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The role of complement in mAb-based therapies of cancer

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ABSTRACT

The ability of complement to promote lysis of antibody-opsonized cells is well-established. Virtually all of the molecular details of this reaction have been elucidated and numerous points of regulation have also been delineated. Use of this information, along with the techniques that were first applied in the fundamental studies of complement, has allowed for investigations of the role of complement in mAb-based immunotherapies of cancer. These studies, which have often combined in vitro investigations with parallel correlative clinical measurements, have revealed that several FDA-approved mAbs make use of complement as an effector function in promoting opsonization and killing of targeted malignant cells. We describe the key methods used in this work, and discuss how the results of these studies provide rational approaches for making more effective use of complement in mAb-based cancer immunotherapy.

1. Introduction and historical background

The complement system has been exhaustively reviewed by many of the leading investigators in the field [1–6]. Therefore we will focus our presentation on relevant complement methodologies and the results obtained with these protocols that allow for an examination of the mechanism of action of mAbs used in the immunotherapy of cancer. We will discuss the important role for complement in the cytotoxic mechanism of action of alemtuzumab (ALM), ofatumumab (OFA) and rituximab (RTX). It should be clear that the methods that have been used to study these mAbs can be readily applied to investigate other future cancer-specific immunotherapeutic mAbs that may make use of the complement system.

The Antibody-opsonized sheep Erythrocyte (EA) was initially the most important in vitro target of complement [7]. Use of this substrate led to the elucidation of many of the complex and interesting details of the classical pathway (CP) of the complement cascade [1]. One of the outcomes of the work on EA, based on detailed quantitative studies, suggested that a single "hit" of what was later demonstrated to be the membrane attack complex(es) (MAC) of complement would be adequate to lyse the targeted cell [8]. That is, a single MAC-induced lesion in the cell membrane would allow the uncontrolled influx of water and extracellular ions and lead to rapid cell death. Erythrocyte substrates are relatively easy to kill with human complement, and if the same were true for malignant nucleated cells then it would seem reasonable that complement could be employed in the immunotherapy of cancer.

However, it is considerably more difficult to kill mAb-opsonized human cancer cells with complement, because they can make use of several very effective defense mechanisms. Normal as well as malignant nucleated cells can internalize or remove membrane-associated MAC, and it is now established that multiple independent MAC hits (likely more than one hundred, but difficult to quantitate) are needed to lyse these cells [9–14]. Moreover, due to its potential to induce rapid inflammation and high levels of cellular cytotoxicity, the complement cascade is under very tightly regulated controls, which serve to protect normal tissue and cells from "innocent bystander" complement-mediated damage [1,3–6]. In particular, several membrane-associated complement regulatory proteins (mCRP), which down-regulate complement at both the C3 activation step (MCP (CD46), and DAF (CD55)) and during MAC assembly (membrane inhibitor of reactive lysis, CD59), are expressed on normal cells and on malignant cells [15–17]. In addition, several soluble factors, in particular factors H and I, can function cooperatively with each other or with certain mCRP to down-regulate complement activation on mAb-targeted cells [18–21]. These phenomena raise the bar for cell lysis even higher and suggest that during the course of a malignancy tumor cells may evolve to overcome natural immune mechanisms, including complement, that would otherwise eliminate these cells. Indeed, there is considerable evidence in support of this phenomenon [16,22,23].

2. Effector mechanisms: complement and Fcγ receptors

Despite these considerable challenges, over the past 17 years several mAbs have been developed, tested and approved for the immunotherapy of certain forms of cancer, and multiple lines of evidence indicate an important role for complement in the mech-

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List of abbreviations

7-AAD	7-aminoactinomycin D
ALM	alemtuzumab
β2-M	β2-microglobulin
CDC	complement-dependent cytotoxicity
CLL	chronic lymphocytic leukemia
CP	classical pathway of complement
CVF	cobra venom factor
EA	antibody-opsonized sheep erythrocyte
HBSS	Hanks buffered saline solution
HI-NHS	heat inactivated NHS
MAC	membrane attack complex
mCRP	membrane-associated complement regulatory proteins
NHS	normal human serum
OFA	ofatumumab
PI	propidium iodide
RTX	rituximab

anism of action of certain of these mAbs [24–31]. We will present selected examples to illustrate the various methods used to study complement in this context. Moreover, numerous synergistic approaches, many of which target mCRP, or factors H and I, or make use of deposited C3b, are now under investigation with the goal of *increasing* the ability of such immunotherapeutic mAbs to induce complement-dependent cytotoxicity (CDC) of malignant, but not normal cells [14,18–21,26,32–36]. Although these later adjunctive strategies have not yet reached the clinic, the methods that have been used to test and develop these novel reagents provide additional valuable instruction.

