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Role and mechanism of action of complement in regulating T cell immunity

Jason R Dunkelberger and Wen-Chao Song^{*}

Institute for Translational Medicine and Therapeutics and Department of Pharmacology,
University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Abstract

Complement is a part of the innate immune system that contributes to first-line host defense. It is also implicated in a number of human inflammatory conditions and has attracted interest as a potential therapeutic target. Understanding the basic biology of complement and its mechanism(s) of action is imperative for developing complement-based treatments for infectious and autoimmune diseases. One of the exciting new developments in this regard is the revelation that complement plays an important role in T cell immunity. In this review, we highlight recent published studies implicating complement in models of CD4⁺ and CD8⁺ T cell immune responses, and discuss its potential mechanism(s) action in these processes. We also comment on issues that may impact data interpretation and draw attention to their consideration in future studies.

Complement and the innate immune response

The mammalian immune system provides defense against pathogenic invasion by way of detection, prosecution, and clearance of those entities which threaten host viability. Immune processes have traditionally been divided into two broad subsystems, innate and adaptive immunity. The former is composed of immunological effectors that provide robust, immediate, and relatively non-specific immune responses and constitutes the ‘front-line’ of host defense (Medzhitov and Janeway, 2000). The adaptive immune system is an evolutionarily younger and far more tailored system organized around two classes of specialized cell types; B and T cells. These cells display an extremely diverse repertoire of antigen-specific recognition receptors that enable specific identification and elimination of pathogens and generation of long-lived immunological memory which serves to curtail re-infection by the same pathogen (Janeway *et al.*, 2005). Despite the intellectual distinction between these arms of immunity, over the past decades it has become increasingly clear that successful elimination of most pathogens requires concerted efforts on the part of the various immune responses and that crosstalk between innate and adaptive immunity plays a vital role in efficient host defense.

Complement is a part of innate immunity that was identified more than a century ago on the basis of its ability to ‘complement’ the lysis of bacteria by antibodies (Bordet, 1895). It is a significant protein component of serum, amounting to more than 3 g/L and constituting

^{*}To whom all correspondence should be addressed at: Wen-Chao Song Ph.D., University of Pennsylvania School of Medicine, Room 1254 BRBII/III, 421 Curie Blvd., Philadelphia, PA 19104, Tel: 215-573-6641, Fax: 215-746-8941, songwe@upenn.edu.

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more than 15% of the globular fraction of plasma. Activation of complement can occur through three distinct pathways termed the classical, lectin, and alternative pathways (Walport, 2001a, Walport, 2001b, Dunkelberger and Song, 2010). Like other components of innate immunity, such as the Toll-like receptors (TLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLR) and nucleotide-binding oligomerization domain (NOD) receptors (Takeuchi and Akira, 2010), complement can recognize nonself through common and evolutionarily conserved pathogen-associated molecular patterns (PAMPs) and therefore provide immediate responses to common pathogenic surfaces. The classical and lectin pathways activate complement through recognition of PAMPs by natural (complement-fixing; generally IgM or IgG₁) antibodies and lectins (e.g. mannan-binding lectin; MBL), respectively (Walport, 2001a, Walport, 2001b). In contrast, the alternative pathway (AP) is constitutively active by virtue of low-level, hydrolytic ‘tick-over’ of C3 and differentiation between self and nonself by the AP is thought to be achieved by membrane and plasma complement regulators functioning on the host cell but not foreign surfaces (Walport, 2001a, Walport, 2001b, Dunkelberger and Song, 2010, Zipfel and Skerka, 2009). It should be noted however that this ‘textbook’ view of AP activation is currently being expanded with evidence showing that properdin, a component of AP complement, may function as a pattern-recognition molecule for certain PAMPs such as lipopolysaccharide (LPS), lipooligosaccharide (LOS), zymosan and viral double-stranded RNAs to direct AP activation (Spitzer *et al.*, 2007, Kimura *et al.*, 2008, Kemper, Atkinson and Hourcade, 2010, Zhang *et al.*, 2010).

Complement activation initiates a cascade of proteolytic reactions involving more than 30 proteins in the serum and on cell surfaces. Cleavage of early complement components (C4, C2, C3, factor B) leads to the formation of C3 convertases, C4bC2a or C3bBb (Fig 1). Generation of C3 convertases is the keystone event in the activation of complement regardless of the specific activation pathways. C3 convertase assembly results in further downstream proteolytic events, including the formation of C5 convertases, that serve to generate the three major effectors of the complement cascade (i) anaphylatoxins, which are potent pro-inflammatory molecules that alert and prime multiple aspects of the immune system (ii) opsonins, which serve to coat the pathogenic surface and aid in targeting and clearance by the phagocytic system (iii) membrane attack complex (MAC), the terminal assembly of multiple complement proteins that directly lyses targeted (opsonized) pathogens (Fig 1). These effectors allow the complement system to fulfill its three major biological imperatives (i) defense against common pyrogenic infection (ii) disposal of immune complexes and cellular ‘wastes’, including apoptotic cells and other forms of altered self (iii) bridging the innate and adaptive immune systems (Walport, 2001a, Walport, 2001b). This review focuses on the role and mechanism of action of complement in the latter, especially as it relates to T cell biology.

