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# Holding T cells in check – a new role for complement regulators?

**M. Paula Longhi, Claire L. Harris, B. Paul Morgan and Awen Gallimore**

Medical Biochemistry and Immunology, Henry Wellcome Building, Cardiff University, Heath Park, Cardiff, UK, CF14 4XN

**Complement is not only part of the innate immune system, but has also been implicated in adaptive immunity. The role of complement and its regulatory proteins in modulating T cell activity has been the focus of several recent studies. These, which have included work on the membrane co-factor protein (MCP or CD46), decay accelerating factor (DAF or CD55) and CD59, indicate that complement regulators can influence the proliferative capacity of T cells and their ability to produce cytokines, influencing the outcome of a T cell response to a given antigen. Here we review these studies, which reveal another important link between the innate and the adaptive immune system.**

## Regulation of complement

Complement (C) has long been considered an arm of the innate immune system, as it is activated immediately in the presence of pathogen and provides a rapid and efficient means for opsonising or killing pathogens and triggering inflammation. As far back as the 1970s, however, there were indicators that C might have a role in adaptive immunity, particularly in the humoral immune response (reviewed in [1]). C activation is triggered via either the alternative pathway, which ticks over constantly in plasma providing an immediate means of defence against pathogens, or the classical pathway, triggered by binding of C1 to antibody or of mannan-binding lectin (MBL) to bacterial carbohydrate moieties (Box 1). Products of activation include the pro-inflammatory fragments C3a and C5a, which recruit leukocytes to sites of infection and activate these and other cells, and the opsonic fragments C3b and C4b, which label targets for elimination by phagocytes. All routes of activation culminate in the formation of a lytic transmembrane pore, the membrane attack complex (MAC). The C system is in a constant state of low-level tick-over activation, poised to amplify on targets and pepper adjacent innocent bystander cells with C fragments and complexes. To survive this potent threat, an armoury of regulatory proteins has evolved that protect self cells from damage by homologous C. Some of these C regulators (CRegs) are soluble, plasma proteins, whereas others are expressed on cell membranes; the latter group is the subject of this review.

The activation pathways are controlled by several membrane CRegs that belong to the 'regulators of C activation' gene family and include decay accelerating factor (DAF or CD55) and membrane co-factor protein (MCP or CD46; reviewed in [2,3]). In common with all members of this family, they contain a structural module comprising ~60 amino acids, termed the short consensus repeat (SCR); the C3b-binding and functional activities of the proteins reside in this region [4]. CD46 and CD55 each contain four SCR modules (reviewed in [5]; Figure 1). On cell membranes, CD55 regulates C by binding to the C3 and C5 convertases, accelerating the decay of the enzyme subunits (decay accelerating activity) and thereby interfering with both the classical and alternative arms of the C cascade [(d) in Box 1] [6]. CD46 binds either C3b or C4b, freshly deposited or left on the membrane from decayed convertases, enabling a plasma serine protease, factor I (fI), to proteolytically cleave C3b or C4b (co-factor activity), preventing regeneration of the convertase (reviewed in [7]). In rodents, CD55 is broadly expressed and performs a similar CReg function [8,9]; by contrast, CD46 is absent from most murine tissues, expressed only in testis [10]. Instead, rodents have another broadly expressed membrane CReg, C receptor related protein-y (Crry) [11,12]. Crry is also built from SCR domains and performs the functions of both CD55 and CD46 [12,13].

Although several proteins collaborate to regulate the activation pathways of C, only one membrane CReg, CD59, inhibits the terminal pathway [14]. CD59, a small, globular glycoprotein (~20 kDa), is broadly expressed in tissues in humans and rodents and associated with the plasma membrane through a glycosyl phosphatidylinositol (GPI) anchor [15]. CD59 blocks formation of the lytic MAC by binding C8 within the C5b–8 complex, preventing unfolding and polymerisation of the final component, C9 [(d) in Box 1] [16]. Here, we review novel roles of these Cregs in modulating adaptive immune responses.

## Modulation of T cell activation by CRegs

### *CD46 and Crry*

Several recent publications and reviews have highlighted a role for CD46 in modulating T cell activity ([17] and reviewed in [18–20]). Simultaneous antibody ligation of CD3 and CD46 triggers induction of T cells showing characteristics of T regulatory 1-type cells (Tr1 cells). These cells secrete large amounts of interleukin-10 (IL-10) and inhibit proliferation of conventional $CD4^+$ T cells. Later studies revealed that CD46-induced Tr1 cells express high levels of granzyme B and perforin and can

*Corresponding author:* Gallimore, A. (gallimoream@cardiff.ac.uk).
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directly kill autologous targets [21]. Collectively, these observations suggest that Tr1 cells impinge upon other immune responses either through cell lysis or through immunosuppressive effects of IL-10. Importantly, these and other studies also showed that triggering with physiologically relevant ligand, such as C3b dimer or pathogen, induced the same phenotype [22,23].

As noted above, CD46 expression in rodents is restricted to testis and its C regulatory function is subsumed by the rodent-specific CReg, Crry. Like CD46, Crry might also influence T cell activation. Although studies using natural ligands to ligate Crry have not been performed, antibody crosslinking of Crry on murine T cells promotes T cell activation and the production of Th2 cytokines [24,25].

### *CD55 and CD59*

There are two genes for CD59 in the mouse, encoding the widely distributed CD59a (the homologue of human CD59) and the testis-restricted CD59b [26,27]. Similarly, two genes for CD55 have been characterised in mice (named Daf1 and Daf2). Daf1 is GPI anchored, widely distributed and is the human homologue of CD55, whereas Daf2 is transmembrane anchored and is expressed only in testis and spleen [28,29]. Mice deficient in Daf1 or CD59a have been engineered and used to examine the influence of these proteins on T cell activity *in vitro* and *in vivo*.