A substantial literature documents the ability of three FDA-approved mAbs, ALM, OFA and RTX, to promote CDC of opsonized cell lines and primary cells in vitro, thus strongly implicating complement in their immunotherapeutic action [24–31]. However, it is also well-established that these three mAbs can also eliminate tumor cells by other immune effector mechanisms, principally based on the interaction of the mAb-opsonized cells with effector cells that express Fcγ receptors, such as macrophages, NK cells and neutrophils [37–44]. In the case of RTX, there is very good evidence, based on analyses of Fcγ receptor polymorphisms that have been correlated with the results of both vitro measurements as well as with examinations of RTX clinical efficacy, that these effectorcell-based mechanisms likely play the predominant role in RTX efficacy in certain forms of B cell lymphoma [45–47]. However, in chronic lymphocytic leukemia (CLL), a disease in which malignant cells are found in several compartments exposed to complement, results reported from numerous laboratories indicate that complement plays an important role in the immunotherapeutic action of ALM, OFA, and RTX [24–31,48–50].

Over the past decade several studies have documented the importance of cross-talk between the complement system and FcγR [51–55]. In particular, it is now clear that complement activation fragment C5a increases the inflammatory "poise" of effector cells by up-regulating expression of the activating receptors FcγRI and FcγRIII while down-regulating the inhibitory receptor FcγRIIb. Therefore, if an immunotherapeutic mAb activates complement, it can indirectly increase elimination of mAb-opsonized cells by fixed tissue macrophages, even if the mAb itself can not directly promote CDC. Moreover, new pathways have been defined in which IgG immune complexes promote the production of C5a even in the absence of C3 [54]. On this basis it would certainly seem reasonable to measure complement split products, including C5a, when patients receivemAb therapies. These measurements can best be accomplished by ELISAs, many of which are commercially available, and detailed protocols to measure complement activity and split products have been reported [56,57]. Due to the labile nature of many of these products, we recommend that blood samples be taken soon after the mAb infusions are started, and then immediately processed and the plasma/sera frozen so as to have the best chance of preserving and properly identifying the split products. Finally, we suggest that analysis of FcγR levels on circulating monocytes and neutrophils before, during and after RTX or OFA infusion is warranted. If the levels increase, it would suggest that indeed the "cross-talk" is relevant in this system as well.

3. An analogy to Koch's postulates for examination of the role of complement in mAb-based therapy of cancer

It can be quite difficult to obtain definitive *proof* of the in vivo immunotherapeutic mechanism(s) of action of an unconjugated mAb used to target tumor cells in humans. Careful in vitro experiments that attempt to model the in vivo situation, combined with relevant correlative clinical studies have the best chance to reach reasonably clear answers. However, there is now evidence that

Table 1
Selected polyclonal and monoclonal antibodies specific for C components cited in this review.

Poly/mAb	Specificity	Source[a]	Reference
Rabbit	C1q	Dako	[24,70,71]
Sheep	C1q	Serotec	[61]
Sheep	C1q	Biodesign	[99]
Goat	C3	ICN	[83]
mAb 1H8	C3b, iC3b, C3d	Cedarlane	[27,30,34,50,65,71,94,104]
mAb 7C12	C3b, iC3b	Cedarlane	[19,27,31,50,65,71,94,104]
mAb	C3d	Quidel	[33]
Rabbit	C4c	Dako	[50,70]
Eculizumab	C5	Alexion	[44]
mAb aE11	C5b-9	Dako, Abcam, Hycult Quidel & others	[30,60,91]
Rabbit	C5b-9 neo	Comp. Tech.	[31]
mAb	C6	Quidel	[91]

[a] Many of these monoclonal and polyclonal antibodies are available from a number of suppliers. Widely cited vendors include: Cedarlane, Complement Technology, Dako, Hycult, ICN, Quidel, Serotec.