Role of complement in T cell immunity

The functions of the complement system in opsonization, lysis, and generation of an inflammatory response are paradigmatic and represent a well-characterized component of innate host defense. However, the role of complement is not limited to these innate immune responses and it has become increasingly appreciated that complement also plays an important role in adaptive immunity. The controversial (at the time) finding that B cells bound C3 raised questions as early as the 1970’s as to whether complement was involved in adaptive immunity (Nussenzweig *et al.*, 1971). Works from the past several decades have validated this concept and it is now well established that complement acts as a natural adjuvant for the humoral immune response. Complement regulates B cell immunity at multiple levels. It facilitates antigen trapping and retention in lymphoid tissues, lowers the B

cell activation threshold, promotes memory B cell survival and influences induction of tolerance (Carroll, 2004, Holers and Kulik, 2007, Carroll, 2008)

In contrast to the well characterized role of complement in antibody production, the relevance of complement to the development and maintenance of T cell immunity has been less well studied and a consensus on its mechanism(s) of action in this arm of the adaptive immune system is yet to emerge. Nevertheless, many studies using different models of T cell responses have appeared in the literature and it is now undisputed that complement does play a role in T cell immunity, evident especially at the organismal level (Kemper and Atkinson, 2007). In the sections below, we will briefly summarize some of these studies and then discuss possible mechanisms of action of complement in these processes. The studies highlighted here are meant to be illustrative examples rather than an exhaustive citation of all relevant literature.

Viral infections

A role for the complement system in T cell-mediated antiviral immune responses was illustrated in a study by Kopf *et al* in 2002 using an influenza infection model. It was shown that C3-deficient mice had delayed viral clearance and increased viral titers, due to a defect in migration of CD4⁺ and CD8⁺ T cells to the lung, in response to pulmonary influenza infection (Kopf *et al.*, 2002). Influenza, like many other pulmonary infections, requires the host to maintain efficient T cell responses in order to successfully clear the invading pathogen and therefore the ability of complement to augment T cell responses could be of critical importance in defense against viral infection in the respiratory tract (Hikono *et al.*, 2006).

Further support for the role of complement in general T cell responses to viral infection was obtained following systemic acute infection of C3-deficient mice with lymphocytic choriomeningitis virus (LCMV), the causative agent of aseptic (non-bacterial) meningitis (Suresh *et al.*, 2003). This study showed defects in antigen-specific CD8⁺ T cell expansion in C3^{-/-} mice in response to multiple LCMV protein epitopes and revealed an influence of the mouse genetic background on the phenotype. Furthermore, it was established that these effects were largely independent of complement receptors (CR) 1 and 2, suggesting that the lack of C3 altered T cell functions in a manner different from the way complement augments B cell responses, at least in this model. West Nile virus (WNV), a RNA virus of the *Flaviviridae* family known to cause fever and neurological inflammation (encephalitis) in humans as well as several other vertebrate species, is another viral infection vitally contingent on T cells to successfully control (Shrestha and Diamond, 2004). In a murine model of WNV infection, mice deficient in C3 or CR1/2 presented with defects in the ability to prevent central nervous system (CNS) infection and were susceptible to lethal infection (Mehlhop *et al.*, 2005). The dependence of this phenotype on CR1/2, as well as the observed defects in humoral immune responses, pointed to a role of complement in modulating the B cell response, potentially through augmentation of antigen presentation. However, a follow-up study utilized mice deficient in specific activation pathways and found that different complement pathways contributed to disparate aspects of the adaptive immune response (Mehlhop and Diamond, 2006). For example, while mice deficient in C4, C1q, Factor B (fB) or Factor D (fD) all exhibited increased mortality during infection, C4- and fB-deficient mice showed impaired CD8⁺ T cell activation and trafficking but normal antibody responses (Mehlhop and Diamond, 2006). This suggests that complement's ability to modulate B or T cell adaptive immune responses is dependent on the specific pathogen being encountered and that the relative contribution of each complement pathway to the various aspects of the adaptive immune response will vary accordingly.

In other studies, treatment of mice with a peptide antagonist to C5aR prior to influenza type A challenge reduced the total number of virus-specific CD8⁺ cells and attenuated antiviral activity in the lung, confirming that complement in general, and C5aR in specific, plays a role in the T cell-dependant clearance of influenza infection (Kim *et al.*, 2004). A critical role for C3 and C5aR in antiviral CD8⁺ T cell immunity has also been demonstrated both in acute and chronic LCMV infection of decay-accelerating factor (DAF) knockout mice (Fang *et al.*, 2007). DAF is a membrane complement regulatory protein which serves to prevent unchecked C3 convertase formation by inhibiting both the assembly of new C3 convertases and decreasing the half-life of pre-formed convertases (Lublin and Atkinson, 1989, Kim and Song, 2006). Presumably, virus-induced complement activation is increased in the absence of DAF and this may amplify any complement-mediated effect on T cell immune responses. Compared with wild-type mice, DAF^{-/-} mice had markedly increased expansion in the spleen of total and viral Ag-specific CD8⁺ T cells after acute or chronic LCMV infection. Splenocytes from LCMV-infected DAF^{-/-} mice also displayed significantly higher killing activity than cells from wild-type mice toward viral Ag-loaded target cells, and DAF^{-/-} mice cleared LCMV more efficiently (Fang *et al.*, 2007). Importantly, deletion of the complement protein C3 or C5aR from DAF^{-/-} mice reversed the enhanced CD8⁺ T cell immunity phenotype (Fang *et al.*, 2007). These results demonstrated that DAF is an important regulator of CD8⁺ T cell immunity in viral infection and that it fulfills this role by acting as a complement inhibitor to prevent virus-triggered complement activation and C5aR signaling.

