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REVIEW

Complement in Cancer and Cancer Immunotherapy

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Abstract Recently, there has been an increase of interest in the use of biological or immune-based therapies for patients with malignancies. This has been informed by the deeper understanding of the crosstalk between the host immune system and malignant tumours, as well as the potential advantages of immunotherapy—high specificity and less toxicity compared to standard approaches. The particular emphasis of this article is on the role of the complement system in tumour growth and antibody-based cancer immunotherapy. The functional consequences from overexpression of complement regulators by tumours and the development of strategies for overcoming this are discussed in detail. This review discusses these issues with a view to inspiring the development of new agents that could be useful for the treatment of cancer.

Keywords Immunotherapy · Cancer · Complement · Complement regulators · Complement-dependent cytotoxicity (CDC) · Antibody-dependent cell-mediated cytotoxicity (ADCC)

Complement Overview

Complement is a very complex system consisting of more than 30 membrane-bound and soluble plasma proteins. It consists of proteins that recognise each other upon activation of the complement system and form complexes with proteases that cleave and activate other enzymes in a cascade like manner similar to the activation of caspases and blood coagulation. Complement is an integral part of innate immunity and plays an important role in infections (bacterial and fungal), clearance of immune complexes and apoptotic cells (Walport 2001a). In this review we discuss the role of complement in anti-cancer defence and its role in cancer immunotherapy, particularly utilising monoclonal antibodies (mAbs), and strategies developed in last few decades to increase the complement-mediated clearance of tumours.

Pathways of Complement Activation on Cancer Cells

The complement system has three main activation pathways—classical, lectin (MBL/Ficolin) and alternative. The lectin pathway is initiated upon binding of mannose-binding lectin (MBL) or one of the ficolins (M, L and H) to patterns of *N*-acetyl residues (ficolins) and patterns of carbohydrate surfaces (Thiel 2007). The classical pathway is initiated by the binding of C1q to either IgG or IgM bound to antigen in immune complexes (Kishore and Reid 2000). C1q, MBL and the ficolins are soluble pattern-recognition molecules with collagen-like regions (Thiel 2007).

C3, a central component of the complement system, could be auto-activated on the surface of microorganisms or by different proteases such as kinin-generating proteases kallikrein and thrombin (Kemper et al. 2010), known as alternative pathway activation. It has also been reported that properdin bound to microbial surfaces can activate the complement system (Spitzer et al. 2007). All complement pathways converge on the C3 molecule, which upon cleavage by proteases form C3a (a potent anaphylatoxin) and C3b (opsonin). (Spitzer et al. 2007; Walport 2001a).

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Complement activation is very tightly regulated in vivo and C3b is rapidly cleaved by various regulators to iC3b, and C3c or C3dg (Liszewski et al. 2008). C3 convertase can generate large amounts of C3b through the alternative pathway amplification loop, some of which binds to nearby cell/microorganisms surfaces (Ollert et al. 1994) or to the C3 convertase forming the C5 convertase (shown in Fig. 1). C5 convertase cleaves C5 to produce C5a (anaphylatoxin) and C5b (Walport 2001a, b). C5b associates with C6 and C7 to form C5b67 complex which inserts into the cell membrane and then binds to C8 and C9 (Bubeck et al. 2011) to form the membrane attack complex (MAC) which promotes lysis of cells and microorganisms when in sufficient quantities (Walport 2001b).

When focussing on complement in the context of cancer and transformed cells it is important to establish whether they are capable of activating endogenous complement in vivo. Some of the first evidence of complement activation due to transformed cells was found using immunohistochemistry of breast cancer tissue which showed deposition of the MAC and the components IgG, C3 and C4, in contrast to the lack of such components in benign tissue. These data show transformed cells are capable of complement activation as far as the terminal pathway and also suggests that this is due to the classical pathway (Niculescu et al. 1992). Other examples include similar results found in papillary thyroid carcinoma on which deposits of C3d, C4d, C5, IgG1 and IgG4 were found (Lucas et al. 1996). The concentration and activity of MBL present in the serum of colorectal cancer patients was higher when compared with normal controls, suggesting MBL may activate upon transformation (Ytting et al. 2004). The

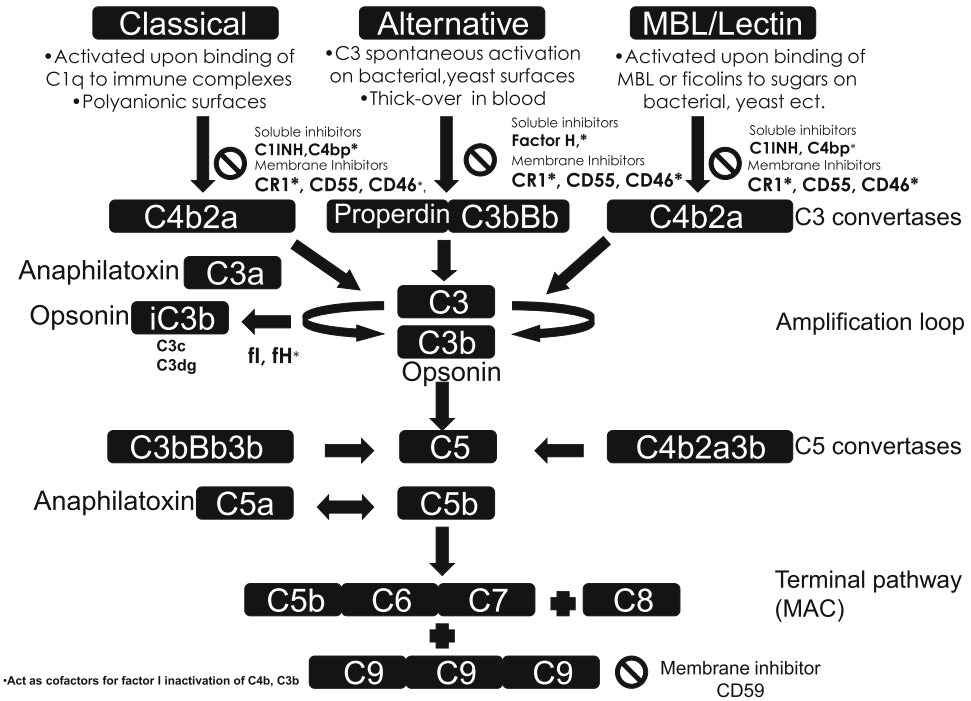

Fig. 1 Activation pathways of the complement system. *Classical pathway* activation is initiated by aggregation of immunoglobulins on cell surface. This permits multiple coincident interactions of globular heads of the C1q with immunoglobulin (usually IgM and IgG), and consequent activation of C1r and C1s serine proteases. C1q can also interact directly with polyanionic surfaces (e.g. DNA), leading to antibody-independent activation of the classical pathway. C1s then cleaves C4 to C4a and C4b and C2 to C2a and C2b which results in the generation of C4bC2a molecule, termed C3 convertase of the classical pathway. *The alternative pathway* can be activated via the C3b generation from the classical pathway where it serves as an amplification loop to the classical pathway. C3 is cleaved by C4bC2a enzyme and the generated C3b binds to factor B, which is then cleaved by factor D to generated C3bBb C3 convertase of the alternative pathway. *The lectin pathway* starts by recognition of acetylated sugars on the surface of bacteria by MBL or ficolins. This activates MASP-2, one of the three human MASPs (MASP-1, MASP-2 and MASP-3), a main effecter of the lectin pathway, which then activates C4 and C2 to generate C3 convertase of the lectin pathway (C4bC2a). By the addition of the C3b molecule to C4bC2a or C3bBb they are now able to cleave C5 to C5b and C5a and initiate *the terminal pathway* which leads to the formation of a pore of polymerised C9 molecules and thus generation of membrane attack complex (MAC) comprising of C5b6-9. Complement activation results in formation of C3a and C5a, small peptide-like molecules that act as anaphilatoxins and can recruit immune cells (e.g. neutrophils, T cells) to sites where complement activation occurs. There are number of regulatory molecules that control each step of the cascade and act in solution or on cell surfaces. C1INH (C1 inhibitor), C4bp (C4-binding protein), fH (factor H), fI (Factor I) are an example of soluble regulators, although factor H can also act on surfaces of cells. The group of membrane-bound regulators consists of CD35, CD46, CD55 and CD59. CD59 inhibits the formation of MAC and is the sole inhibitor of the terminal pathway. CD46 and CD55 both act on formation of the C3 convertase

presence of soluble C3a and MAC in the ascetic fluid taken from ovarian cancer patients also indicates activation of the alternative and classical pathways in this tumour type. This also results in deposition of some activation products, for example C3d and C1q, on the surface of tumour cells (Bjørge et al. 2005). The exact trigger for complement activation upon transformation is still not fully understood although activation through immune complexes, antibody binding or C1q binding to necrotic/apoptotic cellular components are all possibilities. The relative contribution made by each activation pathway is unclear, but as these data suggest each of the three main pathways may be implicated.

