Published in final edited form as:

Arch Immunol Ther Exp (Warsz). 2011 February ; 59(1): 49–59. doi:10.1007/s00005-010-0109-7.

CD46 Plasticity and its Inflammatory Bias in Multiple Sclerosis

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Abstract

Known as a link to the adaptive immune system, a complement regulator, a 'pathogen magnet' and more recently as an inducer of autophagy, CD46 is the human receptor that refuses to be put in a box. This review summarizes the current roles of CD46 during immune responses and highlights the role of CD46 as both a promoter and attenuator of the immune response. In MS patients, CD46 responses are overwhelmingly pro-inflammatory with notable defects in cytokine and chemokine production. Understanding the role of CD46 as an inflammatory regulator is a distant goal considering the darkness in which its regulatory mechanisms reside. Further research into the regulation of CD46 expression through its internalization and processing will undoubtedly extend our knowledge of how the balance is tipped in favor of inflammation in MS patients.

Keywords

CD46; Multiple Sclerosis; T cell; regulation

Introduction

Multiple Sclerosis is a chronic inflammatory disease of the brain. It is a complex disease that is thought to involve genetic, environmental and immunological aspects. T cells are central players in the induction of chronic inflammation, demyelination and axonal damage (Zozulya and Wiendl, 2008). Recent studies have highlighted the importance of T-regulatory cells (Tregs) in maintaining immunological homeostasis in MS (Viglietta et al., 2004; Venken et al., 2010). There are several types of Treg cells: CD4+CD25+ T regulatory cells which are either thymic derived (natural Treg or nTreg) or induced in the periphery (iTregs) and exert their suppressive effects primarily through cell to cell contact. Other iTregs exert their effects through the secretion of anti-inflammatory cytokines IL-10 (Tr1) (Battaglia et al., 2006) or TGF- β (Th3) (Faria and Weiner, 2005). CD46 is a co-stimulatory receptor for T cells (Astier et al, 2000; Zaffran et al, 2001) that can induce a Tr1- like phenotype characterized by the secretion of large amounts of IL-10 (Kemper et al., 2003). This pathway was shown to be defective in MS patients (Astier et al., 2006; Astier and Hafler,

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2007) and this report has recently been confirmed in two other independent studies, one using an *in vivo* MS monkey model (Martinez-Forero et al., 2008; Ma et al., 2009).

CD46 is a type 1 transmembrane protein expressed on all nucleated cells. The extracellular domain consists of four short consensus repeats or complement control proteins CCP1-4, followed by three exons rich in serine, threonine and proline (STP A, B and C), a transmembrane segment and a intracytoplasmic tail. Multiple isoforms are produced as a result of alternative splicing of the STP region and cytoplasmic tails (Liszewski et al., 1991). There are two distinct cytoplasmic isoforms Cyt1 (16 aa) and Cyt2 (23 aa). Both cytoplasmic tails are co-expressed with equivalent expression patterns in all cell types except for the salivary glands, kidney and brain where a preferential expression of Cyt2 was observed (Johnstone et al., 1993).

Biological roles of the complement regulator CD46

Over 20 years ago, CD46 was first identified as the membrane cofactor protein (MCP) and a member of the regulators of complement gene cluster (RCA) located on chromosome 1,1q32 (Seya et al., 1999). Complement regulators negatively regulate the complement activation cascade. CD46 acts as a cofactor for cleavage of C3b and C4b on the surface of cells, protecting autologous cells from complement attack. Human mutations in CD46 predispose individuals to atypical hemolytic uremic syndrome (Richards et al., 2003), and deficiency of the mouse homolog Crry results in embryonic lethality (Xu et al., 2000). During pregnancy, increased expression levels of CD46 are present in the placenta and protect the fetus from complement attack (Seya et al., 1999). Tumor cells may also hijack the function of complement regulators to shield themselves from complement attack and promote tumor metastasis. Some cancers show increased levels of surface CD46 (Gorter and Meri, 1999) and soluble CD46 shed from cancer cells retains the ability to bind C3b (Hakulinen et al., 2004). Indeed, recombinant soluble forms of CD46 can prevent malignant cells from complement attack (Gorter and Meri, 1999). In addition to a protective function, binding of CD46 to complement facilitates the fusion of spermatozoa and oocyte (Anderson et al., 1993). Indeed, the role of CD46 in fertilization is thought to extend beyond direct complement binding (Taylor et al., 1994). Of note, rodents only express CD46 in the testis (Tsujimura et al., 1998).

CD46 a 'Pathogen magnet' and an obliging host?