Allergic asthma

Complement's ability to modulate T cell responses has also been studied in the progression of allergic asthma. Although the genesis of asthma remains unclear, it is generally accepted that the condition results from inappropriate immunological reactions to common environmental antigens in a genetically-defined responder group. CD4⁺ T cells play a critical role in the effector phase of allergic asthma through the production of T helper type-2 (T_H2)-biased cytokines, including interleukin (IL)-4, which contributes to airway hyperresponsiveness (AHR), eosinophilic infiltration, excessive mucus production and other pathogenic features of asthma (Wills-Karp, 2007, Barrett and Austen, 2009). C3aR-deficient mice and guinea pigs display decreased T_H2 responses, along with protection from AHR, following allergic antigenic stimulation (Humbles *et al.*, 2000, Bautsch *et al.*, 2000, Drouin *et al.*, 2002). These results are supported by works using a C3-deficient mouse in both *Aspergillus fumigatus*- and ovalbumin-induced allergic asthma (Drouin *et al.*, 2001). Likewise, when C5a is inhibited pharmacologically, the severity of AHR is reduced during the effector phase of allergic asthma in both mice and rats which agrees with the pro-allergic roles that are characteristic of the anaphylatoxins (Abe *et al.*, 2001, Baelder *et al.*, 2005). However, identification of the C5 gene as a susceptibility locus for murine allergic asthma has seemingly contradicted the view that complement upregulates T cell responses during asthma (Karp *et al.*, 2000, Drouin *et al.*, 2006). Further studies have suggested that complement plays a multi-faceted role in allergic asthma: on one hand it serves to exacerbate AHR and other pathological markers of asthma and increase the T_H2 response during the effector phase of allergic asthma, and on the other it seems to have immunomodulatory function and induces tolerance during allergen presentation to T cells (Kohl *et al.*, 2006, Zhang *et al.*, 2009) (for more comprehensive reviews on the topic, see Wills-Karp, 2007, Kohl and Wills-Karp, 2007).

Experimental autoimmune encephalomyelitis

Another widely used model in the study of CD4⁺ T cell immunity and in which complement has been implicated is experimental autoimmune encephalomyelitis (EAE). EAE, most often induced in rodents, reproduces the pathology of human multiple sclerosis (MS) which is the

most common cause of neurological disability in young adult Caucasian populations (Compston and Coles, 2002). The etiology of the disease remains unclear; but it is likely that a complex and diverse set of immunological and genetic factors contribute to initiation and progression of the demyelination and axonal damage that characterize the condition and result in a variety of neurological deficits (McFarland and Martin, 2007). In EAE, demyelination and other pathological hallmarks of MS are induced through the introduction of known central nervous system (CNS) antigens such as myelin basic protein (MBP) or myelin oligodendrocyte glycoprotein MOG (Lassmann, 2007). Pathology is perpetuated by auto-reactive CD4⁺ T cells which are primed in the periphery and subsequently migrate to the CNS where they encounter myelin antigens presented by local antigen presenting cells (APCs), in turn causing them to become activated and release pro-inflammatory cytokines and stimulate microglia (Heppner *et al.*, 2005). This inflammatory cascade sets up the effector mechanisms that lead to demyelination. The effector mechanisms may include CD8⁺ T cells, macrophages, glial cells and other cytotoxic mediators such as the terminal complement cascade, among many others (Ingram *et al.*, 2009). Despite the complexity of the EAE model, it appears that pathology is driven by T cells. It was originally thought that CNS injury was due to myelin-specific, CD4⁺ T helper type-1 (T_H1) cells, which are a major source of IFN- γ (Williams, Ulvestad and Hickey, 1994). In more recent years, both IFN γ -producing T_H1 and IL-17-producing T_H17 T cells have been shown to contribute to pathology in EAE, and the autoimmune-promoting T_H17 cells may play a greater role in this regard (Komiyama *et al.*, 2006, Kroenke *et al.*, 2008).

Many studies have implicated complement in the pathogenesis and progression of EAE. Early experiments in which complement was depleted through cobra venom factor (CVF) showed that complement depletion was protective in this model, results that were duplicated in part through the use of soluble CR1 (sCR1) as a complement inhibitor (Pabst *et al.*, 1971, Vriesendorp *et al.*, 1997). However, studies with C3^{-/-} mice have produced conflicting data. In one study, C3 deficiency was found to attenuate murine EAE, a conclusion that was reaffirmed in a subsequent study by the same group (Nataf *et al.*, 2000, Smith *et al.*, 2008). In contrast, a study by a different group found no difference between WT and C3^{-/-} mice in the development of EAE (Calida *et al.*, 2001). The role of C3aR and C5aR in EAE has also been studied using genetic and pharmacological tools. Deficiency of C3aR in mice reduced the clinical severity of EAE as well as T cell and macrophage infiltration into the CNS (Boos *et al.*, 2004). The same study showed the converse was also true; targeted overexpression of C3a in the CNS (under the control of the glial fibrillary acidic protein [GFAP] promoter) exacerbated disease pathology considerably. Surprisingly, C5aR^{-/-} mice were fully susceptible to EAE and treatment with a C5aR antagonist failed to protect from disease progression in rats (Reiman *et al.*, 2002, Morgan *et al.*, 2004). These latter results would suggest that C5aR does not play a role in EAE. The involvement of anaphylatoxin receptors in EAE was obscured somewhat by a recent report utilizing a C3aR^{-/-}C5aR^{-/-} double knockout mouse which suggested a certain degree of cross-modulation between the receptors in EAE as the double knockout seemed to lose at least a portion of the protective effect seen in the C3aR single knockout (Ramos, Wohler and Barnum, 2009).

