Review TRENDS in Immunology Vol.25 No.3 March 2004

Complement function in mAb-mediated cancer immunotherapy

Kyra A. Gelderman[1], Stephen Tomlinson[2], Gordon D. Ross[3] and Arko Gorter[1]

[1]Department of Pathology L1-Q, Leiden University Medical Center, Postbox 9600, 2300 RC Leiden, The Netherlands
[2]Department Microbiology and Immunology, Medical University of South Carolina, 173 Ashley Ave, BSB-201 Charleston, SC 29425, USA
[3]Tumor Immunology Program, James Graham Brown Cancer Center, 529 South Jackson St., Room 319, University of Louisville, Louisville, KY 40202, USA

Complement activation by mAbs can cause direct tumor cell lysis or enhance antibody-dependent cell-mediated cytotoxicity. However, tumor cells are protected from complement-mediated injury by membrane-bound complement regulatory proteins (mCRP) that are often expressed at elevated levels on tumor cells. Recent studies indicate that blocking or overwhelming the function of tumor cell mCRP might substantially improve the efficacy of monoclonal antibody (mAb) immunotherapy. In addition, the use of β-glucan as an adjuvant for mAb immunotherapy enables iC3b deposited on tumor cells by mAbs to activate complement receptor 3 (CR3) on effector cells, thus inducing CR3-dependent cellular cytotoxicity. These strategies provide novel cell-mediated mechanisms of tumor cytotoxicity that are additive to all other mAb effector mechanisms.

Immunotherapy using monoclonal antibodies (mAbs) holds great promise as an anti-cancer therapeutic strategy because of its ability to target cancer cells specifically and spare surrounding normal tissue. This is an important advantage over relatively non-specific chemo- and radiotherapeutic treatments. This Review will describe antibody-induced complement-mediated effector mechanisms, evasion of complement injury by tumor cells and how complement activation or regulation can be manipulated to enhance the clinical efficacy of therapeutic mAbs. Until now, there has been some success with the treatment of hematological malignancies with mAbs, although, in general, clinical results have fallen short of expectations. Only seven mAbs have been approved for use in the clinic and five of these are directed against hematological tumors. This Review summarizes data that explain why results might have been disappointing and how improvements might be achieved through modifications of therapy that better use the complement system.

mAb-induced complement-mediated effector mechanisms

Most mAbs that mediate antibody-dependent cellular cytotoxicity (ADCC) also activate the complement system (Box 1 and Figure I in Box 1) [1]. In particular, chimerized or humanized mouse mAbs containing the human IgG1 Fc-region trigger both ADCC and complement activation. Complement initiates three mechanisms that can be used against mAb-coated tumor cells (Figure 1). The first is direct complement killing of tumor cells by the

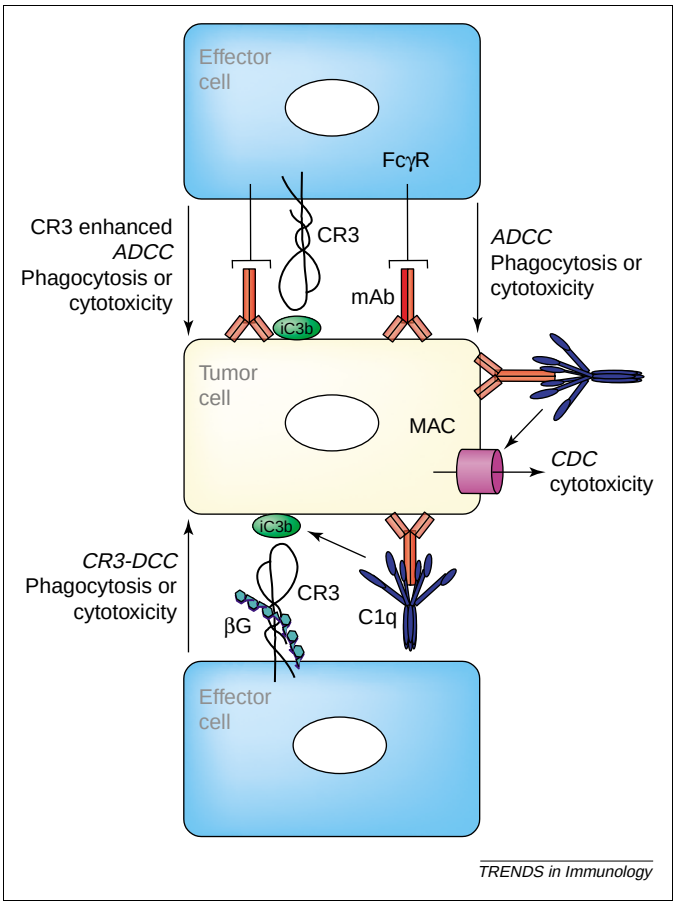

Figure 1. Effector mechanisms of monoclonal antibodies (mAbs). mAbs bound to target antigen can be recognized by Fc-receptors (FcRs) expressed on immune cells, such as granulocytes, natural killer (NK) cells, monocytes and macrophages, leading to antibody-dependent cellular cytotoxicity (ADCC). mAbs can also activate the complement system following binding of C1q to the mAb Fc region. Complement activation results in the deposition of C3b, which is subsequently converted to iC3b, and can lead to the formation of the cytolytic membrane attack complex (MAC or C5b-9), resulting in complement-dependent cytotoxicity (CDC). ADCC can be enhanced by complement receptor 3 (CR3) binding to iC3b, thus enhancing FcγR-mediated effector-cell binding. Ligation of iC3b by CR3 can, on CR3 priming with β-glucan (βG), induce CR3-dependent cellular cytotoxicity (CR3-DCC).