CD46 has been labeled a pathogens' magnet (Cattaneo, 2004), as it has the ability to bind to various viruses and bacteria. CD46 was first identified as the measles virus (MV) receptor gp57/67, a glycoprotein with a molecular weight of 57-67kDa (Dorig et al., 1993; Nanche et al., 1993). Since then, CD46 has been identified as a receptor for several other human pathogens; *Neisseria gonorrhoeae* (Kallstrom et al., 1997; Santoro et al., 1999), herpes virus 6 (HHV-6) (Santoro et al., 1999), various group B adenovirus (Segerman et al., 2003; Gaggari et al., 2003) and *streptococcus pyogenes* (Okada et al., 1995; Giannakis et al., 2002). Binding of pathogens to CD46 can trigger inflammatory reactions that aid host defense by inducing CD46 downregulation and sensitivity to complement attack (Schnorr et al., 1995), inflammatory cytokine production (Ghali and Schneider-Schaulies, 1998), antibody class

switching (Imani et al., 1999) and autophagy (Joubert et al., 2009). However, pathogens can also manipulate CD46 signaling in favor of their own survival, by utilizing signaling pathways that to date remain largely unknown. Binding of *Neisseria gonorrhoeae* to epithelial cells induces a calcium flux that is blocked by CD46 antibodies (Kallstrom et al., 1998) and is involved in the adhesion of these bacteria to epithelial cells. Adhesion appears to be an active process involving CD46 tail signaling, as it is not supported in CD46 mutants with truncated tails (Kallstrom et al., 1997; Santoro et al., 1999). Upon infection of human epithelial cells, tyrosine phosphorylation of Cyt2 occurs involving the src kinase c-Yes. PP2, a src family kinase inhibitor reduces adhesion abilities of the bacteria (Lee et al., 2002). Antibody ligation of CD46 in Jurkat cells also results Cyt2 tyrosine phosphorylation by the src kinase Lck (Wang et al., 2000b). Additionally, group A *Streptococcus* can manipulate the adaptive immune system by inducing an immunosuppressive Tr1 phenotype in T cells associated with IL-10 and granzyme B production (Price et al., 2005).

MV infection is followed by immune suppression that contributes to a high mortality rate after infection. Down-regulation of IL-12 as a result of infection is thought to play a role in immuno-suppression. Upon ligation of CD46 with MV, C3b or antibodies, human monocytes decrease IL-12 production (Karp et al., 1996). However, a later study demonstrated that human macrophages infected with the wild-type MV strain Kohnno (KO) or ligated with CD46 antibodies induce the upregulation of IL-12 p40 and nitric oxide. The authors suggest that pro-inflammatory or anti-inflammatory responses may be CD46 ligand dependent (Kurita-Taniguchi et al., 2000). Differences in monocyte/macrophage responses may also be due to differences in their maturation stages. Indeed, upon MV infection, mouse macrophages expressing human CD46 (especially the Cyt1 isoform) also promote inflammation. In the presence of IFN γ , CD46 tails induce signaling that increases IFN α/β production, resulting in increased nitric oxide production and decreased viral replication (Hirano et al., 1999; Katayama et al., 2000). Other cell types also elicit proinflammatory responses upon MV infection. Astrocytes produce IL-6 after treatment with MV proteins and CD46 crosslinking (Ghali and Schneider-Schaulies, 1998). In the presence of IL-4, infection with MV or CD46 crosslinking increases IgE class switching in B cells (Imani et al., 1999).

In MS, it is hypothesized that T cells become activated in the periphery via non-self antigens in a process known as 'molecular mimicry', whereby viral proteins with similar epitopes to Myelin basic protein (MBP) can trigger an autoimmune response via cross-reactive T cells. Cross-reactive epitopes are found in Epstein-Barr virus, Hepatitis B virus, HHV-6, and Haemophilus influenza (Miller and Eagar, 2001; Croxford et al., 2002; Markovic-Plese et al., 2005; Tejada-Simon et al., 2003). HHV-6 is also linked to MS, albeit controversially, and may play a role in skewing T cell responses. HHV-6 is linked to MS due to its neurotropism, latency and periodic reactivation (Clark, 2004). CD46 is a receptor for HHV-6 (Santoro et al., 1999) and is relocated to lipid rafts upon viral entry (Tang et al., 2008). In dendritic cells (DCs), HHV-6 infection suppresses IL-12 production normally induced by IFN γ or LPS and is believed to be the result of CD46 engagement (Smith et al., 2003). CD46 expression on oligodendrocytes and astrocytes facilitates cell-cell fusion with infected T cells and may support transmission of HHV-6 from the periphery to the central

nervous system (CNS) (Cassiani-Ingoni et al., 2005). Active HHV-6 replication has been associated with relapses and increased EDSS in RRMS patients (Alvarez-Lafuente et al., 2006). Elevated levels of HHV-6 DNA have also been reported in MS plaques and serum that correlated with clinical exacerbations (Cermelli et al., 2003; Berti et al., 2002). As CD46 T cell co-stimulation promotes inflammation in MS patients, increased levels of its ligands in the periphery such as HHV-6 are likely to increase the probability of CD46 co-stimulation.