Key Molecules of Complement Cascade Playing a Critical Role in Cancer Immunotherapy

The biological function of the complement system is much more diverse than simple lysis of foreign cells. C3b for instance is a major opsonin and is responsible for clearance of immune complexes (antigen–antibody) and opsonisation of bacteria. C3d, a product of C3b degradation by complement regulators is known to lower the threshold for B-cell activation by 1,000-fold by binding to the CD19/CD21 complex (Cherukuri et al. 2001; Fearon and Carroll 2000). C3a and C5a as mentioned above are very important anaphylatoxins acting to recruit immune cells (neutrophils, phagocytic cells and more) to the site of inflammation (reviewed in Klos et al. 2009). C3a further promotes serotonin release from guinea pig platelets (Fukuoka and Hugli 1988) and modulates synthesis of interleukin (IL)-6 and tumour necrosis factor-α from B cells and monocytes (Fischer and Hugli 1997). C3a also exerts a direct bactericidal activity (Klos et al. 2009). C5a is a powerful chemoattractant for macrophages (Aksamit et al. 1981), neutrophils (Ehrengruber et al. 1994), activated B (Ottonello et al. 1999) and T cells (Nataf et al. 1999), basophils (Lett-Brown and Leonard 1977) and mast cells, the latter of which also migrate towards a C3a gradient (Hartmann et al. 1997). In addition, both C3a and C5a induce smooth muscle cell contraction and increased vascular permeability as well as degranulation of endothelial cells, mast cells (histamine release) and phagocytes leading to a protective acute inflammatory reaction, another major function of complement activation (Guo and Ward 2005). MAC is a transmembrane pore complex that can increase the membrane permeability and cause colloid-osmotic swelling (through cation permeability) and lysis of the target cell or bacteria (Gram-negative only). In sublytic concentrations, however, MAC can result in Ca^{2+} influx leading to signalling events (Halperin et al. 1993; Nicholson-Weller and Halperin 1993). This deposition also has the effect of inducing pro-inflammatory mediators, causing entry into the cell cycle, induction of expression of adhesion molecules and production and release of arachidonic acid derivatives (Kilgore et al. 1997; Rus et al. 1996; Tedesco et al. 1997). Sub-lytic doses of MAC are known to have anti-apoptotic and pro-survival effects on several cell types by directly signalling through G-protein mediated pathways causing cell-cycle activation and apoptosis inhibition (Rus et al. 2001).

Link Between Complement and Cancer Immunotherapy

The classical pathway and the amplification loop of the alternative pathway have been shown to play role in cancer immunotherapy with various mAbs (Weiner et al. 2010). For instance the antitumour effect of rituximab (an anti-CD20 antibody initially used to treat non-Hodgkin lymphoma) in $C1q^{-/-}$ mice is completely abolished (Di Gaetano et al. 2003). Depletion of complement with cobra venom factor (inhibition of the alternative pathway) decreases the therapeutic activity of rituximab in a xenograft model of human B-cell lymphoma (Cragg and Glennie 2004).

Deposition of MAC on the cell surface is often regarded as complement-dependent cytotoxicity (CDC) and is one of the mechanisms of tumour clearance in monoclonal cancer immunotherapy. Complement activation and deposition of C3b on B cells and subsequent lysis have been shown for rituximab (Manches et al. 2003). However, levels of CDC of primary lymphoma cells treated with rituximab in vitro correlate with the level of expression of complement regulatory proteins on the tumour cells (Kennedy et al. 2003). Complement is readily available in the serum and with the tick-over of C3 (constant low level C3 activation) and/or low level of classical pathway activation (Manderson et al. 2001) it requires tight regulation to protect host cells from destruction.

Complement Regulators

Nearly half of all proteins that form the complement system are regulators of its activation (Halperin et al. 1993). They can be generally divided into two groups: soluble/secreted and membrane-bound complement regulators (mCRegs).

Secreted/Soluble Complement Regulators

The group of secreted complement regulators consists of C1 inhibitor, C4-binding protein, Factor I, Factor H, Factor H-related proteins and S protein.

C1 inhibitor (C1INH) is a plasma protein from the serine protease inhibitors family (Cicardi et al. 2005). C1INH binds and inactivates complement proteases C1s, C1r (part of C1 complex with C1q), MASP-1 and MASP-2 (associates with MBL to activate the lectin pathway, see Fig. 1). C1INH inhibits both the classical and lectin pathways (reviewed in Davis et al. 2008).

C4-binding protein (C4bp) is a large 500-kDa glycoprotein composed of seven α-chains and one β-chain (Blom et al. 2004b). C4bp acts as a cofactor to factor I (FI) in the proteolytic inactivation of C4b in the inhibition of the classical pathway activated by antibodies (Blom et al. 2004a; Scharfstein et al. 1978). It also prevents the assembly of C4b2a, (the classical and lectin pathway C3-convertase complex) and accelerates the decay of the already formed complex. It circulates in complex with the vitamin K-dependent protein S, which provides C4bp with the ability to interact with negatively charged phospholipid membranes (Blom et al. 2004a).

Factor I is a 67-kDa heterodimer of a light and heavy chains linked by disulfide bonds (Catterall et al. 1987). Its primary function is to cleave the α-chains of C4b and C3b in the presence of the cofactors C4bp and factor H (FH), respectively (Minta et al. 1998). It also cleaves C3b into C3f and iC3b in conjunction with its cofactors FH and CD46 and then further cleaves iC3b into C3c and C3dg using CR1 as cofactor (Ross et al. 1982). As mentioned above, FI also inactivates C4b by cleaving it to C4c and C4d fragments (Nagasawa et al. 1980).

FH is a 150-kDa secreted plasma glycoprotein that consists of 20 short consensus repeat (SCR) domains (Ripoche et al. 1988; Sim and DiScipio 1982). FH can act as a fluid phase regulator and also on the cell surface (by binding to glycosaminoglycans on the host cells). FH functions as a cofactor in the inactivation of C3b by FI. It also increases the rate of dissociation of the C3bBb complex (C3-convertase) and the C5-convertases of both alternative [(C3b)2Bb] and classical pathway [C4b2a3b] (Heinen et al. 2009; Jokiranta et al. 1996).

Membrane-Bound Complement Regulators

The group of membrane-bound regulators (mCRegs) consists of CD35 (Complement Receptor 1, CR1), CD46 (membrane cofactor protein), CD55 (decay-accelerating factor) and CD59 (protectin).

CD35 is a 223-kDa transmembrane glycoprotein that consists of 30 SCRs (Smith et al. 2002). It binds to C3b and C4b with high affinity and iC3b and C3dg with lower affinity (Coburn et al. 2005; Cooper 1969; Smith et al. 2002). It inhibits both the classical and the alternative pathways via C3 and C5 convertases due to its decay-accelerating activity and cofactor activity for FI (Ross et al. 1982). CD35 is expressed on the surface of erythrocytes, T-cell subsets, mature B cells, follicular dendritic cells and monocytes. Due to recognition of C3b, iC3b and C4b and its expression on erythrocytes, CD35 plays major role in clearance of complement-opsonised immune complexes from the bloodstream (Nielsen et al. 1997b). CD35 on macrophages and neutrophils binds to complement-opsonised immune complexes (or cells) and mediate phagocytosis (Nielsen et al. 1997b). It has also been proposed that CR1 in whole blood promotes the degradation of immune complex-bound iC3b to C3dg, thereby rendering the immune complex inaccessible for binding to CR3 preventing the oxidative burst (Nielsen et al. 1997a).

CD46 is a transmembrane glycoprotein that consists of four extracellular SCR domains (Purcell et al. 1990). CD46 acts as a cofactor for complement FI and cleaves C3b and C4b deposited on host tissue. It also may be involved in the fusion of the spermatozoa with the oocyte during fertilisation. CD46 also acts as a costimulatory factor for T cells along with CD3 and IL-2 in vitro which induces the differentiation of $CD4^+$ cells into T-regulatory cells. These T-regulatory cells suppress immune responses by secreting IL-10, transforming growth factor (TGF)-β, and, therefore, are thought to prevent autoimmunity (Astier et al. 2000; Kemper et al. 2003). A number of viral and bacterial pathogens seem to exploit this property and directly induce an immunosuppressive phenotype in T cells by binding to CD46 (Santoro et al. 2003; Segerman et al. 2003).