In most of these studies, the question of whether complement influenced EAE directly, e.g. through MAC-mediated cellular injury (Mead *et al.*, 2002, Barnum and Szalai, 2006), or indirectly through effects on T cell priming, differentiation and trafficking was not specifically addressed. It is likely that the complement effect reflected a combination of mechanisms whose relative importance may depend on the experimental procedure and nature of antigens used, e.g. MBP vs MOG peptides. The role of complement in EAE and T cell immunity was investigated in other studies using mice deficient in DAF. When immunized with MOG peptides, DAF^{-/-} mice showed heightened pathology in EAE, concomitant with increased development of antigen-specific T_H1 and T_H17 cells (Liu *et al.*,

2005, Liu *et al.*, 2008). In re-stimulation assays *in vitro*, splenocytes from DAF^{-/-} mice immunized with MOG peptides contained more IFN- γ and IL-17-producing T cells and secreted more of these cytokines into the culture medium (Liu *et al.*, 2005, Liu *et al.*, 2008). In one strain of DAF^{-/-} mice, both the EAE disease phenotype and heightened T cell immunity were rescued by C3 gene deletion (Liu *et al.*, 2005), suggesting involvement of the complement system in these responses. In the same study, neutralization of C5 with a mAb also rescued the T cell immunity phenotype, although its effect on EAE was not tested. Studies of a second DAF^{-/-} mouse strain showed that EAE and enhanced T_H1 and T_H17 cell differentiation was rescued by either C3aR or C5aR deficiency (Liu *et al.*, 2008). The latter result, as well as the finding by the same group that C3aR^{-/-}C5aR^{-/-} mice developed attenuated EAE compared with WT mice (Strainic *et al.*, 2008), thus differed from the data cited above showing a lack of involvement of C5aR in EAE (Reiman *et al.*, 2002, Morgan *et al.*, 2004, Ramos, Wohler and Barnum, 2009).

Allograft rejection

Immune-mediated rejection of allografts is comprised of both innate and adaptive immune systems response to alloantigen and the adaptive immune responses can be further divided on the basis of whether B or T cells are predominant. Complement and other aspects of the innate immune response play a large role in cases of ischemia/reperfusion (I/R) injury, which in a clinical sense is a function of organ storage and organ condition upon transplant. Deficiency of a variety of complement components is protective in the various models of I/R-mediated injury (Riedemann and Ward, 2003). Likewise, complement is a major effector perpetuating the alloantibody response to allografts and leading to rejection and organ failure (Rocha *et al.*, 2003).

More relevant to this discussion is the role complement plays in mediating the T-cell dependant allograft rejection pathways. Both CD8⁺ and CD4⁺ T cells are involved in T cell-mediated allograft rejection. CD8⁺ cytotoxic T lymphocytes (CTLs) are primed and activated by recognition of donor major histocompatibility complex (MHC) class I antigens displayed on donor-derived APCs (Rocha *et al.*, 2003). CD4⁺ T cells are also involved in transplantation rejection and are activated either by recognizing allo-MHC class II directly on donor cells or through an indirect pathway by which either MHC class I or II are ingested and processed by recipient APCs before being presented to CD4⁺ T cells (Le Moine, Goldman and Abramowicz, 2002). Like EAE, allograft rejection was initially thought to be mediated by IFN γ -producing T_H1 effector cells before the role of the T_H17 lineage became increasingly appreciated in this process (Benghiat *et al.*, 2009).

Complement's influence on T cell-mediated rejection was first illustrated in a pivotal paper in 2002 using an allogeneic kidney transplantation model (Pratt, Basheer and Sacks, 2002). This study showed that kidneys from C3-deficient donors survived longer than normal kidneys in allogeneic recipients, suggesting that local production of C3 was vital to graft rejection. In mixed lymphocyte reactions, T cells from mice receiving WT allogeneic kidneys proliferated more vigorously and contained a higher number of CR1/2⁺ cells among the CD4⁺ population than those from mice receiving C3^{-/-} kidneys after allogeneic antigen re-stimulation (Pratt, Basheer and Sacks, 2002). Furthermore, macrophages from C3-deficient mice were shown to express less MHC class II, and allogeneic CD4⁺ T cell responses, as characterized by IL-2 and IFN- γ production, were impaired in a mixed lymphocyte culture system (Zhou *et al.*, 2006). The role of complement in allograft rejection was also addressed in heart and skin transplantation studies using DAF^{-/-} mice (Pavlov *et al.*, 2008). In a fully allogeneic heart transplantation model, DAF^{-/-} mouse hearts were shown to be rejected faster than WT hearts in MHC mismatched recipients and this phenotype tracked with DAF expression on bone marrow-derived cells in the allografts. Accelerated rejection of DAF^{-/-} mouse hearts was dependent on C3 as DAF^{-/-}C3^{-/-} hearts

survived as long as WT hearts in allogeneic recipients (Pavlov *et al.*, 2008). When re-stimulated with allogeneic APCs, alloantigen-primed CD8⁺ T cells from mice receiving DAF^{-/-} hearts proliferated more vigorously and secreted more IFN- γ than CD8⁺ cells from mice receiving WT allografts. Similar results demonstrating a role of complement in T cell-mediated allograft rejection were obtained using DAF^{-/-} mice in a minor Ag-disparate male skin transplantation model (Heeger *et al.*, 2005). Of note, DAF expression in both the donor organ and in the recipients was shown to influence graft survival in the skin transplantation model, whereas only DAF expression in the donor was found to be important in the setting of allogeneic heart transplantation (Pavlov *et al.*, 2008, Heeger *et al.*, 2005).