Corresponding author: Arko Gorter (A.Gorter@lumc.nl).

 1471-4906/$ - see front matter doi:10.1016/j.it.2004.01.008

Box 1. Activation and regulation of the complement system

The classical pathway of the complement system is activated following binding of monoclonal antbodies (mAbs) to tumor cells (Figure I). This pathway is initiated on binding of the C1 complex to mAbs (in close proximity) on the tumor-cell membrane. The lectin pathway and alternative pathways are generally activated by pathogens and not by cell-bound mAbs and are therefore not further discussed. Complement activation on a cell surface results in the formation of the membrane bound C3- and C5-convertases, which are enzyme complexes that cleave and activate C3 and C5, respectively. The cleavage of C3 results in the generation of C3b, a small number of which become covalently attached to the cell surface. Cell-surface bound C3b is involved in amplifying the complement cascade, however, because complement activation is tightly regulated on host tissue, including host-derived tumor cells, C3b is rapidly degraded into the fragments iC3b and C3dg. These cell-bound fragments can function to promote complement receptor-enhanced antibody-dependent cellular cytotoxicity (ADCC) through binding to CR3 on leukocytes [2]. C5 cleavage by the C5-convertase results in fragmentation into C5a and C5b. C5a is the most important inflammatory mediator of the complement system and it functions to recruit and activate immune effector cells at the site of complement activation. C5b binds to the cell membrane and initiates formation of the membrane attack complex (MAC or C5b-9). This complex can cause direct complement-dependent cytotoxicity (CDC) through the formation of membrane-penetrating pores.

To prevent uncontrolled activation and consumption of complement components, complement activation is tightly regulated by complement regulatory proteins (CRPs). CRPs are present as soluble proteins in the fluid phase and as membrane-bound complement regulatory proteins (mCRPs) on most cell types. The mCRPs comprise CR1, CD46, CD55 and CD59 (Figure I). CR1 and CD46 are cofactors for factor I-mediated degradation of C3b and C4b (to iC3b and iC4b, respectively), preventing formation of new convertases, whereas CD55 accelerates the decay of C3- and C5-convertases, thereby preventing activation of downstream complement proteins [1]. Rodents express an additional mCRP, termed Crry, a structural and functional analogue of CR1 but with a wider cell type distribution [35]. Thus, by inhibiting complement activation, CR1, CD46 and CD55 (and Crry in rodents) limit the generation of the opsonic fragments C3b and iC3b and of the chemotactic factors C3a and C5a. Finally, CD59 binds to complement proteins C8 and C9 during MAC assembly and prevents complement (MAC)-dependent cell lysis (CDC).

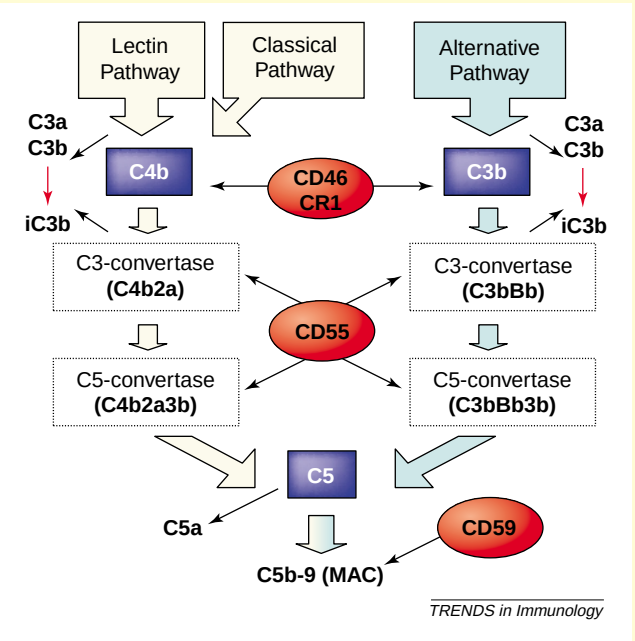

Figure I. The human complement system and complement regulatory proteins CR1, CD46, CD55 and CD59.

membrane attack complex (MAC), a process usually called 'complement-dependent cytotoxicity' (CDC). The second mechanism is complement receptor-dependent enhancement of ADCC. In this case, CR3 binds to iC3b, thus enhancing FcγR-mediated effector cell binding. A third mechanism used for killing microorganisms, CR3-dependent cellular cytotoxicity (CR3-DCC), is usually not activated with tumors [2]. Nevertheless, as outlined later, specific interventions can be made that make both CDC and CR3-DCC important mechanisms of mAb-mediated tumor therapy.