CD55 is a membrane glycosylphosphatidylinositol (GPI) anchored glycoprotein that has four SCRs (Nakano et al. 1992). It recognises C4b and C3b fragments that condense with cell-surface hydroxyl or amino groups when nascent C4b and C3b are locally generated during C4 and C3 activation. Interaction of CD55 with cell-associated C4b and C3b polypeptides interferes with their ability to catalyse the conversion of C2 and factor B to enzymatically active C2a and Bb and thereby prevents the formation of C4b2a and C3bBb, the amplification convertases of the classical and alternative pathway of the complement cascade. It is also a receptor for certain viruses (Shafren et al. 1995; Ward et al. 1994). In mice deficient of the *daf1* gene (mouse analogue of human CD55), a more vigorous T-cell response was observed (more IL-2, interferon γ, less IL-10) which was shown to be complement dependent when this effect was almost completely abolished in $daf^{-/-}C3^{-/-}$ double knockout mice (Liu et al. 2005). Other studies in $daf^{-/-}$ mice indicate a possible role of CD55 in priming by antigen-presenting cells of naïve T cells (Heeger et al. 2005).

CD59 is a small GPI-anchored glycoprotein with globular structure (Sugita et al. 1993). CD59 has a molecular weight of 19 kDa, is heavily glycosylated, and can be found in a soluble and membrane-bound state (Meri et al.

1996). The soluble form from urine retains its specific complement binding activity but exhibits a greatly reduced ability to inhibit MAC assembly on cell membranes (Meri et al. 1991). CD59 is the only membrane-bound inhibitor of the complement MAC. It acts by binding to the C8 and/or C9 components of the assembling MAC thereby preventing incorporation of the multiple copies of C9 required for complete formation of the osmolytic pore (Bodian et al. 1997; Meri et al. 1990). This inhibitor appears to be species-specific (Yu et al. 1997). CD59 has been shown to downmodulate $CD4^+$ T cells in response to polyclonal and Ag-specific stimulation in both mice (Longhi et al. 2005) and humans (Sivasankar et al. 2009). CD59 on cell surfaces localises within lipid rafts where, upon crosslinking with anti-CD59 antibodies, it associates with kinases leading to phosphorylation of TCRζ/ZAP70 (Murray and Robbins 1998). In a recombinant vaccinia virus model of infection in *cd59*$^{-/-}$ mice, $CD4^+$ T cells, but not $CD8^+$, were found to proliferate more when compared to wild types. The addition of GPI-anchored CD59 returned proliferation to normal levels (Longhi et al. 2005). The non-complement roles played by the mCRegs have been extensively reviewed (Longhi et al. 2006).

Implications of Complement Regulators for Cancer

With evidence suggesting that complement activation occurs on the surface of tumour cells, the associated deposition of C3b and generation of anaphilatoxins (C3a and C5a) could potentially lead to the activation of the immune system and recruitment of immune cells to the tumour site. Complement activation inevitably leads to MAC formation that could lyse the tumour cells in a process termed CDC. Opsonisation of tumour cells by C3b/iC3b on the other hand leads to engagement of leucocyte complement receptors (e.g. $CR3^-$ $CD11b/CD18^-$ (Gelderman et al. 2004)) which enhance cell-mediated cytotoxicity via a process called CR3-DCC (CR3-dependent cell cytotoxicity). CR3-DCC requires β-glucan (Yan et al. 1999).

In the presence of mAbs, FcγIIIR expressing immune cells, such as NK cells, can kill tumour cells through antibody-dependent cell-mediated cytotoxicity (ADCC) which is enhanced by the anaphilatoxins (C5a, C3a) generated during complement activation (Gancz and Fishelson 2009). However, most tumour cell types (as with all nucleated cells) express complement regulators on their surface. These regulators protect them from complement attack. There are numerous examples published in the literature of tumours overexpressing at least one of the mCRegs (reviewed in Fishelson et al. 2003; Yan et al. 2008). This is a great hurdle which needs to be overcome if we are to successfully treat using mAb immunotherapy.

Overexpression of mCReg on Tumour Surface

Tumour Protection

In addition to the evidence suggesting complement activation occurs surrounding cancer cells in vivo, activation was also shown in anti-cancer immunotherapy with mAbs (Idusogie et al. 2001; Zhou et al. 2008). Taken together this strongly suggests a role of the complement system in anti-cancer defence. However, most tumours have been shown to overexpress either soluble regulators or mCRegs, thus suppressing activation of the complement system on tumour surface and diminishing its role in tumour clearance (Fishelson et al. 2003; Gorter and Meri 1999; Kumar et al. 1993). Much attention has been focussed upon the mCRegs: CD46, CD55 and CD59 as their overexpression on tumours protects them from direct complement lysis. Moreover, CD59 has been shown to promote tumour growth and decrease survival in vivo. In this study, human LAN-1 neuroblastoma cell line was transfected with rat CD59 (Chen et al. 2000) and inoculated in immune deficient rats. Rat CD59 was directly responsible for the increase of the onset and rate of tumour growth in LAN-1 CD59-positive cells. Interestingly, endogenous expression of CD55 was also upregulated in these cells compared to normal LAN-1 cells. Increased CD55 expression correlated with decreased C3 and C9 deposition on the surface of these cells.

Most tumours express at least one mCRegs on their surface. Some tumours like glioblastoma cell lines express all three (CD59 in higher amounts and CD46 and CD55 in lower amounts) (Juhl et al. 1997a; Junnikkala et al. 2000). Breast cancers seem to have a moderate to high expression of all three mCRegs in both tumours and their associated cell lines and even secrete soluble CD55 and CD59 (Donin et al. 2003; Hakulinen and Meri 1994; Hofman et al. 1994; Niehans et al. 1996; Thorsteinsson et al. 1998). Colorectal tumours have also been shown to express all three mCRegs (membrane and soluble variants) in both cell lines and primary tumours (Gelderman et al. 2002a; Mizuno et al. 1995; Niehans et al. 1996; Thorsteinsson et al. 1998). For a comprehensive table of mCRegs expression in primary tumours and tumour cell lines see Fishelson et al. (2003). Data published in the literature on mCReg expression suggests much higher overall expression of mCRegs in solid tumours when compared to haematological malignancies (Donin et al. 2003). A few tumour types seem to lack expression of any of the mCRegs. For example, small-lung carcinoma and some renal tumours have been reported to have no staining for any of the mCReg (Niehans et al. 1996).

A good example of how the tumours resist the complement system is the elimination of the MAC from the

plasma membrane by tumour cells such as U937 and K562 cells (Morgan 1992). At lower MAC concentrations on the cell surface, tumours are able to escape lysis either by shedding their membrane (ectocytosis or vesiculation) or by endocytosis of the MAC. This process is rapid and involves Ca^{2+} influx in cytosol, activation of protein kinase C and extracellular signal-regulated protein kinase (ERK) (Pilzer et al. 2005). Using fluorescently labelled C9 molecules Moskovich and Fishelson (2007) found that 5–10 min after complement challenge, K562 cells started either shedding or endocytosing MAC that was deposited on their surface. Interestingly CD59 level was found to inversely correlate with vesiculation/shedding, meaning more vesiculation was observed when amount of CD59 was less on the cell surface and vice versa. Soluble complement inhibitors released by tumour cells to the microenvironment can also protect the cell against complement activation and lysis. Junnikkala et al. (2000) tested H2-glioblastoma cells and found that although these cells express a significant amount of CD59 and moderate levels of CD46 and CD55, soluble regulators FH and FH-like protein 1 (FHL1) actually confer their resistance to complement lysis in vitro. Similarly, lung, ovarian, glial and colon cancer cells show enhanced expression and surface binding of soluble regulators, including FH, FHL1, C4bp and FI (Zipfel and Skerka 2009). Second messengers (Ca^{2+}, cAMP) and heat shock proteins can desensitise tumours to MAC, by helping cell repair processes (Fishelson et al. 2003). The mechanisms of this protection are generally poorly understood although there are some mechanisms established. For example Kraus et al. (2001) found that sublytic doses of complement to K562 and COS7 cells can lead to the resistance of tumour cells to MAC due to activation of ERK-mitogen activated kinases 1 and 2. Similar desensitising of tumour cells to complement lysis was shown to be due to calcium influx and protein kinase C activation as induced by sublytic complement attack (Kraus and Fishelson 2000). Sublytic complement has also been shown to activate protooncogenes such as c-fos, c-jun and junD and thus influencing the cell cycle and proliferation of tumour (or normal) cells (Rus et al. 2001). Another effect of sublytic complement is inhibition of apoptosis by inhibition of caspases-3 and -9 in OLG rat cells (Rus et al. 2001). Phosphorylation of BCL-2 antagonist of cell death (BAD) and BAD/BCL-Xl complexes was found to be essential. These findings suggest that with the higher the expression of mCRegs on tumour surface the chance of sublytic MAC attack is greater and thus it may lead to tumour survival and growth instead of tumour death. Therefore, a strategy for inhibiting expression of the mCRegs on tumours and/or their activity would have a high stimulating effect in tumour immunoclearance.