CD46 in acquired immunity: a molecule that swings both ways

The simplified view of CD46 as a membrane co-factor protein was cast aside 10 years ago when CD46 was discovered to be a novel T-cell co-stimulatory molecule (Astier et al., 2000). CD46 was revealed as a new link between innate and adaptive immunity. Initially, ligation of CD46 was shown to induce tyrosine phosphorylation of p120^{CBL} and LAT, two adaptor molecules that are activated by T cell activation, and CD46 co-stimulation induced strong levels of proliferation (Astier et al., 2000). Further studies identified signaling molecules Vav, Rac, Erk and MAPK, important molecules in T cell activation, in CD46 co-stimulation and demonstrated morphological changes and actin relocalization in T cells (Zaffran et al., 2001). Later, it was demonstrated that ZAP70 and TCR/CD3 zeta chain were also phosphorylated upon CD46 activation (Sanchez et al., 2004). Interestingly, it was reported that Crry, the murine autologous molecule of CD46, also acts as a co-stimulatory molecule for murine T cells, highlighting the role of these co-stimulatory molecules in regulating adaptive responses (Fernandez-Centeno et al., 2000; Longhi et al., 2006).

Efforts to identify the phenotype of CD46 co-activated T cells became clearer and yet more complex when a contact hypersensitivity reaction in CD46 transgenic mouse model demonstrated antagonistic roles on inflammation of CD46 cytoplasmic isoforms Cyt1 and Cyt2. Cyt1 was shown to promote CD4+ T cell proliferation, to decrease CD8+ cytotoxicity and IL-2 production and overall to suppress the inflammatory reaction while Cyt2 increased CD8+ cytotoxicity and decreased IL-10 production with an overall increase in inflammation (Marie et al., 2002). Costimulation of human CD4+ cells by CD46 monoclonal antibodies or C3b in the presence of IL-2 was later shown to acquire a Tr1-like phenotype, producing large amounts of IL-10 and inhibiting bystander T cell activation (Kemper et al., 2003). Interestingly, CD46-induced Tr1s also express granzyme B and perforin and have the ability to destroy autologous activated T cells, DCs and monocytes in a contact dependent manner (Grossman et al., 2004).

Sanchez et al confirmed that upon CD46 co-stimulation CD4+ T cells enhance production of IL-10. However, in contrast to Kemper et al, CD4+ T cells stimulated with anti-CD28/CD46/CD3 resulted in a Th1 response characterized by a large production of IL-2 and IFN γ (Sanchez et al., 2004). The exact reason for this inconsistency is unclear, however, differences in antibody clones and their concentrations may be responsible. Sanchez et al also observed that, upon CD46 costimulation, CD4+ T cell blasts (obtained by activation of PBMC with PHA) produced IFN γ and IL-5 but failed to increase IL-10 production compared to CD3 stimulation alone. Addition of PMA increased IFN γ , IL-2 and IL-5 with no change in IL-10 production. Thus, Sanchez et al conclude that both T cell blasts and

CD4⁺ T cells co-stimulated with CD28 are predisposed to a Th1 phenotype, but that CD4⁺ T cells co-stimulated with CD46 in the absence of CD28 stimulation differentiate in Tr1 cells (Sanchez et al., 2004). Importantly, the authors do not preclude the induction of Tr1 cells but rather highlight the importance of understanding the mechanism of CD46 ligation. Further roles have been identified for CD46-induced Tregs. They can promote B cell antibody production through cell-cell contact and IL-10 production (Fuchs et al., 2009). In addition, CD46-induced Tregs secrete CD40L and GM-CSF that promote DC maturation in spite of IL-10 production (Barchet et al., 2006).