CR3-DCC as an anti-tumor effector mechanism

The C3 receptors on human leukocytes, CR1 and CR3, do not trigger the killing of tumor cells coated with their respective ligands, C3b and iC3b. However, considerable evidence has now been reported that CR3 (CD11b–CD18), expressed by phagocytes and natural killer (NK) cells, can be manipulated in such a way that it will trigger cytotoxicity of iC3b-coated tumor cells. CR3-DCC is normally reserved for yeast and fungi that bear β-glucan as an exposed component of their cell wall. Induction of cytotoxicity by iC3b-coated yeast requires the dual ligation of CR3 to both iC3b and cell wall β-glucan. Yeast cell wall β-glucan binds to a C-terminal lectin domain of CD11b, priming CR3 for efficient cytotoxic degranulation responses after it also binds iC3b by a N-terminal I-domain binding site of CD11b [2]. In contrast to microorganisms, tumor cells, as well as other host cells, lack β-glucan as a surface component and therefore cannot trigger CR3-DCC, even when heavily coated with iC3b. Soluble β-glucan binds to CR3 and primes the receptor in a way that enables it to trigger cytotoxic degranulation of iC3b-coated tumors that lack endogenous β-glucan [3,4]. Mice injected intravenously with soluble yeast β-glucan exhibit tumor regression and long-term survival [5,6]. This activity of β-glucan requires that tumor cells be coated with iC3b through the action of either naturally occurring anti-tumor Abs or intravenously administered anti-tumor mAbs that activate complement. This adjuvant activity of β-glucan did not occur with a mouse IgG1 mAb that did not activate complement but did occur with an IgM complement-activating mAb [7]. Also, tumor regression by combined mAb and yeast β-glucan therapy did not occur in mice deficient in either C3 or CR3, highlighting the requirement for both iC3b deposited on tumor cells (by complement-activating mAbs) and its receptor CR3 on the phagocytes [5,6].

Anti-tumor mAbs in clinical use

Seven anti-cancer mAbs are now approved for clinical use and another ten are currently being tested in Phase III clinical trials [8,9] (Table 1). Each of these mAbs has been examined to determine their mechanism of action in mediating therapeutic responses. Many mAbs have been modified with the objective of improving ADCC mediated

Table 1. Currently approved mAbs for treating cancer and mAbs tested in Phase III clinical trials[a]

Isotype[b]/origin	Target	Mab names	Conjugated	Cancer type	Approved
hIgG1	HER2/neu	Trastuzumab (Herceptin®)	No	Breast cancer	Yes (US)
hIgG1	VEGF	Bevacizumab	No	Breast cancer, colorectal cancer, renal cell carcinoma, non-small cell lung cancer	Phase III
hIgG1	CD33	Zamyl™	No	Acute myelogenous leukemia	Phase III
hIgG1	CD52	Alemtuzumab (Campath®)	No	Chronic lymphatic leukemia	Yes (US)
hIgG1	CD22	Epratuzumab		Non-Hodgkin lymphoma	Phase III
hIgG4	CD33	Gemtuzumab (Mylotarg®)	Yes (cali-cheamicin)	Acute myelogenous leukemia	Yes (US)
cIgG1	EGFR1	Cetuximab	No	Colorectal cancer	Yes (Switzerland)
cIgG1	CD20	Rituximab (Rituxan®)	No	Non-Hodgkin lymphoma	Yes (US)
cIgG1	DNA-associated antigens	Cotara™	Yes (^{131}I)	Glioma	Phase III
mIgG2a	Ep–CAM	Edrecolomab	No	Colorectal cancer	Yes (Germany)
mIgG2a	GD3	Mitomomab	No	Small cell lung cancer	Phase III
mIgG2a	CD20	Tositumomab (Bexxar®)	Yes (^{131}I)	Non-Hodgkin lymphoma	Yes
mIgG1	CEA	CeaVac™	No	Colorectal cancer	Phase III
mIgG1	CD20	Ibritumomab (Zevalin™)	Yes (^{90}Y)	Non-Hodgkin lymphoma	Yes (US)
mIgG1	CA125	OvaRex®	No	Ovarian carcinoma	Phase III
mIgG1	MUC1	Pemtumomab	Yes (^{90}Y)	Ovarian carcinoma	Phase III

[a]Abbreviations: CEA, carcino-embryonic antigen; cIg, chimerized Ig; EGFR, epithelial growth factor receptor; Ep–CAM, epithelial cell adhesion molecule; HER2/neu, EGFR2; hIg, humanized Ig; mAbs, monoclonal antibodies; mIg, mouse Ig; MUC1, human mucin 1 (episialin) VEGF, vascular endothelial growth factor.
[b]In general, the following isotypes efficiently fix human complement: mIgG2a, mIgG2b, mIgG3, mIgM, hIgG1, hIgG4, hIgM.

by Fcγ-receptors (FcγR) expressed on leukocytes. The importance of ADCC as a cytotoxic mechanism of anti-tumor mAbs has been demonstrated in animal studies. Ravetch *et al*. showed that the tumoricidal effect of a humanized anti-HER2/neu mAbs (epithelial growth factor receptor 2; Trastuzumab) was significantly reduced in FcγR knockout nude mice as compared to wild-type nude mice [10]. Similarly, the tumor regression activity of a chimeric anti-CD20 mAb (Rituximab) was significantly reduced in FcγR deficient mice as compared to wild-type mice [10,11]. Further support for an important role for ADCC was provided by a study of Cartron *et al*., who found that in patients with a polymorphism in FcγRIIIa leading to increased binding of IgG1, therapy with an anti-CD20 mAb produced a 90% response rate (patients with complete remission or partial response) at 12 months, compared to a 51% response rate in individuals not expressing this polymorphism of FcγRIIIa [12]. Others have shown that this FcγRIIIa polymorphism and also a polymorphism in FcγRIIa are associated with the response rate to therapeutic mAbs [13]. In contrast to ADCC, there is a paucity of information regarding complement-activation by these clinically used mAbs.