Functional Consequences for Cancer Immunotherapy

The importance of mCRegs overexpression can be illustrated by using blocking antibodies to CD55 and CD59 that helped increase the rituximab induced CDC in various lymphoma cell lines and fresh chronic lymphocytic leukaemia samples from patients (Golay et al. 2000; Harjunpää et al. 2000; Treon et al. 2001). However, complement activation is not only about lysis of target cells. It also helps attract immune cells via C3a and C5a anaphylatoxin fragments, opsonisation of target cells with C3b and iC3b that could be later recognised by CR1 or CR3 and lead to complement-dependent cell cytotoxicity (CDCC) via phagocytic cells or NK cells (see Fig. 2 for different modes of action of mAbs). That is why complement activation could potentially be a very important event in anti-cancer immunity and immunotherapy. Most of the currently approved antibodies for cancer immunotherapy have IgG1 isotype, which activates effectively both the complement system and can also be recognised by FcγIII receptor on the surface of neutrophils, macrophages, NK- and T cells and can activate them to kill the tumours via ADCC (Wang and Weiner 2008). A number of in vitro studies have shown that rituximab is highly capable of activating complement and killing tumours via CDC (Flieger et al. 2000; Reff et al. 1994). Higher CD59 expression on B-cell lymphoma cell lines was found to correlate with lower CDCC mediated by rituximab (Flieger et al. 2000).

In recent in vivo studies using murine models, mCRegs have also been shown to have role in ADCC. Varela and colleagues utilised murine bladder cancer cell line MB49 and were able to show that downregulation of Crry (mouse analogue of human CD46 mCReg) had a positive effect not only on CDC in vitro but also increased survival of mice in syngeneic model (Varela et al. 2008). Downmodulation of Crry was thought to be the reason why $CD8^+$ T cells were found to be more active in this model. The effect was dependent on activation of the complement system, as it was lost in C3 deficient animals. In another study, Macor et al. (2007) used biotinylated miniantibodies against CD55 and CD59 and showed increased survival of SCID mice when these were injected in conjunction with biotinylated rituximab and avidin treatment. When all three antibodies were used, CDC was significantly higher than rituximab alone in all lymphoma cell lines tested. Interestingly blocking of CD55 and CD59 on the surface of tumour cells using rituximab as primary ADCC targeting antibody resulted in an increase in tumour cell lysis when compared with rituximab alone. These data clearly show that the overexpression of mCRegs on tumour surface diminishes the therapeutic effect of antibodies used in cancer immunotherapy.

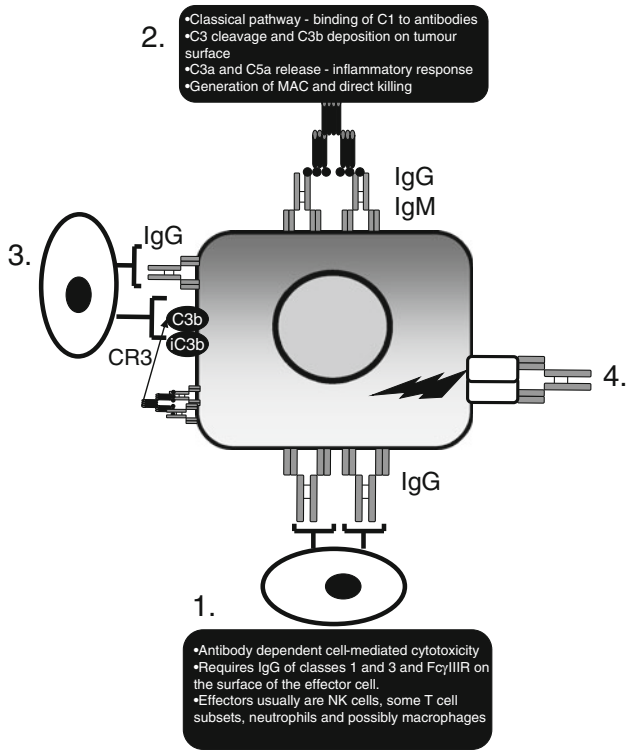

Fig. 2 Mechanisms of action of monoclonal antibodies (mAbs) in cancer immunotherapy. mAbs bound to target antigen on the tumour cell surface can be recognised by Fcγ-receptors (FcRs) expressed on immune cells, such as granulocytes, natural killer (NK) cells, monocytes and macrophages, or some T-cell subsets. This mode of action of the therapeutic mAbs is known as antibody-dependent cellular cytotoxicity (ADCC) (*1*). mAbs can also activate complement system through the classical pathway, following binding of C1q to the mAb Fc region. Complement activation results in deposition of C3b or iC3b on cell surface. Both molecules serve as opsonising agents for cells having CR3 (CD11b/CD18). C3R recognises iC3b but C3b is usually rapidly cleaved to iC3b by factor I and CD46 or factor I and factor H. Complement activation can also lead to the formation of the MAC, resulting in complement-dependent cytotoxicity (CDC) (*2*). ADCC can be further enhanced by complement receptor 3 (CR3) binding to iC3b deposited on cell surface, thus enhancing FcgR-mediated effector-cell binding and combining the above described mechanisms (*3*). Some mAbs are targeting pro-apoptotic factors such as the TNF receptor, TRAIL-R and could induce apoptosis in tumour cell via Fas or TRAIL pathways (*4*). Growth arrest is caused by mAbs targeting receptors for growth factors (e.g. cetuximab, rituximab)

Strategies to Overcome mCReg Expression

It has been demonstrated that tumours can be sensitised to complement by removal of CD55 and CD59 by phosphatidylinositol-phospholipase C treatment (PI-PLC) (Brasoveanu et al. 1996; Gelderman et al. 2006). Brasoveanu and colleagues showed that using increasing doses of PI-PLC, progressively decreased cell-surface expression of CD59 and increased C-mediated lysis of cells sensitised with mAb R24. A different approach was taken by Blok et al. (2003). Using cytokines, they were able to downregulate the mCRegs expression in renal tumours. They found that IL-1β downregulated the expression of CD46 and CD59, IL-4 on the other hand downregulated the expression of CD46 and TGF-β downregulated the expression of both CD46 and CD55. However, treatment with both IL-1β and IL-4 also decreased the expression of G250 TAA. The decrease in the expression of CD55 and CD59 were associated with increase in the amount of C3 deposited and, therefore, with CDC. These studies demonstrate the need to design strategies for targeted neutralisation of mCReg in order to increase the role of complement system in cancer immunotherapy. There are different strategies that have been proposed to overcome the overexpression mCRegs and to more fully utilise the therapeutic potential of anti-cancer mAbs. A summary of different strategies for the improvement of tumour immunoclearance is given in Table 1 and some of these are discussed below in details.

Bispecific Abs

One way to block the mCRegs induced complement inhibition is by using neutralising mAbs against them. A major drawback in this strategy, however, is the ubiquitous expression of the mCReg in the body (Gorter and Meri 1999). Monoclonal antibodies with one Fab directed against tumour-associated antigen (TAA) and the other against overexpressed mCRegs have been proposed. These are known as bi-specific antibodies. Junnikkala et al. (1994) were among the first to propose a biotinylated anti-CD59 antibody to crosslink it to avidin conjugated R24 anti-GD3 ganglioside. CD59-biotin was used as neutralising antibody to CD59 and R24 was used as complement-fixing antibody. Addition of CD59 Fab fragment greatly improved lysis of tumour cells (Junnikkala et al. 1994). Harris et al. showed that chemically engineered bispecific antibody against CD38 $\times$ CD59 significantly increased CDC in Raji cells when compared to anti-CD59 (BRIC229) or antiCD55 (BRIC216) antibodies alone. It has been postulated that mCReg neutralising Fab fragment should have lower affinity for their target than that of the TAA Fab (Harris et al. 1997). Bispecific antibody against G250 renal tumour TAA and CD55 did show increased C3 deposition on renal tumours and increased CDC compared to the two parental antibodies alone (Blok et al. 1998). A more recent attempt was made by Gelderman and colleagues who generated two bispecific antibodies against HLA and CD55 and anti-EpCam anti CD55. Both bispecific antibodies were able to increase C3b deposition on the surface of cervical and colorectal carcinoma cell lines substantially (Gelderman et al. 2002a, b) In a subsequent study using anti-EpCamxCD55 bispecific mAb, Gelderman et al. (2006) showed threefold increase in CR3-DCC by

