down-regulation of protein expression and removal of the protein from the cell surface.

Specific inhibition of mCRPs can be achieved with monoclonal antibodies directed to regulatory proteins such as CD46, CD55 and CD59. The majority of evidence shows that anti-mCRP antibodies enhance complement-mediated lysis. The neutralisation of CD55 in

different cancer cells such as Burkitt lymphoma cells, melanoma and breast cancer cells increases their sensitivity to complement [4, 8, 24]. In the second approach, the level of mCRP expression can be modulated by expression of cytokines or growth factors. Only a few studies directed at cancer therapy have been reported. For example, the expression of CD59 and CD46 was reduced in hepatoma cells on treatment with INF gamma [44]. In the third approach, GPI-phospholipase C (GPI-PLC) is clinically used for removal of regulatory proteins. Lysis of melanoma cells, lung cancer cells and cervical carcinoma cells increased following treatment [12]. Yet GPI-PLC is likely to remove other surface proteins as well as complement regulatory proteins. It is hard to establish the causal link between removals of complement regulatory proteins and enhanced sensitivity, requiring more extensive research.

Another approach to immunotherapy would be to utilise anti-idiotypic antibodies that mimic mCRPs. Human anti-idiotypic antibody 105AD7 from a colorectal cancer patient mimics CD55 [1]. In recent studies where patients were given 105AD7 at diagnosis, increased T cell CD4, CD8 and increased tumour apoptosis were seen compared to controls [43].

Discussion

Since the discovery of complement proteins, their role in the immune response has been known. However, a wide range of studies have reported different aspects of the complement system. Especially, the relationship between cancer promotion and complement regulation has been raised as an important tissue. Recent controversy has changed scientists' point of view and provided a wider range of potential complement therapeutics including both complement inhibition and enhancement. In particular, inhibition of C5a by eculizumab is waiting for its blockbuster effect in the drug markets. There is also valid evidence that complement regulatory proteins are used by tumour cells to escape complement damage. Therefore, drug discovery based on the inhibition of complement regulators is a prime development for anticancer therapies.

There are many aspects of complement therapeutics that still have to be discussed. Commercialization of drugs must cover cost, administration routes and infec-

tion risks as well as other adverse effects. Although short-term complement inhibition in a controlled clinical environment may not be a major issue, it could be a substantial risk during long term treatment required for cancer patients. In addition, selection of the right agents would be crucial for trials as complement pathology is still unknown in many cancer tissues. The development of effective biomarkers could enable the selection of the appropriate drug for the patient and allow monitoring of the response.

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