Complement recruitment by clinically approved mAbs

Mouse-anti-Ep–CAM (epithelial cell adhesion molecule) (Edrecolomab) and humanized anti-CD52 (Alemtuzumab) activate the complement system *in vitro* and mediate ADCC [9,14]. Also, anti-HER2/neu and anti-epithelial growth factor receptor 1 (EGFR1) mAbs activate complement *in vitro* [15]. Both chimeric and mouse anti-CD20 mAbs mediate their tumoricidal effects *in vivo* through both ADCC and CDC [11,16,17]. In addition, anti-CD20 mAbs induce apoptosis by cross-linking CD20 [12,18]. However, the primary mechanism of action of the other mAbs currently in use in the clinic does not appear to involve complement as a mechanism of tumor reduction. Nevertheless, because several of these mAbs activate complement on tumor cells *in vitro*, there is potential for improving the efficiency of CDC or CR3-DCC *in vivo*, and these effects would be additive to all the existing effector mechanisms of these mAbs. Other than isotype, one reason why a mAb might not efficiently activate complement is that the tumor cell antigen density might be too low to enable the formation of IgG dimers, which are required for the attachment of C1q and activation of the classical pathway. In this context, a recent publication showed that low HER2/neu antigen density could be circumvented by the use of a cocktail of mAbs that were specific for multiple epitopes on the HER2/neu antigen [19]. This strategy elicited a more efficient CDC *in vitro* and an increase in tumor reduction *in vivo*. For other mAbs, such as humanized anti-vascular endothelial growth factor (VEGF; Bevacizumab), the role of complement activation has not yet been properly investigated, although these mAbs are of a complement activating isotype and activate complement *in vitro*.

Complement regulation on tumor cells

Complement activation seems not to be an important effector mechanism of mAbs, which can be explained by the expression of mCRPs. Both normal and tumor cells are highly resistant to homologous complement attack owing to the expression of mCRPs (Box 1 and Figure I in Box 1). An important role for tumor mCRPs in immune evasion is indicated by the finding that tumor cells often over-express one or more of the mCRPs [20,21]. The ability of mCRPs to protect tumor cells from complement-mediated effector mechanisms has been studied extensively on numerous types of cancer cell lines *in vitro*. These studies have demonstrated the potent inhibitory effect of mCRP on mAb-induced C3b deposition, C5a generation and MAC-mediated lysis [22–25]. Tumor cells might also protect themselves by binding soluble complement inhibitory factors from serum, such as complement factor H (fH), a protective mechanism also used by some microorganisms [26,27]. Sialic acid-rich proteins that bind fH are upregulated by many tumors and overexpression of sialic acid has

been associated with clinical severity [27]. It is also interesting that fH or a related protein is a marker for bladder cancer, suggesting a link between complement resistance and escape from immune surveillance [28].

Overexpression of a particular mCRP has been reported for each tumor type against which a therapeutically approved mAb is directed [20,29,30]. Furthermore, CDC mediated by four of the seven approved mAbs is inhibited *in vitro* by mCRPs expressed on tumor cells. In the case of anti-HER2/neu-mediated cytotoxicity (Trastuzumab), there was an increase from 10% to 80% in CDC following inhibition of mCRP function on tumor cells [25]. Similarly, CD55 and CD59 limit complement-mediated injury and their expression on tumor cells determines the response rate *in vitro* for mAbs against Ep–CAM and CD20 [24,31]. These data suggest that mCRPs indeed inhibit therapeutic effect of mAbs.

Role of mCRPs expressed on tumor cells in experimental animal models and in clinical immunotherapy

Although *in vitro* studies indicate a role for mCRPs in determining the outcome of mAb immunotherapy, only a few studies have investigated the role of mCRPs in appropriate experimental animal cancer models. Because mCRPs act in a species selective fashion [32,33], heterologous animal models involving complement and mCRPs of different species might not be clinically relevant. For example, an anti-tumor mAb might be effective in a rodent model of human cancer because the human mCRPs expressed on the tumor cell are not effective against rodent complement. Such protocols that mix human tumors and rodent complement [e.g. human tumors in nude or severe combined immunodeficient (SCID) mice] might explain why the same antibody that was active against tumors in mice is ineffective in a clinical (homologous) setting. Thus, to investigate mAb efficacy and the effect of mCRP expression, a syngeneic model is more clinically relevant [32–34].