Table 1 Strategies for improvement of tumour immunoclearance

Type	Reagent	References
Improvement of CDC	mAb or Fab conjugated with cobra venom factor	(Juhl et al. 1990, 1995, 1997b)
	mAb conjugated with C3b	(Reiter and Fishelson 1989a, b)
	Bi-specific mAb one Fab against TAA, the other against mCReg	(Blok et al. 1998; Harris et al. 1997; Junnikkala et al. 1994)
	Secondary mAb directed against either the anti-tumour mAb or against the iC3b deposited on tumour cells by the primary mAb	(Gelderman et al. 2002b)
	Jumbling of the heavy chain by shuffle IgG1 and IgG3 sequences within a heavy chain constant region, Point mutation of Fcγ region (improve binding to FcIIIRa), removal of fucose from Asn297-linked oligosaccharides	(Kroesen et al. 2002; Sokoloff et al. 2000)
	Cocktail of mAbs against TAA to improve C1q binding and complement activation	Reviewed in Natsume et al. (2009)
	Use of cytokines that modulate mCRegs expression on tumours	(Spiridon et al. 2002)
	Pharmacological agents downregulating mCReg expression	(Bjørge et al. 1995; Bjørge and Matre 1995; Blok et al. 2003)
	shRNA or siRNA targeting expression of Crry (mouse model)	(Geis et al. 2010; Varela et al. 2008)
Improvement of CDC and ADCC	Anti-sense phosphorothioate oligonucleotides (S-ODNs) downmodulation of CD46 and CD55	(Zell et al. 2007)
	REST-derived peptide for modulation of CD59 expression and cell survival genes	(Donev et al. 2008; Tediose et al. 2010)
Improvement of CR3-DCC	Treatment with phosphatidylinositol-specific phospholipase C to remove GPI-anchored CD55 and CD59	(Brasoveanu et al. 1996)
	CR2-Fc fusion in conjunction with MUC1 mAb (ADCC)	(Imai et al. 2007)
	Miniantibodies against CD55 and CD59 in conjunction with therapeutic mAb	(Macor et al. 2007)
	β-glucan	(Vetvicka et al. 1997; Yan et al. 1999)

CR3-positive effector cells in the presence of β-glucan when compared to anti-EpCam antibody alone.

Ziller et al. (2005) produced a single chain Fv (ScFv) ''miniantibodies'' recognising CD55 and CD59. They found that these ScFv miniantibodies used in conjunction with rituximab increased CDC in vitro twofold compared to rituximab alone. The same miniantibodies were used in a mouse model of human B-cell lymphoma and mice treated with both of them and rituximab showed increased survival compared to mice treated with rituximab alone (Macor et al. 2007). In order to avoid binding of these miniantibodies to CD55 and CD59 on normal mouse cells the authors employed very interesting three-ways system by first injection with rituximab–biotin followed by avidin and then biotinylated miniantibodies. Interestingly they found that these miniantibodies also increase ADCC (Ziller et al. 2005).

Bi-specific antibodies showed promising results increasing tumour immunoclearance in vitro. However, there are difficulties with their targeting in vivo, which probably is the major reason for the relatively low number of clinical trials with these antibodies (source: http://clinicaltrials.gov/ct2/results?term=cancer+AND+bispecific+antibodies). Currently there are no clinical trials using bi-specific antibodies blocking complement regulators on tumour surface.

RNA Interference

Ever since the discovery of RNA interference was made in the early nineties it attracted attention for its therapeutic potential as it can be used to knock-down protein expression. Loberg et al. (2006) described short interfering RNA (siRNA) that suppressed CD55 expression in PC-3 and DU145 cells and thus increased CDC against these cell lines and attenuated the overall tumour burden in vivo. Zell et al. (2007) used anti-sense phosphorothioate oligonucleotides (nuclease resistant oligonucleotides) to knock-down up to 80% of CD46 and CD55 expression in breast, lung and prostate carcinoma cell lines. The knock-down resulted in enhanced C3 deposition and improved CDC on some of the treated cell lines.

Some lung tumour cell lines such as A549 secrete FH, which protects them from complement cytotoxicity. Ajona et al. (2007) used siRNA to knock-down the expression of FH and observed a greater C3 deposition and C5a release than controls. Interestingly, combined inhibition of CD59 and FH with antibodies yielded further tumour sensitivity to CDC. When using the A549 cell line manipulated in vitro with siRNA against FH and injected in athymic mice, the growth of FH-deficient cells was reduced due to enhanced complement activation on these cells.

siRNA could also be used in vivo after cloning into a retroviral vector (Shi et al. 2009). In this study silencing CD59 in A2780 cancer ovary cells lead to a significant increase in apoptosis, shown by caspases-3 activity, and complement sensitivity in vivo. The size of the tumours was also significantly smaller than controls. However, there are moral and epidemiological concerns when using retroviral vectors in humans. So far, there are only four completed clinical trials using siRNAs, however, none of these siRNAs targets tumour immunoclearance (source: http://clinicaltrials.gov/ct2/results?term=cancer+sirna).

Targeting Transcription Factors: A New Approach in Tumour Immunology

Transcription factors (TF) have been implicated in many diseases including cancer. A number of studies have identified them as potential therapeutic targets in cancer (Marigo et al. 2010). TFs lie at the centre of how a cell responds to its environment. They are usually downstream of many cell stimuli and thus targeting them could potentially have a similar effect to targeting the molecules upstream. Targeting them could also result into a better disease response, as they are usually involved in controlling a number of cell processes (Brennan et al. 2008). There are many hurdles that need to be overcome before targeting TFs can be a viable treatment option. First they are in the cytoplasm or in the nucleus that makes them more difficult to target unlike membrane expressed molecules. Because of their involvement in many different processes interference may lead to much lower specificity and unwanted side effects.

Strategies which target TFs could involve either indirect targeting by influencing more upstream molecules like kinases or by a more direct approach. Direct strategies include: (a) peptides that specifically block transcription factor dimerisation, (b) peptides that target specific TF DNA-binding sequences (c) or decoy oligonucleotides containing the TF specific response element sequence. These strategies are discussed in detail in Brennan et al. (2008). Here we would like to discuss a few recent papers that used this strategy in the context of cancer immunotherapy and the complement system.

There are only a handful of papers seeking to target TFs that control the expression of the mCRegs thus sensitising them for complement attack. In a study from 2008 we designed peptides that target neural restrictive silencer factor (REST). REST has been identified as a TF that controls the expression of CD59. Using peptides derived from the DNA-binding domain of REST (called REST68), endogenous transcription activators were removed from the CD59 promoter. This lowered significantly the expression of CD59 on the surface of a number of neuroblastoma cell lines. This resulted in higher sensitivity to complement-mediated lysis as triggered by the classical pathway using anti-TAA GD2-disialoganglioside antibody (Donev et al. 2008). This work was recently extended and it was demonstrated that this peptide also sensitises tumours to ADCC by peripheral blood mononuclear cells by instigating apoptosis (Kolev et al., in preparation).

Peptide-based approaches to target TFs hold good promise as an adjuvant for cancer immunotherapy but clearly a good understanding of the underlying molecular mechanisms is required in order to choose the right target. Targeting TFs could be beneficial because of the wide array of effects expected due to their involvement in number of cellular processes. Unfortunately this could also translate into lower specificity, off-target effects and difficulty in discriminating between normal and malignant cells. For this reason careful target selection and effective tumour delivery strategies may be required. Apparently, when these factors are considered, therapeutic peptides are very promising approach for the treatment of cancer. There are 464 clinical trials either completed or ongoing using therapeutic anticancer peptides (http://clinicaltrials.gov/ct2/results?term=cancer+peptide), however, there are no data for trials with peptides sensitising tumours to immunoclearance.

Concluding Remarks: Inhibition of Complement in Context of Immunotherapy Against Tumours—the Good versus the Bad

Choosing the right target in the context of complement system in cancer immunotherapy might be of great importance. Complement has been shown, not only to help in tumour clearance, but also to promote tumour growth. Recent study from Markiewski et al. (2008) showed that complement activation and C5a anaphylatoxin in particular could promote tumour growth. In this elegant study C5a was found to promote suppressive capacity of myeloid-derived suppressor cells (MDSCs), which in turn suppressed $CD8^+$ T-cell anticancer activity. This resulted in larger tumour volumes in vivo in wild type mice when compared with C3 or C4 deficient knockout animals. C5a receptor (C5aR) deficient animals had smaller tumours than the wild type mice, suggesting that C5aR signalling is important in promoting tumour growth. In line with this result was the observation that therapeutically blocking C5aR yielded a decrease in tumour volume. According to the authors this was due to decreased number and activity (measured by generation of suppressive reactive oxygen species) of MDSCs in the tumours.

On the other hand, cells involved in tumour clearance (NK cells, T cells) are less efficient when complement activation products are missing in vitro (Imai et al. 2007; and our own unpublished data). Therefore, the overall effect of inhibiting a regulator of the complement system on cancer growth and cancer immunotherapy needs to be assessed very carefully. The benefit of inhibiting expression and/or activity of CD59 is less ambiguous because this mCRegs does not control the generation of complement activation products promoting tumour survival. Considering the above issues, we believe that greater results in tumour immunoclearance will be achieved by simultaneous modulation of expression of multiple complement and immunoregulators as well as cell survival/growth genes. The most efficient way to achieve this is by identifying and targeting a single TF controlling expression of the majority of these genes. Our published and unpublished in vitro and in vivo data strongly support this point of view.