Oliaro et al unravel one of the physical mechanisms whereby the timing of CD46 ligation can regulate lymphocyte polarization and cell signaling. In summary, ligation of CD46 on cytotoxic T lymphocytes (CTLs) and NK cells leads to cell polarization at the site of ligation. Similarly, T cells cultured with L cells expressing surface measles hemagglutinin, a CD46 ligand, resulted in CD46, CD3 and the microtubule organization centre (MTOC) localization at the point of contact. However, if CTLs were pre-incubated with soluble CD46 prior to anti-CD3/28 stimulation there was a 55% reduction in polarization, alongside a decrease in IFN γ production. Similarly, incubation of T cells with allogenic DCs after CD46 ligation leads a reduction of IFN γ production, a capping of CD46 and a reduction of CD3 and MTOC at the site of ligation compared to allogenic DC stimulation alone. Additionally, CD46-ligated NK cells cultured with HeLa cells also showed decreased MTOC and perforin at the point of ligation that was associated with decreased HeLa cell death (Oliaro et al., 2006). This paper highlights the role of CD46 in cell polarization and cell function and further underlies the necessity to understand when and how CD46 is ligated *in vivo*.

CD46: A gateway for Inflammation in MS

When the timing and mechanism of CD46 ligation plays such an apparent role in regulating cell responses it is likely that abnormal ligation of CD46 occurs in dysregulated immune states such as MS that can further skew or inflame inherent defects in CD46.

Tr1 cells and IL-10

IL-10 is a potent immunosuppressive cytokine and its importance in regulating autoimmune diseases is well documented in the Experimental Autoimmune Encephalomyelitis (EAE) murine model of MS (Cohen et al., 2010; Bettelli et al., 1998; Cua et al., 1999). Functional defects in CD4⁺CD25^{high} Tregs have been identified in MS (Viglietta et al., 2004; Haas et al., 2005). These defects and the crucial regulatory role of IL-10 led to similar investigations in Tr1 function (Astier et al., 2006; Astier, 2008). An impaired IL-10 production by CD46-induced Tregs was observed. T cells isolated from RRMS patients showed little or no IL-10 production that was specific to CD46 stimulated T cells, as there was no decrease of IL-10 in CD28 stimulated cells (Astier et al., 2006). IFN β treatment had no effect on IL-10 production suggesting its immunomodulatory effects do not alter CD46-induced Tregs. Increased levels of Cyt2 at the RNA level were also observed supporting evidence from the transgenic mouse model that Cyt2 promotes inflammation (Astier et al., 2006). Similarly to Astier et al., Martinez-Forero and collaborators observed a significant reduction in IL-10 production upon CD46 activation in MS patients compared to healthy controls (Martinez-

Forero et al., 2008). Additionally, CD46-induced Tr1 dysfunction has been observed in a mimic monkey model of MS. *Ex vivo* induced Tr1 cells from active MS monkeys had a 9-fold decrease in IL-10 production compared to controls. TGF β production was also diminished especially in active MS. Consistent with Astier et al., reduced IL-10 production was specific to CD46 stimulation and there was no difference in IFN γ production in MS models and healthy controls (Ma et al., 2009). Genomic studies have demonstrated that MS patients have alterations in the alpha chain of the IL-2 receptor, IL-2RA (Hafler et al., 2007). Since IL-2 plays a crucial role in the maintenance of CD46 induced Tr1 cells, it likely contributes to the defects in CD46 signaling.

IL-23 and IL-17

IL-23, a pro-inflammatory cytokine, is critical to the development of autoimmune inflammation in the brain (Cua et al., 2003) and the survival of IL-17-producing effector cells (McGeachy et al., 2009). IL-23 secretion levels are elevated in activated DCs of MS patients (Vaknin-Dembinsky et al., 2006). IL-23 consists of two subunits, the specific p19 subunit and the p40 subunit (shared with IL-12) (Cua et al., 2003). Maturation of myeloid DCs (mDCs) in the presence of CD46 antibodies enhanced the expression of the specific IL-23p19 subunit compared to cells activated with LPS alone. In addition, CD46-activated mDCs supernatant significantly increased IL-17 secretion by CD4 $^{+}$ T cells (Vaknin-Dembinsky et al., 2008). Compared to healthy controls, MS patients showed significant increases in IL-23 production in CD46-activation mDCs, and this was associated with the upregulation of the IL-23p19 subunit. Therefore, increased levels of IL-23 in MS may be partially due to defects in CD46.

CD46 and T Cell Migration

The migration of inflammatory cells to the brain and their transport across the blood brain barrier (BBB) is a crucial factor in the pathogenesis of MS. CD46 is highly expressed at the BBB (Shusta et al., 2002), and transgenic mice expressing human CD46 facilitate transport across the BBB, and subsequently become susceptible to meningococcal disease (Johansson et al., 2003). CD46 co-stimulated cells spread out and form membrane protrusions containing actin, a phenotype typically associated with high cell motility (Zaffran et al., 2001).