However, data on the effect of mCRPs on mAb therapy in syngeneic models are scarce. Di Gaetano *et al*. recently reported the development of a mouse model of human B-cell lymphoma and demonstrated that complement activation was required for the therapeutic activity of Rituximab in this model [16]. It should be noted that rodents express, in addition to the mCRP described above, an additional C3 regulatory protein, Crry/p65 (Crry) [35]. Crry is the major C3 regulatory protein in rodents (Box 1). Using metastatic murine EL4 cells transfected with human CD20, it was shown that humanized anti-CD20 (Rituximab) and a murine anti-CD20 mAb were therapeutic in wildtype syngeneic mice but not in C1q-deficient syngeneic mice. In another report, a rat model was used that incorporated human breast cancer cells expressing rat Crry or CD59. Crry, but not CD59, expression prevented host regression of tumors as compared to mock-transfected cells [36,37]. However, in a rat model of human neuroblastoma homologous for rat CD59, expression of CD59 promotes tumorigenesis [38]. In these models, complement was activated on the tumor cells by naturally occurring rat anti-tumor antibodies, and the data indicated that different complement-dependent mechanisms were involved in controlling growth of the different types of tumors. In another study, populations of a rat hepatoma cell line expressing either low or high levels of Crry were injected into syngeneic rats, and Crry expression levels were found to correlate inversely with tumor-free survival [39]. In this study, inhibition of Crry with anti-Crry F(ab′)2 substantially increased survival rate, confirming an important role for Crry in promoting tumor growth.

There are several clinical studies indicating that mCRPs expressed on tumor cells provides protection from immune attack. On human tumor cells it is believed that CD55 and CD46 perform the same type of complement inhibitory function that Crry does on rodent cells. CD55 has been identified as a tumor-associated antigen and high expression levels of CD55 on colorectal cancer tissue is correlated with a significant decrease in survival [40]. The functional significance of mCRP on tumor cells in humans is also indicated by the correlation of low CD46 expression with high levels of C3 deposition on renal and cervical cancer tissue [41,42]. Furthermore, peripheral blood leukocytes isolated from patients with chronic lymphocytic leukemia (CLL) who had a poor response to anti-CD20 (Rituximab) treatment, were more sensitive to complement lysis on *in vitro* neutralization of CD55 and CD59 than were leukocytes isolated from patients who did respond to Rituximab therapy [43]. In addition, significantly higher levels of CD59 have been found on CLL cells that were not cleared from the circulation following Rituximab therapy [44]. These data suggest that mCRPs have an important role in inhibiting an optimal clinical effect of Rituximab treatment. However, this is controversial. For example, it has been reported that tumor cell expression levels of CD55 and CD59 do not correlate with percentages of lysis [41] and another study reported that mCRP expression levels do not predict the clinical outcome of Rituximab treatment [43]. Nevertheless, the consensus among different reports favors an important role for complement, and therefore probably mCRPs, in the mechanism of action of Rituximab. The precise details and relative contributions of complement relative to ADCC remain unresolved.

In summary, data from experimental models of cancer and clinical studies suggest that modulating complement susceptibility of a tumor cell has the potential to increase therapeutic efficacy of a mAb by triggering complement-dependent effector mechanisms, whether or not the primary mechanism of action is complement-dependent.

Enhancing mAb immunotherapy by improving complement-mediated effector mechanisms

mAb-mediated immunotherapy might be enhanced by improving complement-mediated effector mechanisms through modulating mCRPs or by application of the adjuvant β-glucan.

Overcoming tumor cell mCRPs

Overwhelming mCRP function (Figure 2) could be achieved by increasing the complement activating capacity of mAbs by changing the isotype or by humanization. These approaches increase complement activation