References

Ajona D, Hsu Y, Corrales L et al (2007) Down-regulation of human complement factor H sensitizes non-small cell lung cancer cells to complement attack and reduces in vivo tumor growth. J Immunol 178:5991–5998

Aksamit R, Falk W, Leonard E (1981) Chemotaxis by mouse macrophage cell lines. J Immunol 126:2194–2199

Astier A, Trescol-Biémont M, Azocar O et al (2000) Cutting edge: CD46, a new costimulatory molecule for T cells, that induces p120CBL and LAT phosphorylation. J Immunol 164:6091–6095

Bjørge L, Matre R (1995) Down-regulation of CD59 (protectin) expression on human colorectal adenocarcinoma cell lines by levamisole. Scand J Immunol 42:512–516

Bjørge L, Jensen TS, Ulvestad E et al (1995) The influence of tumour necrosis factor-alpha, interleukin-1 beta and interferon-gamma on the expression and function of the complement regulatory protein CD59 on the human colonic adenocarcinoma cell line HT29. Scand J Immunol 41:350–356

Bjørge L, Hakulinen J, Vintermyr OK et al (2005) Ascitic complement system in ovarian cancer. Br J Cancer 92:895–905

Blok V, Daha M, Tijsma O et al (1998) A bispecific monoclonal antibody directed against both the membrane-bound complement regulator CD55 and the renal tumor-associated antigen G250 enhances C3 deposition and tumor cell lysis by complement. J Immunol 160:3437–3443

Blok VT, Gelderman K, Tijsma OH et al (2003) Cytokines affect resistance of human renal tumour cells to complement-mediated injury. Scand J Immunol 57:591–599

Blom A, Villoutreix B, Dahlbäck B (2004a) Complement inhibitor C4b-binding protein-friend or foe in the innate immune system? Mol Immunol 40:1333–1346

Blom A, Villoutreix B, Dahlbäck B (2004b) Functions of human complement inhibitor C4b-binding protein in relation to its structure. Arch Immunol Ther Exp 52:83–95

Bodian D, Davis S, Morgan B et al (1997) Mutational analysis of the active site and antibody epitopes of the complement-inhibitory glycoprotein, CD59. J Exp Med 185:507–516

Brasoveanu L, Altomonte M, Fonsatti E et al (1996) Levels of cell membrane CD59 regulate the extent of complement-mediated lysis of human melanoma cells. Lab Invest 74:33–42

Brennan P, Donev R, Hewamana S (2008) Targeting transcription factors for therapeutic benefit. Mol Biosyst 4:909–919

Bubeck D, Roversi P, Donev R et al (2011) Structure of human complement C8, a precursor to membrane attack. J Mol Biol 405:325–330

Catterall C, Lyons A, Sim R et al (1987) Characterization of primary amino acid sequence of human complement control protein factor I from an analysis of cDNA clones. Biochem J 242:849–856

Chen S, Caragine T, Cheung N et al (2000) CD59 expressed on a tumor cell surface modulates decay-accelerating factor expression and enhances tumor growth in a rat model of human neuroblastoma. Cancer Res 60:3013–3018

Cherukuri A, Cheng PC, Pierce SK (2001) The role of the CD19/CD21 complex in B cell processing and presentation of complement-tagged antigens. J Immunol 167:163–172

Cicardi M, Zingale L, Zanichelli A et al (2005) C1 inhibitor: molecular and clinical aspects. Springer Semin Immunopathol 27:286–298

Coburn B, Li Y, Owen D et al (2005) *Salmonella enterica* serovar Typhimurium pathogenicity island 2 is necessary for complete virulence in a mouse model of infectious enterocolitis. Infect Immun 73:3219–3227

Cooper N (1969) Immune adherence by the fourth component of complement. Science 165:396–398

Cragg M, Glennie M (2004) Antibody specificity controls in vivo effector mechanisms of anti-CD20 reagents. Blood 103:2738–2743

Davis AE 3rd, Mejia P, Lu F (2008) Biological activities of C1 inhibitor. Mol Immunol 45:4057–4063

Di Gaetano N, Cittera E et al (2003) Complement activation determines the therapeutic activity of rituximab in vivo. J Immunol 171:1581–1587

Donev RM, Gray LC, Sivasankar B et al (2008) Modulation of CD59 expression by restrictive silencer factor-derived peptides in cancer immunotherapy for neuroblastoma. Cancer Res 68:5979–5987

Donin N, Jurianz K, Ziporen L et al (2003) Complement resistance of human carcinoma cells depends on membrane regulatory proteins, protein kinases and sialic acid. Clin Exp Immunol 131:254–263

Ehrengruber MU, Geiser T, Deranleau DA (1994) Activation of human neutrophils by C3a and C5A. Comparison of the effects on shape changes, chemotaxis, secretion, and respiratory burst. FEBS Lett 346:181–184

Fearon DT, Carroll MC (2000) Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. Annu Rev Immunol 18:393–422

Fischer WH, Hugli TE (1997) Regulation of B cell functions by C3a and C3a(desArg): suppression of TNF-alpha, IL-6, and the polyclonal immune response. J Immunol 159:4279–4286

Fishelson Z, Donin N, Zell S et al (2003) Obstacles to cancer immunotherapy: expression of membrane complement regulatory proteins (mCRPs) in tumors. Mol Immunol 40:109–123

Flieger D, Renoth S, Beier I et al (2000) Mechanism of cytotoxicity induced by chimeric mouse human monoclonal antibody IDEC-C2B8 in CD20-expressing lymphoma cell lines. Cell Immunol 204:55–63

Fukuoka Y, Hugli T (1988) Demonstration of a specific C3a receptor on guinea pig platelets. J Immunol 140:3496–3501

Gancz D, Fishelson Z (2009) Cancer resistance to complement-dependent cytotoxicity (CDC): problem-oriented research and development. Mol Immunol 46:2794–2800

Geis N, Zell S, Rutz R et al (2010) Inhibition of membrane complement inhibitor expression (CD46, CD55, CD59) by siRNA sensitizes tumor cells to complement attack in vitro. Curr Cancer Drug Targets 10:922–931

Gelderman KA, Blok VT, Fleuren GJ et al (2002a) The inhibitory effect of CD46, CD55, and CD59 on complement activation after immunotherapeutic treatment of cervical carcinoma cells with monoclonal antibodies or bispecific monoclonal antibodies. Lab Invest 82:483–493

Gelderman KA, Kuppen PJ, Bruin W et al (2002b) Enhancement of the complement activating capacity of 17–1A mAb to overcome the effect of membrane-bound complement regulatory proteins on colorectal carcinoma. Eur J Immunol 32:128–135

Gelderman KA, Tomlinson S, Ross GD et al (2004) Complement function in mAb-mediated cancer immunotherapy. Trends Immunol 25:158–164

Gelderman KA, Lam S, Sier CF et al (2006) Cross-linking tumor cells with effector cells via CD55 with a bispecific mAb induces beta-glucan-dependent CR3-dependent cellular cytotoxicity. Eur J Immunol 36:977–984

Golay J, Zaffaroni L, Vaccari T et al (2000) Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab in vitro: CD55 and CD59 regulate complement-mediated cell lysis. Blood 95:3900–3908

Gorter A, Meri S (1999) Immune evasion of tumor cells using membrane-bound complement regulatory proteins. Immunol Today 20:576–582

Guo RF, Ward PA (2005) Role of C5a in inflammatory responses. Annu Rev Immunol 23:821–852

Hakulinen J, Meri S (1994) Expression and function of the complement membrane attack complex inhibitor protectin (CD59) on human breast cancer cells. Lab Invest 71:820–827

Halperin JA, Taratuska A, Nicholson-Weller A (1993) Terminal complement complex C5b-9 stimulates mitogenesis in 3T3 cells. J Clin Invest 91:1974–1978

Harjunpää A, Junnikkala S, Meri S (2000) Rituximab (anti-CD20) therapy of B-cell lymphomas: direct complement killing is superior to cellular effector mechanisms. Scand J Immunol 51:634–641

Harris CL, Kan KS, Stevenson GT et al (1997) Tumour cell killing using chemically engineered antibody constructs specific for tumour cells and the complement inhibitor CD59. Clin Exp Immunol 107:364–371

Hartmann K, Henz BM, Krüger-Krasagakes S et al (1997) C3a and C5a stimulate chemotaxis of human mast cells. Blood 89:2863–2870

Heeger PS, Lalli PN, Lin F et al (2005) Decay-accelerating factor modulates induction of T cell immunity. J Exp Med 201:1523–1530

Heinen S, Hartmann A, Lauer N et al (2009) Factor H-related protein 1 (CFHR-1) inhibits complement C5 convertase activity and terminal complex formation. Blood 114:2439–2447