Other experimental models

In addition to the experimental models discussed above, many other recent studies have implicated complement and its regulatory proteins in T cell immune responses. These results serve to reinforce the role of complement in T cell biology and further emphasize the importance of complement in human pathologies. For example, deletion of DAF from MRL/lpr mice, a model of human lupus, exacerbated lymphoproliferation and skin disease (Miwa *et al.*, 2002). In a focal and segmental glomerulosclerosis (FSGS) model induced by passively transferring mouse podocyte-specific sheep antibodies into Balb/c mice, DAF^{-/-} but not WT mice developed kidney disease with characteristic features of FSGS (Bao *et al.*, 2009). This phenotype tracked with T cells in the DAF^{-/-} mice as disease was substantially attenuated by depletion of CD4⁺ T cells. Additionally, both WT kidneys transplanted into DAF^{-/-} recipients and kidneys of DAF-sufficient but T cell-deficient Balb/(cnu/nu) mice reconstituted with DAF^{-/-} T cells developed FSGS. In contrast, DAF-deficient kidneys in WT hosts and Balb/(cnu/nu) mice reconstituted with DAF-sufficient T cells did not develop FSGS. In another model with far reaching human health implications, a connection between complement and T cell-mediated tumor rejection has also been established recently (Markiewski *et al.*, 2008). It was found that C5a in a tumor microenvironment enhanced tumor growth by suppressing the antitumor CD8⁺ T cell-mediated response. This activity of C5a was correlated with recruitment of myeloid-derived suppressor cells into tumors and augmentation of their T cell-directed suppressive abilities. Enhancement of the suppressive capacity of myeloid-derived suppressor cells by C5a was mediated by the production of reactive oxygen and nitrogen species (Markiewski *et al.*, 2008).

Mechanism of action of complement in regulating T cell immune responses

While there is ample evidence as cited above for a role of complement in many models of T cell immunity, the mechanism(s) by which complement exerts this effect remains incompletely understood. In principle, complement can affect T cell biology through two pathways that are not mutually exclusive: through effect on T cells themselves or through alteration of APC function, thereby modulating their ability to prime T cells and affect T cell responses. In the sections below, we will discuss the complement effectors that have been shown to regulate T cell and/or APC functions, with a particular emphasis on the anaphylatoxins.

Membrane complement regulatory proteins on T cells

Notwithstanding the examples of DAF affecting T cell immunity through complement regulation, there is a substantial literature on membrane complement regulators playing a role in T cell activation/differentiation independent of their regulator function (Fig 2A). For example, crosslinking of CR1 on human T cells inhibited proliferation and IL-2 production (Wagner *et al.*, 2006). In contrast, crosslinking the MAC-regulator CD59 led to increased IL-2 production and proliferation in human T cells (Korty, Brando and Shevach, 1991). The latter result seemed to contradict data from CD59a (the murine homolog of human CD59)

knockout mice. It has been shown that CD59a-deficient mice presented with enhanced virus-specific CD4⁺ T cell responses in a complement-independent fashion when infected with recombinant vaccinia virus expressing a glycoprotein of LCMV (Longhi *et al.*, 2005). This suggested that CD59a engagement on T cells down-modulated T cell activity. Likewise, in some settings DAF can regulate T cell function via complement-independent mechanisms, in addition to its complement-dependent effects as discussed above. Such a function has been demonstrated on human T cells after antibody crosslinking of DAF (Davis *et al.*, 1988, Shenoy-Scaria *et al.*, 1992, Capasso *et al.*, 2006) and in the MRL/lpr model of murine lupus which showed C3-independent exacerbation of lymphoproliferation by DAF deficiency (Miwa *et al.*, 2007). These activities of DAF may relate to its potential as a GPI-anchored signaling molecule on the T cell surface and/or as a ligand for the 7-transmembrane receptor CD97 (Capasso *et al.*, 2006, Hamann *et al.*, 1996).

CD46, another membrane complement regulatory protein, has also been linked to T cell regulation (Kemper *et al.*, 2005). Crosslinking of CD46 by antibody or natural/pathogenic ligands induced signaling in human T cells as well as in lymphocytes of CD46-expressing transgenic mice (Astier *et al.*, 2000, Marie *et al.*, 2002). Upon treatment with anti-CD3 and co-engagement of CD46, human T cells proliferated nearly as much as with classical costimulation with CD28 and CD3 (Astier *et al.*, 2000). CD46 activation can also lead to morphological changes in T cells that may be indicative of a propensity to migrate to secondary lymphoid tissues following CD46 costimulation (Zaffran *et al.*, 2001). Finally, it was demonstrated that CD46 and CD3 costimulation on human CD4⁺ T cells led to their differentiation into regulatory T cell (Treg) type 1 (Tr1) phenotype characterized by IL-10 and granzyme B secretion (Kemper *et al.*, 2003). These Treg cells are capable of suppressing T cell responses but do not prevent dendritic cell maturation (Barchet *et al.*, 2006).