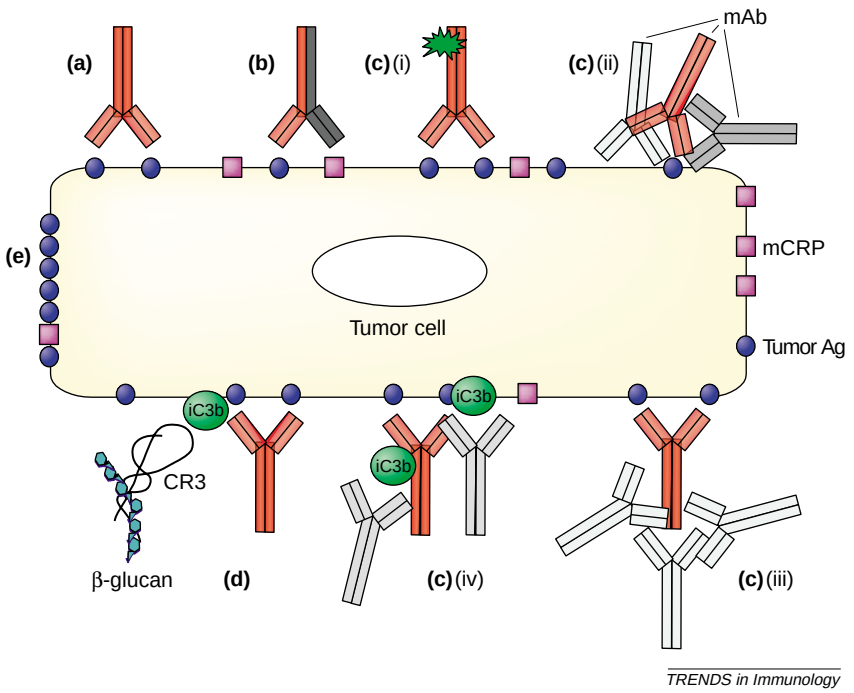

Figure 2. Strategies to improve monoclonal antibody (mAb)-mediated immunotherapy of cancer. **(a)** Engineering of a mAb to improve complement-activating properties. **(b)** Blocking the effect of membrane-bound complement regulatory proteins (mCRPs) with a bispecific mAb directed against a tumor antigen (Ag) and a mCRP. **(c)** Overwhelming the effect of mCRP, by: (i) a mAb conjugated to a complement-activating protein [e.g. cobra venom factor (CVF) or C3b]; (ii) a cocktail of mAbs directed against multiple epitopes of a tumor-associated antigen; (iii) secondary mAbs directed against the tumor-specific mAb; and (iv) mAbs directed against deposited complement fragments (iC3b). **(d)** Enhancing the CR3-dependent cellular cytotoxicity (CR3-DCC) of iC3b-opsonized tumor cells by priming leukocyte CR3 with soluble β-glucan. **(e)** Downregulating the expression of mCRP or increasing the expression of tumor-associated antigens with cytokines or gene therapy.

in vitro [14,45–47]. Other ways to increase complement activation include amplification of the amount of mAb deposited on tumor cells by use of a secondary mAb directed against either the anti-tumor mAb [48] or against the iC3b deposited on tumor cells by the primary mAb [49]. Injection of an anti-iC3b mAb following Rituximab (humanized anti-CD20) treatment in a cynomologous monkey model of lymphoma indeed resulted in enhanced iC3b deposition on the tumor cells [50]. Another potential strategy to enhance complement activation and overwhelm mCRPs is to conjugate an anti-tumor mAb to a complement-activating protein, such as cobra venom factor (CVF) or C3b [51]. The effectiveness of this approach has been shown *in vitro* using either colorectal cancer cells coated with anti-Ep/CAM–CVF conjugates [24], or neuroblastoma cells coated with anti-GD2–CVF conjugates [52,53]. To reduce the expected immunogenic reactions against CVF, the active part of CVF can be genetically inserted in the region coding for the Fc-tail of the appropriate immunotherapeutic mAb [54].

Downregulating the expression of mCRPs has the capacity to enhance mAb-mediated complement activation and some success using various cytokines has been reported *in vitro* [55–57]. Upregulating the expression levels of tumor antigen might also result in increased complement activation. For example, it has been reported that the MUC1 (human mucin 1; episialin) antigen, the target for Pemtumomab, can be upregulated *in vitro* by dexamethasone on myeloma cells [58] and by interferon-γ (IFN-γ) on a broad range of cancer cell lines [59]. Another way to circumvent the effect of mCRP on mAb-induced complement activation is by using mAbs that block the function of mCRPs to enhance the complement susceptibility of tumor cells. However, such an approach cannot be used readily *in vivo* because of the widespread expression of mCRPs on normal tissue. *In vitro* experiments have shown that a bispecific mAb that recognize both a tumor antigen and a mCRP can selectively target tumor cells and enhance their susceptibility to complement deposition and lysis [24,60,61]. Also, *in vivo*, these bispecific mAbs prevent tumor outgrowth in a syngeneic rat model (K.A. Gelderman *et al*., unpublished) Ideally, these bispecific mAbs need to have a higher affinity for the tumor cells than for normal cells expressing the targeted mCRP.

Use of β-glucan to prime CR3-DCC

Soluble yeast β-glucan induces CR3-DCC of tumor cells *in vitro* and *in vivo*. CR3-DCC is additive to all other effector mechanisms of the mAb. Initial reports showed this adjuvant activity with intravenously administered soluble yeast β-glucan [5,6], however, it now appears probable that certain large molecular size β-glucans can have this same complement-dependent adjuvant function when given orally [7]. This is an advantage when being clinically administered. Cheung *et al*. showed that large molecules of barley β-glucan given orally to mice functioned as adjuvants that greatly promoted the tumor regression activity of mAbs that activated complement [7,22,62]. Moreover, studies with mouse tumor models showed that oral β-glucan therapy could greatly augment tumor regression mediated by anti-CD20, anti-HER2/neu and anti-EGFR1 [7]. The combined use of oral or

intravenous β-glucans with mAb therapy recruits granulocytes as tumor killer cells [6,7]. The current data suggest that orally administered β-glucan functions through anti-tumor mAbs and the complement system to recruit $CR3^+$ effector cells that produce tumor regression and tumor-free survival [7,63]. As with intravenously administered soluble yeast β-glucan, the therapeutic benefit of oral β-glucan was lost in C3- or CR3-deficient mice, confirming a similar requirement for iC3b on tumors and CR3 on granulocytes [63]. Clinical trials of the oral barley β-glucan given in combination with an anti-GD2 ganglioside mAb to patients with neuroblastoma are currently in progress at Memorial Sloan-Kettering Cancer Center (New York, USA). This trial has yielded encouraging preliminary results (G.D. Ross, pers. commun.).