Hofman P, Hsi B, Manie S et al (1994) High expression of the antigen recognized by the monoclonal antibody GB24 on human breast carcinomas: a preventive mechanism of malignant tumor cells against complement attack? Breast Cancer Res Treat 32:213–219

Idusogie EE, Wong PY, Presta LG et al (2001) Engineered antibodies with increased activity to recruit complement. J Immunol 166:2571–2575

Imai M, Ohta R, Varela JC et al (2007) Enhancement of antibody-dependent mechanisms of tumor cell lysis by a targeted activator of complement. Cancer Res 67:9535–9541

Jokiranta TS, Zipfel PF, Hakulinen J et al (1996) Analysis of the recognition mechanism of the alternative pathway of complement by monoclonal anti-factor H antibodies: evidence for multiple interactions between H and surface bound C3b. FEBS Lett 393:297–302

Juhl H, Petrella EC, Cheung NK et al (1990) Complement killing of human neuroblastoma cells: a cytotoxic monoclonal antibody and its F(ab′)2-cobra venom factor conjugate are equally cytotoxic. Mol Immunol 27:957–964

Juhl H, Sievers M, Baltzer K et al (1995) A monoclonal antibody-cobra venom factor conjugate increases the tumor-specific uptake of a 99mTc-labeled anti-carcinoembryonic antigen antibody by a two-step approach. Cancer Res 55(23 suppl):5749s–5755s

Juhl H, Helmig F, Baltzer K et al (1997a) Frequent expression of complement resistance factors CD46, CD55, and CD59 on gastrointestinal cancer cells limits the therapeutic potential of monoclonal antibody 17-1A. J Surg Oncol 64:222–230

Juhl H, Petrella EC, Cheung NK et al (1997b) Additive cytotoxicity of different monoclonal antibody-cobra venom factor conjugates for human neuroblastoma cells. Immunobiology 197:444–459

Junnikkala S, Hakulinen J, Meri S (1994) Targeted neutralization of the complement membrane attack complex inhibitor CD59 on the surface of human melanoma cells. Eur J Immunol 24:611–615

Junnikkala S, Jokiranta TS, Friese MA et al (2000) Exceptional resistance of human H2 glioblastoma cells to complement-mediated killing by expression and utilization of factor H and factor H-like protein 1. J Immunol 164:6075–6081

Kemper C, Chan AC, Green JM et al (2003) Activation of human CD4+ cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype. Nature 421:388–392

Kemper C, Atkinson JP, Hourcade DE (2010) Properdin: emerging roles of a pattern-recognition molecule. Annu Rev Immunol 28:131–155

Kennedy AD, Solga MD, Schuman TA et al (2003) An anti-C3b(i) mAb enhances complement activation, C3b(i) deposition, and killing of CD20+ cells by rituximab. Blood 101:1071–1079

Kilgore KS, Schmid E, Shanley TP et al (1997) Sublytic concentrations of the membrane attack complex of complement induce endothelial interleukin-8 and monocyte chemoattractant protein-1 through nuclear factor-kappa B activation. Am J Pathol 150:2019–2031

Kishore U, Reid KB (2000) C1q: structure, function, and receptors. Immunopharmacology 49:159–170

Klos A, Tenner AJ, Johswich KO et al (2009) The role of the anaphylatoxins in health and disease. Mol Immunol 46:2753–2766

Kraus S, Fishelson Z (2000) Cell desensitization by sublytic C5b-9 complexes and calcium ionophores depends on activation of protein kinase C. Eur J Immunol 30:1272–1280

Kraus S, Seger R, Fishelson Z (2001) Involvement of the ERK mitogen-activated protein kinase in cell resistance to complement-mediated lysis. Clin Exp Immunol 123:366–374

Kroesen BJ, McLaughlin PM, Schuilenga-Hut PH et al (2002) Tumor-targeted immune complex formation: effects on myeloid cell activation and tumor-directed immune cell migration. Int J Cancer 98:857–863

Kumar S, Vinci JM, Pytel BA et al (1993) Expression of messenger RNAs for complement inhibitors in human tissues and tumors. Cancer Res 53:348–353

Lett-Brown MA, Leonard EJ (1977) Histamine-induced inhibition of normal human basophil chemotaxis to C5a. J Immunol 118:815–818

Liszewski MK, Fang CJ, Atkinson JP (2008) Inhibiting complement activation on cells at the step of C3 cleavage. Vaccine 26(suppl 8):I22–I27

Liu J, Miwa T, Hilliard B et al (2005) The complement inhibitory protein DAF (CD55) suppresses T cell immunity in vivo. J Exp Med 201:567–577

Loberg RD, Day LL, Dunn R et al (2006) Inhibition of decay-accelerating factor (CD55) attenuates prostate cancer growth and survival in vivo. Neoplasia 8:69–78

Longhi MP, Sivasankar B, Omidvar N et al (2005) Cutting edge: murine CD59a modulates antiviral CD4+ T cell activity in a complement-independent manner. J Immunol 175:7098–7102

Longhi MP, Harris CL, Morgan BP et al (2006) Holding T cells in check—a new role for complement regulators? Trends Immunol 27:102–108

Lucas SD, Karlsson-Parra A, Nilsson B et al (1996) Tumor-specific deposition of immunoglobulin G and complement in papillary thyroid carcinoma. Hum Pathol 27:1329–1335

Macor P, Tripodo C, Zorzet S et al (2007) In vivo targeting of human neutralizing antibodies against CD55 and CD59 to lymphoma cells increases the antitumor activity of rituximab. Cancer Res 67:10556–10563

Manches O, Lui G, Chaperot L et al (2003) In vitro mechanisms of action of rituximab on primary non-Hodgkin lymphomas. Blood 101:949–954

Manderson A, Pickering M, Botto M et al (2001) Continual low-level activation of the classical complement pathway. J Exp Med 194:747–756

Marigo I, Bosio E, Solito S et al (2010) Tumor-induced tolerance and immune suppression depend on the C/EBPbeta transcription factor. Immunity 32:790–802

Markiewski MM, DeAngelis RA, Benencia F et al (2008) Modulation of the antitumor immune response by complement. Nat Immunol 9:1225–1235

Meri S, Morgan BP, Davies A et al (1990) Human protectin (CD59), an 18,000–20,000 MW complement lysis restricting factor, inhibits C5b-8 catalysed insertion of C9 into lipid bilayers. Immunology 71:1–9

Meri S, Vakeva A, Laari T et al (1991) Soluble forms of CD59-antigen: distribution in body fluids and functional activity. Comp Inflam 8:193

Meri S, Lehto T, Sutton CW et al (1996) Structural composition and functional characterization of soluble CD59: heterogeneity of the oligosaccharide and glycophosphoinositol (GPI) anchor revealed by laser-desorption mass spectrometric analysis. Biochem J 316(Pt 3):923–935

Minta JO, Fung M, Turner S et al (1998) Cloning and characterization of the promoter for the human complement factor I (C3b/C4b inactivator) gene. Gene 208:17–24

Mizuno M, Nakagawa M, Uesu T et al (1995) Detection of decay-accelerating factor in stool specimens of patients with colorectal cancer. Gastroenterology 109:826–831

Morgan BP (1992) Effects of the membrane attack complex of complement on nucleated cells. Curr Top Microbiol Immunol 178:115–140

Moskovich O, Fishelson Z (2007) Live cell imaging of outward and inward vesiculation induced by the complement c5b-9 complex. J Biol Chem 282:29977–29986

Murray EW, Robbins SM (1998) Antibody cross-linking of the glycosylphosphatidylinositol-linked protein CD59 on hemato-poietic cells induces signaling pathways resembling activation by complement. J Biol Chem 273:25279–25284

Nagasawa S, Ichihara C, Stroud RM (1980) Cleavage of C4b by C3b inactivator: production of a nicked form of C4b, C4b′, as an intermediate cleavage product of C4b by C3b inactivator. J Immunol 125:578–582

Nakano Y, Sumida K, Kikuta N et al (1992) Complete determination of disulfide bonds localized within the short consensus repeat units of decay accelerating factor (CD55 antigen). Biochim Biophys Acta 1116:235–240

Nataf S, Davoust N, Ames RS et al (1999) Human T cells express the C5a receptor and are chemoattracted to C5a. J Immunol 162:4018–4023

Natsume A, Niwa R, Satoh M (2009) Improving effector functions of antibodies for cancer treatment: enhancing ADCC and CDC. Drug Des Devel Ther 3:7–16

Nicholson-Weller A, Halperin JA (1993) Membrane signaling by complement C5b-9, the membrane attack complex. Immunol Res 12:244–257

Niculescu F, Rus HG, Retegan M et al (1992) Persistent complement activation on tumor cells in breast cancer. Am J Pathol 140:1039–1043