C1q and cell surface-bound C3 activation fragments

In addition to membrane regulators acting intrinsically on T cells, many studies have shown that T cell activation can also be modulated by C1q and cell surface-bound C3 activation fragments as opsonins (Fig 2B). Deficiency of C1q is a high penetrance risk factor for SLE (Pickering *et al.*, 2000). Although impaired clearance of apoptotic cell antigens is considered a major underlying mechanism, recent studies have demonstrated activities of C1q on DC maturation, cytokine production and costimulatory molecule expression (Csomor *et al.*, 2007, Fraser *et al.*, 2009, Baruah *et al.*, 2009, Hosszu *et al.*, 2010), with potential implications for T cell immunity. C1q binding to human T cells or opsonization of immune complexes have also been shown to influence T cell activation, proliferation and cytokine production, either directly or through enhanced antigen uptake and presentation in APCs (Chen *et al.*, 1994, Jiang *et al.*, 2003, van Montfoort *et al.*, 2007). Interaction between cell surface-deposited C3 activation products and complement receptors on T cells and APCs represents another mechanism of T cell immune regulation. A number of studies have shown that treatment of macrophages, B cells and monocyte-derived DCs with autologous serum *in vitro* led to their opsonization with C3 activation fragments via covalent bonding (Kerekes *et al.*, 1998, Kerekes *et al.*, 2001, Papp *et al.*, 2008, Sandor *et al.*, 2009). This reaction directed the APCs to increase expression of MHCII, CD83 and CD86 and resulted in elevated secretion of TNF- α , IL-6 and IL-8 (Sandor *et al.*, 2009). These phenotypic changes enabled APCs to gain increased capacity to stimulate antigen-specific or allogeneic T cells (Kerekes *et al.*, 1998, Sandor *et al.*, 2009). Interestingly, it has been shown that B cells and macrophages could also release cell membrane deposited C3 fragments in the form of exosomes, and C3 fragment-containing exosomes from antigen-loaded APCs induced a significantly higher T cell response in the presence of suboptimal antigen stimulus (Papp *et al.*, 2008).

Anaphylatoxins

The role of the anaphylatoxins in regulating T cell immunity has received a great deal of attention in the last few years, and works from several laboratories have implicated C3aR and C5aR in APC and T cell functions as well as in crosstalks between complement and TLRs with consequent influence on T cell immune responses (Fang *et al.*, 2007, Strainic *et al.*, 2008, Peng *et al.*, 2008, Zhang *et al.*, 2007, Lalli *et al.*, 2008, Weaver *et al.*, 2010, Fang *et al.*, 2009, Hawlisch *et al.*, 2005). These studies used macrophages and bone marrow (BM)-derived or splenic DCs as APCs and allogeneic or TCR transgenic T cells and probed the role of anaphylatoxins in T cell priming, differentiation and survival. The use of mice deficient in C3 or fB, C4, C3aR, C5aR, DAF and C3aR- or C5aR-blocking reagents has allowed the dissection of specific complement activation pathways and anaphylatoxin receptors in these processes. Based on some of these studies, it has been postulated that local complement activation, via the alternative pathway, produces C3a and C5a which then act on C3aR and C5aR on both APCs and T cells to regulate antigen uptake, costimulatory molecule expression and T cell expansion and differentiation (Fig 3) (Liu *et al.*, 2008, Strainic *et al.*, 2008, Peng *et al.*, 2008, Lalli *et al.*, 2008, Lalli *et al.*, 2007, Li *et al.*, 2008). Some studies have suggested that AP complement activation and C3aR/C5aR signaling was autonomous to APCs, occurring before their contact with T cells (Peng *et al.*, 2008, Peng *et al.*, 2009), whereas others described these events as occurring on both T cells and APCs, triggered by cognate T cell/APC interaction and enhanced in the absence of DAF which was shown to be downregulated on both partners during their engagement (Strainic *et al.*, 2008, Heeger *et al.*, 2005, Lalli *et al.*, 2008). The effects of C3aR and C5aR signaling on APC function were mediated by cAMP production and AKT and MAP kinase phosphorylation (Strainic *et al.*, 2008, Peng *et al.*, 2008, Li *et al.*, 2008). Ablation of C3aR and C5aR signaling, by gene deletion of C3, C3aR or C5aR or pharmacological targeting with C3aR or C5aR antagonists, led to reduced IL-12 and IFN- γ , and increased IL-10 production (Liu *et al.*, 2008, Strainic *et al.*, 2008, Heeger *et al.*, 2005, Lalli *et al.*, 2008, Lalli *et al.*, 2007, Li *et al.*, 2008, Peng *et al.*, 2009, Peng *et al.*, 2006, Lalli *et al.*, 2009). In the context of TLR2 activation by PamC₃CSK₄, lack of C5aR signaling on splenic DCs also promoted induction of Treg and T_H17 cells in addition to inhibiting T_H1 cell polarization (Weaver *et al.*, 2010). Conversely, it was described that lack of DAF on both APCs and T cells facilitated more C3a and C5a production within the immunological synapses and enhanced T cell priming and T_H1 cell differentiation, as well as inhibited T cell apoptosis (Heeger *et al.*, 2005, Lalli *et al.*, 2008).

The above hypothesis raises several interesting questions and spotlights some unresolved issues in the literature. There is still uncertainty as to whether all obligatory complement components are synthesized and secreted in active forms by APCs or T cells, and how and when AP complement is triggered in these cells. Some studies have indicated that C3, fB, fD and C5 were all synthesized by DCs and their synthesis was enhanced by interaction with T cells (Strainic *et al.*, 2008), suggesting that DCs alone could support AP complement activation. In contrast, C5 production by DCs was not detected by RT-PCR in other studies and transcellular production of C5a by cell types other than DCs in the BM cell culture was believed to provide the necessary ligand for C5aR activation on DCs (Peng *et al.*, 2009, Peng *et al.*, 2006). Recently, it was revealed that fD is secreted by cells as an inactive zymogen and proteolytic activation by MASP1/3 in the fluid phase is required for generating active fD (Takahashi *et al.*, 2010). Of interest, adipocytes, the major source of fD biosynthesis *in vivo*, do not express MASP1/3 and fD secreted by cultured adipocytes is unprocessed and inactive (Takahashi *et al.*, 2010). It is not known if MASP1/3 is produced by APCs or T cells and whether fD, if secreted by these cells, is active or not.