Concluding remarks

There is now substantial evidence that the complement system can be manipulated in such a way that it can substantially contribute to the efficient elimination of mAb opsonized tumor cells. Such manipulation could be achieved either through the blockade or overwhelming of mCRP function to enhance CDC, or through the use of β-glucan to enable CR3 positive effector cells to eliminate iC3b-opsonized tumors. The experimental and clinical observations showing that modulating the function of mCRP increased the therapeutic effectiveness of immunotherapeutic mAbs not only improves the prospects of immunotherapeutic treatment but also increases the number of mAbs that can be useful in the clinic. Moreover, mAbs that have virtually no tumor regression activity when used alone are able to mediate complete regression when given in combination with β-glucan. Awareness of the mechanisms responsible for effective mAb-mediated cancer immunotherapy might finally enable mAb immunotherapy to fulfill its expectations and in many cases replace non-specific chemotherapy.

References

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

2 Ross, G.D. (2000) Regulation of the adhesion versus cytotoxic functions of the Mac-1/CR3/αMβ2-integrin glycoprotein. *Crit. Rev. Immunol.* 20, 197–222

3 Vetvicka, V. (1996) Soluble β-glucan polysaccharide binding to the lectin site of neutrophil or natural killer cell complement receptor type 3 (CD11b/CD18) generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells. *J. Clin. Invest.* 98, 50–61

4 Xia, Y. (1999) The β-glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. *J. Immunol.* 162, 2281–2290

5 Yan, J. *et al.* (1999) β-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

6 Hong, F. (2003) β-Glucan functions as an adjuvant for monoclonal antibody immunotherapy by recruiting tumoricidal granulocytes as killer cells. *Cancer Res.* 63, 9023–9031

7 Cheung, N.K. *et al.* (2002) Orally administered β-glucans enhance anti-tumor effects of monoclonal antibodies. *Cancer Immunol. Immunother.* 51, 557–564

8 Carter, P. (2001) Improving the efficacy of antibody-based cancer therapies. *Nat. Rev. Cancer* 1, 118–129

9 Trikha, M. *et al.* (2002) Monoclonal antibodies as therapeutics in oncology. *Curr. Opin. Biotechnol.* 13, 609–614

10 Ravetch, J.V. and Clynes, R.A. (1998) Divergent roles for Fc receptors and complement *in vivo*. *Annu. Rev. Immunol.* 16, 421–432

11 Clynes, R.A. *et al.* (2000) Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. *Nat. Med.* 6, 443–446

12 Cartron, G. *et al.* (2002) Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor *FcγRIIIa* gene. *Blood* 99, 754–758

13 Weng, W.K. and Levy, R. (2003) Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J. Clin. Oncol.* 21, 3940–3947

14 Velders, M.P. *et al.* (1994) New chimeric anti-pancarcinoma monoclonal antibody with superior cytotoxicity-mediating potency. *Cancer Res.* 54, 1753–1759

15 Sliwkowski, M.X. *et al.* (1999) Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Semin. Oncol.* 26, 60–70

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

17 Cragg, M.S. and Glennie, M.J. (2003) Antibody specificity controls *in vivo* effector mechanisms of anti-CD20 reagents. *Blood* 10.1182/blood-2003-06-2037 (www.bloodjournal.org/)

18 Johnson, P. and Glennie, M. (2003) The mechanisms of action of rituximab in the elimination of tumor cells. *Semin. Oncol.* 30, 3–8

19 Spiridon, C.I. *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

20 Niehans, G.A. *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

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

22 Cheung, N-K.V. *et al.* (1988) Decay-accelerating factor protects human tumor cells from complement-mediated cytotoxicity *in vitro*. *J. Clin. Invest.* 81, 1122–1128

23 Juhl, H. *et al.* (1997) 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

24 Gelderman, K.A. *et al.* (2002) 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

25 Jurianz, K. *et al.* (1999) Neutralization of complement regulatory proteins augments lysis of breast carcinoma cells targeted with rhumAb anti-HER2. *Immunopharmacology* 42, 209–218

26 McDowell, J.V. *et al.* (2003) Analysis of the ability of spirochete species associated with relapsing fever, avian borreliosis, and epizootic bovine abortion to bind factor H and cleave c3b. *J. Clin. Microbiol.* 41, 3905–3910

27 Fedarko, N.S. *et al.* (2001) Elevated serum bone sialoprotein and osteopontin in colon, breast, prostate, and lung cancer. *Clin. Cancer Res.* 7, 4060–4066

28 Fedarko, N.S. *et al.* (2000) Factor H binding to bone sialoprotein and osteopontin enables tumor cell evasion of complement-mediated attack. *J. Biol. Chem.* 275, 16666–16672

29 Seya, T. *et al.* (1994) Distribution of C3-step regulatory proteins of the complement system, CD35 (CR1), CD46 (MCP), and CD55 (DAF), in hematological malignancies. *Leuk. Lymphoma* 12, 395–400

30 Hara, T. *et al.* (1992) Levels of complement regulatory proteins, CD35 (CR1), CD46 (MCP) and CD55 (DAF) in human haematological malignancies. *Br. J. Haematol.* 82, 368–373