## Deficiency of Daf1 on T cells increases responses to antigen

Two recent studies describe T cell responses in CD55-deficient ($Daf1^{-/-}$) mice. The first study by Liu *et al.* [30] showed that recall responses measured using spleen cells from mice immunised with ovalbumin (OVA) or an MHC class II restricted myelin oligodendrocyte glycoprotein (MOG)-derived peptide were more vigorous in $Daf1^{-/-}$ mice than in controls. T cells from the $Daf1^{-/-}$ mice produced more interferon γ (IFNγ) and IL-2 upon antigen re-stimulation than their wild-type counterparts, whereas production of the immunosuppressive cytokine IL-10 was reduced. This hyper-responsiveness of T cells from the $Daf1^{-/-}$ mice was due to lack of CD55 on the T cells themselves because antigen presenting cells (APCs) purified from $Daf1^{-/-}$ mice stimulated T cells to produce IFNγ to the same degree as those purified from $Daf1^{+/+}$ mice. The more vigorous response observed in $Daf1^{-/-}$ T cells was not due to a global increase in sensitivity of T cells to antigen stimulation because no difference was observed in the response of $Daf1^{-/-}$ and $Daf1^{+/+}$ T cells to stimulation with superantigen. A key finding was that the enhanced production of IL-2 and IFNγ by $Daf1^{-/-}$ T cells after re-stimulation with antigen was largely dependent upon a functional C system because the effect was lost when $Daf1^{-/-}$ mice were backcrossed onto C3-deficient ($C3^{-/-}$) mice in which C activity is abolished. A residual, C-independent effect of Daf1 deficiency on T cell function was suggested by the observation that T cell responses were more vigorous in $C3^{-/-}Daf1^{-/-}$ mice than in $C3^{-/-}Daf1^{+/+}$ mice. The C-independent effect was minor and unlikely to be of physiological relevance, as shown by studies in a T cell-driven experimental autoimmune encephalomyelitis model. Here disease was exacerbated in $Daf1^{-/-}$ mice, but not in $Daf1^{-/-}C3^{-/-}$ mice. Importantly, the increased T cell activity upon antigen re-stimulation *in vitro* was lost in $Daf1^{-/-}$ mice after neutralisation of C5 *in vivo*. This finding implies that C products downstream of C5, that is, the anaphylatoxin C5a or the MAC, are responsible for the observed phenotype in $Daf1^{-/-}$ mice.

Heeger *et al.* [31] independently reported similar findings in a different line of $Daf1^{-/-}$ mice. In this study, the authors observed that $Daf1^{-/-}$ spleen cells proliferated more vigorously following *in vitro* stimulation with allogeneic cells. In addition, more $Daf1^{-/-}$ spleen cells from female mice immunised with the male antigen (HY*Dby*) produced IFNγ upon restimulation *in vitro* than in controls. $Daf1^{-/-}$ female mice also rejected male skin grafts more efficiently than controls and had more HY-specific T cell activity in IFNγ ELISpot assays.

Although results described in these two studies are superficially similar and support a role for Daf1 in modulating T cell response, there are important differences. Liu *et al.* [30] reported that $Daf1^{-/-}$ and

**Box 1. C activation and regulation**

C is activated via two pathways. The alternative pathway (Figure Ia, see next page) ticks over spontaneously in plasma (left), generating C3b-like molecules or C3b that can deposit on cell membranes. Membrane-bound C3b binds factor B (fB), which is cleaved and activated by factor D (fD) to form the C3 convertase (cleaving enzyme), C3bBb. This enzyme cleaves multiple molecules of C3 to C3b, which deposit on the membrane and form fresh C3 convertase enzymes or associate with the initiating enzyme to form C3bBbC3b, the C5 convertase. The C5 convertase cleaves multiple molecules of C5 to C5a and C5b, initiating the terminal pathway. The classical pathway (Figure Ib) is activated by antibody. The first component of the classical pathway, C1, binds antibody and becomes activated, leading to cleavage of C4 and deposition of C4b on the membrane. C2 associates with C4b and is cleaved and activated by C1 to form the C3 convertase, C4b2a. As with the alternative pathway, multiple molecules of C3b are cleaved and these associate with C4b2a to form the C5 convertase, C4b2a3b. The lectin pathway is identical to the classical pathway except that it is initiated by mannan-binding lectin (MBL) binding to carbohydrate moieties. Enzymes associated with MBL (MBL-associated serine proteases; MASPs) then cleave C4 and C2 in a manner similar to C1. The activation pathways converge at the point of C5 cleavage (Figure Ic). C5b remains associated with the C5 convertase, where it binds C6 and C7. The C5b67 complex releases from the convertase and binds loosely to the membrane; it rapidly binds C8, forming the C5b678 complex. C9 binds to this complex and polymerises to form a lytic pore, termed the membrane attack complex (MAC). Regulation of the activation and terminal pathways is brought about through the actions of the membrane associated CReg: CD55, CD46 and CD59 (Figure Id). CD55 has decay accelerating activity and acts by binding the C3 and C5 convertases of all activation pathways (the alternative pathway C3 convertase is illustrated here). Binding of CD55 accelerates the decay of the enzyme by promoting the release of the enzyme subunit Bb or C2a. By dissociating the enzymes CD55 prevents amplification of the cascade by further C3 cleavage and enzyme formation. CD46 has cofactor activity: it binds C3b or C4b following decay of the convertase and enables a plasma serine protease, factor I (fI), to cleave and inactivate the protein. CD59 regulates the terminal pathway by binding the C5b678 complex, preventing correct C9 polymerisation and pore formation.