In discussing the mechanism(s) of action of anaphylatoxins in T cell immunity, the term 'APC' has often been used nonspecifically to refer to thioglycollate-elicited peritoneal

macrophages as well as splenic or BM-derived DCs. While it is well established that elicited murine peritoneal macrophages express high levels of C5aR (Chenoweth, Goodman and Weigle, 1982, Soruri *et al.*, 2003), there is conflicting data on the expression of C5aR on splenic and BM-derived DCs. Expression of C3aR and/or C5aR on murine BM DCs and T cells, either before or in response to T cell/DC interaction during antigen stimulation, has been documented by RT-PCR and antibody staining in some studies (Strainic *et al.*, 2008, Peng *et al.*, 2008, Peng *et al.*, 2009). However, other experiments, including one using a new C5aR-green fluorescence protein (GFP) knock-in mouse, failed to observe C5aR or C3aR expression on T cells and splenic DCs, and detected only a fraction (5–20%) of GFP (C5aR)-positive cells among CD11c+ BM DCs (Soruri *et al.*, 2003, Martin *et al.*, 1997, Zwirner, Begemann and Kirchhoff, 1999, Zwirner, Fayyazi and Gotze, 1999, Dunkelberger *et al.*, 2010). Thus, depending on the type of APCs used and their purity, effect of C5aR/C3aR on T cell immune responses may be different and results obtained in one type of APCs may not always be extrapolated to another.

It should also be pointed out that phenotypic deficiencies in elicited peritoneal macrophages or splenic DCs isolated from C3^{-/-}, C3aR^{-/-} or C5aR^{-/-} mice may not necessarily reflect an intrinsic function of the corresponding protein on such cells during APC/T cell engagement. It is possible that such a phenotype is pre-existing, developed *in vivo* as a result of systemic complement deficiency or receptor deletion from other cell types which in turn produces the observed phenotype through transcellular regulation. Indeed, while thioglycollate-elicited peritoneal macrophages from DAF^{-/-} mice, but not from DAF^{-/-}C3^{-/-} mice, have been shown to be more potent stimulators of T_H1 cell responses than WT macrophages (Strainic *et al.*, 2008, Lalli *et al.*, 2007 and C Fang and W.-C. Song, unpublished data), no difference in T cell stimulation could be found between resident peritoneal macrophages from naïve WT and DAF^{-/-} mice (C Fang and W.-C. Song, unpublished data). Furthermore, DAF was down-regulated on elicited peritoneal macrophages of WT mice to the degree that they essentially became DAF-deficient, similar to elicited macrophages of DAF^{-/-} mice (C Fang and W.-C. Song, unpublished data). Thus, at least in the setting of cognate interaction between T cells and elicited peritoneal macrophages, increased T cell stimulation by DAF^{-/-} mouse macrophages may not have been caused by the lifting of restraint on AP complement activation to increase local anaphylatoxin production within the immunological synapses, as has been proposed (Strainic *et al.*, 2008, Heeger *et al.*, 2005, Lalli *et al.*, 2008, Lalli *et al.*, 2007). More likely, it reflected a phenotype acquired *in vivo*, presumably as a consequence of challenging with thioglycollate which may have activated complement in the peritoneal cavity. A similar explanation may be offered to account for the association between DAF^{-/-} CD4+ T cells and the development of FSGS in DAF^{-/-} mice as described above (Bao *et al.*, 2009). Rather than DAF playing an intrinsic role on mouse CD4+ T cells during FSGS induction, it is possible that autoreactive CD4+ T cells developed spontaneously in the mutant mice due to chronic complement activation and inflammation. These autoimmune CD4+ T cells may be stimulated and further expanded to cause FSGS when the mice are challenged with pathogenic sheep anti-mouse podocyte antibodies (Bao *et al.*, 2009).

The possibility of transcellular regulation of APC function by anaphylatoxins, either directly or through cytokines, chemokines and other soluble mediators, should be considered not only for macrophages and splenic DCs *in vivo* prior to their isolation, but also for DCs in culture. In some studies, DCs were purified by FACS sorting (e.g. Strainic, Liu *et al.*, 2008) which is more desirable; while in other reports splenic or BM-derived DCs were enriched by CD11c+ microbeads to >80% purity (Peng *et al.*, 2008, Li *et al.*, 2008, Peng *et al.*, 2009, Weaver *et al.*, 2010). In the latter cases, the possibility cannot be entirely excluded that exogenously added C3a, C5a or C3aR and C5aR antagonists acted on other C3aR- or C5aR-bearing cells instead of, or in addition to, DCs, thus potentially confounding data

interpretation. This is especially true if the pharmacological agents are added to crude BM cultures early on in the DC differentiation process (Peng *et al.*, 2008, Peng *et al.*, 2009). The same caveat applies to RT-PCR detection of complement protein or C3aR/C5aR receptor expression in enriched but not completely pure DC populations (Peng *et al.*, 2008, Peng *et al.*, 2009). Of relevance to this discussion, we have routinely observed a substantial number of CD11c-/GFP+ cells at all stages of C5aR-GFP knock-in mouse BMDC culture (Dunkelberger *et al.*, 2010).