31 Golay, J. *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

32 Shin, M.L. *et al.* (1986) Membrane factors responsible for homologous species restriction of complement-mediated lysis: evidence for a factor

other than DAF operating at the stage of C8 and C9. *J. Immunol.* 136, 1777–1782
33 Harris, C.L. *et al.* (2000) Human and rodent decay-accelerating factors (CD55) are not species restricted in their complement-inhibiting activities. *Immunology* 100, 462–470
34 Zhang, H. *et al.* (1998) Antibodies against GD2 ganglioside can eradicate syngeneic cancer micrometastases. *Cancer Res.* 58, 2844–2849
35 Takizawa, H. *et al.* (1994) Complement inhibitor of rat cell membrane resembling mouse Crry/p65. *J. Immunol.* 152, 3032–3038
36 Caragine, T.A. *et al.* (2002) A tumor-expressed inhibitor of the early but not late complement lytic pathway enhances tumor growth in a rat model of human breast cancer. *Cancer Res.* 62, 1110–1115
37 Yu, J. *et al.* (1999) Protection of human breast cancer cells from complement-mediated lysis by expression of heterologous CD59. *Clin. Exp. Immunol.* 115, 13–18
38 Chen, S.H. *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
39 Baranyi, L. *et al.* (1994) Cell-surface bound complement regulatory activity is necessary for the *in vivo* survival of KDH-8 rat hepatoma. *Immunology* 82, 522–528
40 Durrant, L.G. *et al.* (2003) Enhanced expression of the complement regulatory protein CD55 predicts a poor prognosis in colorectal cancer patients. *Cancer Immunol. Immunother.* 52, 638–642
41 Blok, V.T. *et al.* (2000) A possible role of CD46 for the protection *in vivo* of human renal tumor cells from complement-mediated damage. *Lab. Invest.* 80, 335–344
42 Gelderman, K.A. *et al.* (2002) 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
43 Golay, J. *et al.* (2001) CD20 levels determine the *in vitro* susceptibility to rituximab and complement of B-cell chronic lymphocytic leukemia: further regulation by CD55 and CD59. *Blood* 98, 3383–3389
44 Bannerji, R. *et al.* (2003) Apoptotic-regulatory and complement-protecting protein expression in chronic lymphocytic leukemia: relationship to *in vivo* rituximab resistance. *J. Clin. Oncol.* 21, 1466–1471
45 Idusogie, E.E. *et al.* (2001) Engineered antibodies with increased activity to recruit complement. *J. Immunol.* 166, 2571–2575
46 Ellis, J.H. *et al.* (1995) Engineered anti-CD38 monoclonal antibodies for immunotherapy of multiple myeloma. *J. Immunol.* 155, 925–937
47 Pullyblank, A.M. *et al.* (1994) m17-1A-, c17-1A- and cSF25-mediated antibody-dependent cell-mediated cytotoxicity in patients with advanced cancer. *Br. J. Cancer* 70, 753–758
48 Kroesen, B.J. *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
49 Sokoloff, M.H. *et al.* (2000) Targeting of cancer cells with monoclonal antibodies specific for C3b(i). *Cancer Immunol. Immunother.* 49, 551–562
50 Kennedy, A.D. *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
51 Reiter, Y. and Fishelson, Z. (1989) Targeting of complement to tumor cells by heteroconjugates composed of antibodies and of the complement component C3b. *J. Immunol.* 142, 2771–2777
52 Juhl, H. *et al.* (1997) Additive cytotoxicity of different monoclonal antibody-cobra venom factor conjugates for human neuroblastoma cells. *Immunobiology* 197, 444–459
53 Juhl, H. *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
54 Fecke, W. *et al.* (1998) Expression of factor I-resistant mutants of the human complement component C3 in heterologous systems. *Xenotransplantation* 5, 29–34
55 Blok, V.T. *et al.* (2003) Cytokines affect resistance of human renal tumour cells to complement-mediated injury. *Scand. J. Immunol.* 57, 591–599
56 Andoh, A. *et al.* (1996) Interleukin 4 acts as an inducer of decay-accelerating factor gene expression in human intestinal epithelial cells. *Gastroenterology* 111, 911–918
57 Schmitt, C.A. *et al.* (1999) Expression and regulation by interferon-gamma of the membrane-bound complement regulators CD46 (MCP), CD55 (DAF) and CD59 in gastrointestinal tumours. *Eur. J. Cancer* 35, 117–124
58 Treon, S.P. *et al.* (1999) Muc-1 core protein is expressed on multiple myeloma cells and is induced by dexamethasone. *Blood* 93, 1287–1298
59 Reddy, P.K. *et al.* (2003) Interferon-γ upregulates MUC1 expression in haematopoietic and epithelial cancer cell lines, an effect associated with MUC1 mRNA induction. *Eur. J. Cancer* 39, 397–404
60 Blok, V.T. *et al.* (1998) 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
61 Harris, C.L. *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
62 Cheung, N.K. and Modak, S. (2002) Oral (1 → 3),(1 → 4)-β-D-glucan synergizes with antiganglioside GD2 monoclonal antibody 3F8 in the therapy of neuroblastoma. *Clin. Cancer Res.* 8, 1217–1223
63 Allendorf, D.J. *et al.* (2003) Orally administered β-glucan functions via anti-tumor mAbs and the complement system to recruit $CR3^+$ neutrophils and macrophages that produce tumor regression and tumor-free survival. *Mol. Immunol.* 40, 195–196