Niehans GA, Cherwitz DL, Staley NA et al (1996) Human carcinomas variably express the complement inhibitory proteins CD46 (membrane cofactor protein), CD55 (decay-accelerating factor), and CD59 (protectin). Am J Pathol 149:129–142

Nielsen CH, Antonsen S, Matthiesen SH et al (1997a) The roles of complement receptors type 1 (CR1, CD35) and type 3 (CR3, CD11b/CD18) in the regulation of the immune complex-elicited respiratory burst of polymorphonuclear leukocytes in whole blood. Eur J Immunol 27:2914–2919

Nielsen CH, Matthiesen SH, Lyng I et al (1997b) The role of complement receptor type 1 (CR1, CD35) in determining the cellular distribution of opsonized immune complexes between whole blood cells: kinetic analysis of the buffering capacity of erythrocytes. Immunology 90:129–137

Ollert MW, Kadlec JV, David K et al (1994) Antibody-mediated complement activation on nucleated cells. A quantitative analysis of the individual reaction steps. J Immunol 153:2213–2221

Ottonello L, Corcione A, Tortolina G et al (1999) rC5a directs the in vitro migration of human memory and naive tonsillar B lymphocytes: implications for B cell trafficking in secondary lymphoid tissues. J Immunol 162:6510–6517

Pilzer D, Gasser O, Moskovich O et al (2005) Emission of membrane vesicles: roles in complement resistance, immunity and cancer. Springer Semin Immunopathol 27:375–387

Purcell DF, Deacon N, Andrew SM et al (1990) Human non-lineage antigen, CD46 (HuLy-m5): purification and partial sequencing demonstrates structural homology with complement-regulating glycoproteins. Immunogenetics 31:21–28

Reff ME, Carner K, Chambers KS et al (1994) Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 83:435–445

Reiter Y, Fishelson Z (1989a) Killing of human tumor cells by antibody C3b conjugates and human complement. Targeted Diagn Ther 2:119–135

Reiter Y, Fishelson Z (1989b) Targeting of complement to tumor cells by heteroconjugates composed of antibodies and of the complement component C3b. J Immunol 142:2771–2777

Ripoche J, Day AJ, Harris TJ et al (1988) The complete amino acid sequence of human complement factor H. Biochem J 249:593–602

Ross GD, Lambris JD, Cain JA et al (1982) Generation of three different fragments of bound C3 with purified factor I or serum. I. Requirements for factor H vs CR1 cofactor activity. J Immunol 129:2051–2060

Rus HG, Niculescu F, Shin ML (1996) Sublytic complement attack induces cell cycle in oligodendrocytes. J Immunol 156:4892–4900

Rus HG, Niculescu FI, Shin ML (2001) Role of the C5b–9 complement complex in cell cycle and apoptosis. Immunol Rev 180:49–55

Santoro F, Greenstone HL, Insinga A et al (2003) Interaction of glycoprotein H of human herpesvirus 6 with the cellular receptor CD46. J Biol Chem 278:25964–25969

Scharfstein J, Ferreira A, Gigli I et al (1978) Human C4-binding protein. I. Isolation and characterization. J Exp Med 148:207–222

Segerman A, Atkinson JP, Marttila M et al (2003) Adenovirus type 11 uses CD46 as a cellular receptor. J Virol 77:9183–9191

Shafren DR, Bates RC, Agrez MV et al (1995) Coxsackieviruses B1, B3, and B5 use decay accelerating factor as a receptor for cell attachment. J Virol 69:3873–3877

Shi XX, Zhang B, Zang JL et al (2009) CD59 silencing via retrovirus-mediated RNA interference enhanced complement-mediated cell damage in ovary cancer. Cell Mol Immunol 6:61–66

Sim RB, DiScipio RG (1982) Purification and structural studies on the complement-system control protein beta 1H (Factor H). Biochem J 205:285–293

Sivasankar B, Longhi MP, Gallagher KM et al (2009) CD59 blockade enhances antigen-specific CD4+ T cell responses in humans: a new target for cancer immunotherapy? J Immunol 182:5203–5207

Smith BO, Mallin RL, Krych-Goldberg M et al (2002) Structure of the C3b binding site of CR1 (CD35), the immune adherence receptor. Cell 108:769–780

Sokoloff MH, Nardin A, Solga MD et al (2000) Targeting of cancer cells with monoclonal antibodies specific for C3b(i). Cancer Immunol Immunother 49:551–562

Spiridon CI, Ghetie MA, Uhr J et al (2002) Targeting multiple Her-2 epitopes with monoclonal antibodies results in improved antigrowth activity of a human breast cancer cell line in vitro and in vivo. Clin Cancer Res 8:1720–1730

Spitzer D, Mitchell L, Atkinson J et al (2007) Properdin can initiate complement activation by binding specific target surfaces and providing a platform for de novo convertase assembly. J Immunol 179:2600–2608

Sugita Y, Nakano Y, Oda E et al (1993) Determination of carboxyl-terminal residue and disulfide bonds of MACIF (CD59), a glycosyl-phosphatidylinositol-anchored membrane protein. J Biochem 114:473–477

Tedesco F, Pausa M, Nardon E et al (1997) The cytolytically inactive terminal complement complex activates endothelial cells to express adhesion molecules and tissue factor procoagulant activity. J Exp Med 185:1619–1627

Tediose T, Kolev M, Sivasankar B et al (2010) Interplay between REST and nucleolin transcription factors: a key mechanism in the overexpression of genes upon increased phosphorylation. Nucleic Acids Res 38:2799–2812

Thiel S (2007) Complement activating soluble pattern recognition molecules with collagen-like regions, mannan-binding lectin, ficolins and associated proteins. Mol Immunol 44:3875–3888

Thorsteinsson L, O'Dowd GM, Harrington PM et al (1998) The complement regulatory proteins CD46 and CD59, but not CD55, are highly expressed by glandular epithelium of human breast and colorectal tumour tissues. APMIS 106:869–878

Treon SP, Mitsiades C, Mitsiades N et al (2001) Tumor cell expression of CD59 is associated with resistance to CD20 serotherapy in patients with B-cell malignancies. J Immunother 24:263–271

Varela JC, Imai M, Atkinson C et al (2008) Modulation of protective T cell immunity by complement inhibitor expression on tumor cells. Cancer Res 68:6734–6742

Vetvicka V, Thornton BP, Wieman TJ et al (1997) Targeting of natural killer cells to mammary carcinoma via naturally occurring tumor cell-bound iC3b and beta-glucan-primed CR3 (CD11b/CD18). J Immunol 159:599–605

Walport MJ (2001a) Complement. First of two parts. N Engl J Med 344:1058–1066

Walport MJ (2001b) Complement. Second of two parts. N Engl J Med 344:1140–1144

Wang SY, Weiner G (2008) Complement and cellular cytotoxicity in antibody therapy of cancer. Expert Opin Biol Ther 8:759–768

Ward T, Pipkin PA, Clarkson NA et al (1994) Decay-accelerating factor CD55 is identified as the receptor for echovirus 7 using CELICS, a rapid immuno-focal cloning method. EMBO J 13:5070–5074

Weiner LM, Surana R, Wang S (2010) Monoclonal antibodies: versatile platforms for cancer immunotherapy. Nat Rev Immunol 10:317–327

Yan J, Vetvicka V, Xia Y et al (1999) Beta-glucan, a "specific" biologic response modifier that uses antibodies to target tumors for cytotoxic recognition by leukocyte complement receptor type 3 (CD11b/CD18). J Immunol 163:3045–3052

Yan J, Allendorf DJ, Li B et al (2008) The role of membrane complement regulatory proteins in cancer immunotherapy. Adv Exp Med Biol 632:159–174

Ytting H, Jensenius JC, Christensen IJ et al (2004) Increased activity of the mannan-binding lectin complement activation pathway in patients with colorectal cancer. Scand J Gastroenterol 39:674–679

Yu J, Dong S, Rushmere NK et al (1997) Mapping the regions of the complement inhibitor CD59 responsible for its species selective activity. Biochemistry 36:9423–9428

Zell S, Geis N, Rutz R et al (2007) Down-regulation of CD55 and CD46 expression by anti-sense phosphorothioate oligonucleotides (S-ODNs) sensitizes tumour cells to complement attack. Clin Exp Immunol 150:576–584

Zhou X, Hu W, Qin X (2008) The role of complement in the mechanism of action of rituximab for B-cell lymphoma: implications for therapy. Oncologist 13:954–966

Ziller F, Macor P, Bulla R et al (2005) Controlling complement resistance in cancer by using human monoclonal antibodies that neutralize complement-regulatory proteins CD55 and CD59. Eur J Immunol 35:2175–2183

Zipfel PF, Skerka C (2009) Complement regulators and inhibitory proteins. Nat Rev Immunol 9:729–740