Not mutually exclusive with the above postulation that C3aR/C5aR signaling within the immunological synapses imparts a direct regulatory effect on T cell activation, we propose that in an integrated system C3aR/C5aR signaling could also regulate T cell immunity indirectly through changes in the cytokine milieu ("Signal 3") (Fig 3B). Such an activity of anaphylatoxins may involve crosstalk with TLRs and other innate immune systems and require the participation of other cell types in addition to T cells and APCs (Zhang *et al.*, 2007, Fang *et al.*, 2009). This hypothesis is certainly compatible with our data on DAF^{-/-} mice in two separate *in vivo* models of T cell immunity, one involved soluble antigen (OVA and MOG peptides) immunization in Complete Freund's Adjuvant to assess CD4+ T cell response and the other modeled CD8+ T cell immunity to viral (LCMV) infection (Fang *et al.*, 2007, Liu *et al.*, 2005). In both cases, the phenotype of DAF^{-/-} mice was dependent on C3 and C5 or C5aR and most likely involved TLR signaling as well. In a direct test of this paradigm, sera from mice with coincidental activation of C5aR and TLR4, TLR2 and TLR9 were shown to promote T_H17 cell differentiation when CD4+ T cells were activated by plate-bound anti-CD3/CD28 (Fang *et al.*, 2009). Furthermore, activity in mouse serum was critically dependent on IL-6 and TGF- β , the levels of which were synergistically elevated by C5aR and TLR signaling. This was further reinforced by the observation that the effect of C5aR-TLR4 crosstalk on T_H17 cell differentiation was recapitulated in co-cultures of mouse CD4+ T cells and peritoneal macrophages, with soluble anti-CD3/CD28 as activators (Fang *et al.*, 2009). Importantly, regulation of T_H17 cell differentiation by C5aR was mediated by cytokines released into the cell culture medium by the macrophages rather than through reciprocal C5aR signaling between T cells and macrophages during their cognate interaction. Thus, increased T_H17 differentiation was reversed if a C5aR antagonist was added at the time of macrophage co-stimulation with C5a and the TLR4 ligand LPS (blocking cytokine production), but not if it was added just prior to T cell/macrophage mixing and stimulation with soluble anti-CD3/CD28 (Fang *et al.*, 2009).

Concluding remarks

There seems to be a general agreement now on the conclusion that complement contributes to both CD4+ and CD8+ T cell immune responses in many disease and viral infection models. The complement proteins/effectors involved in these processes may be multiple but recent studies have highlighted the importance of the anaphylatoxins. Two potential mechanisms of action for the anaphylatoxins, not mutually exclusive, have been put forward (Fig 3A, B): 1) they play an intrinsic role on T cells and APCs through a self-sustaining circuit of local production and signaling within the immunological synapses, 2) they are produced either locally or systemically and regulate APC function and T cell immunity, perhaps in collaboration with TLRs, RLRs and NOD-like receptors, through the proxy of cytokines, chemokines and virus-induced interferons. Validation or rejection of these hypotheses requires additional and more vigorous testing in future experiments. Several points should be considered in such efforts. First, the trigger(s) for local AP complement activation in T cells and APCs needs to be established. Non-specific protease activity may play a role but this remains to be examined experimentally. If impaired complement regulation on the cell surface is responsible, then T cells and APCs deficient in Crry, another critical membrane complement regulator, might be expected to display a similar phenotype

as DAF-deficient cells; Second, inconsistencies in the literature on C3aR and C5aR expression on T cells and APCs need to be reconciled. Here, potential differences between human and mouse, resting and activated states, cell purity, and use of proper controls (IgG isotype and gene knockout) are variables that need to be considered; Third, different types of APCs (e.g. macrophages vs. DCs, resident vs. elicited macrophages, splenic vs. BM-derived DCs) may have different C3aR/C5aR expression and signaling machinery and therefore should not be viewed the same; Fourth, distinctions should be made between local (cell intrinsic) and systemic (trans regulation) effects of C3aR and C5aR signaling, particularly with regard to the use of primary cells isolated from gene knockout mice; Finally, in real-life settings such as during viral infection, it is unlikely that complement would regulate T cell immunity in isolation and interaction with other innate immune systems will most probably occur.

In conclusion, the revelation of a role of complement in T cell immunity represents an exciting development in the field but many gaps in our understanding of this phenomenon remain to be filled. Continued interest and study on this topic will not only contribute to our basic knowledge of complement biology but also have therapeutic relevance to T cell-mediated human diseases.

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Fig 1. Complement activation cascade

The complement system can be activated by the classical, lectin or alternative pathway; activated complement fulfills three types of effector function: inflammation, target opsonization and ingestion, and cell lysis. MBL, mannose binding lectin; PAMP, pathogen-associated molecular pattern; MASP; mannose binding lectin-associated serine protease; C3aR, C3a receptor; C5aR, C5a receptor; CR, complement receptor(s); CR1g, complement receptor of the immunoglobulin superfamily.

Fig 2. Regulation of T cell immune response by membrane complement regulators, C1q and C3 activation fragments

A. Both transmembrane and GPI-anchored complement regulators can regulate T cell proliferation and differentiation via complement-independent mechanism. **B.** C1q may regulate T cell immune response through direct binding to T cells or through modulation of APC function via receptor engagement or antigen (immune complex) opsonization. Surface deposition of activated C3 fragments (C3b and further cleavage products) on APCs has also been shown to regulate APC function and enhance T cell/APC interaction. DAF, decay accelerating factor; CR, complement receptor(s); C1qR, C1q receptor; APC, antigen presenting cell; TCR, T cell receptor; MHC, major histocompatibility complex.

Fig 3. Regulation of T cell immunity by anaphylatoxins

A. Anaphylatoxins are produced and act locally within the immunological synapses. Both T cells and APCs synthesize C3a and C5a and express their cognate receptors. **B.** Systemically or locally produced C3a and C5a regulate APC function through the production of cytokines, chemokines and interferons. C3aR/C5aR signaling may occur on APCs as well as other cell types and synergize with TLRs, NLRs and NOD-like receptors. AP, alternative pathway; APC, antigen presenting cell; TLR, Toll-like receptor; RLR, retinoic acid-inducible gene I-like receptors; NLR, nucleotide-binding oligomerization domain (NOD)-like receptors; IFNAR, interferon α receptor; MHC, major histocompatibility complex; CCR5; C-C chemokine receptor type 5.