CD46-activation of mDCs in presence of LPS leads to increased amounts of CCL3 and CCL5 produced compared to LPS alone, while the production of CCL2 is decreased (Vaknin-Dembinsky et al., 2008). CCL5 and CCL3 are both chemokines that promote the recruitment of inflammatory cells and are increased in the cerebrospinal fluid (CSF) of MS patients during relapse (Bartosik-Psujek and Stelmasiak, 2005; Miyagishi et al., 1995). Inhibitors of CCL3 prevent monocellular influx into the CNS and subsequent EAE induction (Karpus et al., 1995). CD46-activated DCs from MS patients secrete increased amounts of CCL3 and CCL5 compared to healthy controls, while the production of CCL2 is abrogated (Vaknin-Dembinsky et al., 2008). In astrocytes, the engagement of CD46, by measles virus induces dose dependent secretion of CCL5 (Noe et al., 1999). HHV-6, previously linked to

MS, induces secretion of both CCL3 and CCL5 by astrocytes (Meeuwsen et al., 2005; Noe et al., 1999).

CXCR3 is typically expressed on T cells upon activation and is associated with a Th1 phenotype (Rot and von Andrian, 2004). Increased expression patterns of CXCR3 have been identified in progressive MS (Balashov et al., 1999), during MS relapses (Mahad et al., 2003), and it has also been found in MS lesions (Balashov et al., 1999). We have observed an increased expression of CXCR3 upon CD46 T cell co-stimulation (unpublished data). In order for T cells to cross the BBB firstly they must attach to the BBB endothelium. This is facilitated by integrins, such as $\alpha 4 \beta 1$ (very late activation antigen 4; VLA-4) and $\alpha 4 \beta 7$ that bind to counter receptors on endothelium cells. Natalizumab, used as a treatment for MS, is a monoclonal antibody that binds to the $\alpha 4$ chain of $\alpha 4 \beta 1$ and $\alpha 4 \beta 7$ (Davenport and Munday, 2007). VLA-4 binds to the epithelial receptor, VCAM-1 (vascular cell adhesion molecule-1). By disrupting the interaction between $\alpha 4 \beta 1 / \alpha 4 \beta 7$ and VCAM-1, Natalizumab is believed to inhibit T cell binding to the BBB endothelium, T cell migration into the CNS and subsequent T cell inflammatory responses (Mellergard et al., 2010). Under certain conditions, CD46-activated T cells upregulate $\alpha 4 \beta 7$ and CCR9 which is indicative of a gut homing phenotype (Alford et al., 2008). However, T cells isolated from the CSF also express $\alpha 4 \beta 7$ and CCR9 (Kivisakk et al., 2006).

CD46 Regulation

As CD46 is clearly involved in T cell activation and MS pathogenesis, the complete understanding of its regulation of expression and function is crucial. Moreover, it is likely that CD46 might be defective in other diseases, as indeed, a recent paper reports the dysfunction of CD46 in asthma patients (Xu et al., 2010).

CD46 Surface Downregulation

CD46 is downregulated at the cell surface by adenovirus (Sakurai et al., 2007), *Neisseria gonorrhoeae* (Gill et al., 2003), measles virus (Naniche et al., 1993), Group A streptococcus (Lovkvist et al., 2008) and gingivalis (Mahtout et al., 2009). CD46 sequence contains the YXXL motif that is required for downregulation of other membrane proteins (Yant et al., 1997). The mode of downregulation varies and it is not limited to pathogen ligation. At the surface level, CD46 is internalized and/or downregulated by at least four mechanisms. Firstly, in DCs and other non-lymphoid cells, CD46 is constitutively internalized via clathrin-coated pits to the golgi or golgi vicinity and recycled to the surface. This process is absent in peripheral blood lymphocytes and the Jurkat cell line. Secondly, as shown by the same group, multivalent cross-linking of CD46 induces a process similar to macropinocytosis and results in the down regulation of CD46 at the surface. This process was observed in lymphoid and non-lymphoid cells (Crimeen-Irwin et al., 2003). Thirdly, apoptotic cells have been shown to shed entire CD46 molecules in vesicles or microparticles (Hakulinen et al., 2004; Elward et al., 2005). Finally, CD46 undergoes proteolytic cleavage in necrotic cells (Elward et al., 2005) and apoptotic cells (Hakulinen and Keski-Oja, 2006; Cole et al., 2006). There is accumulating evidence that matrix metalloproteinases (MMP) and their closely related family of a disintegrin and metalloproteinase (ADAM) play a role

in regulating CD46 expression. Vesicular forms of CD46 are cleaved by ADAM10 after release from apoptotic epithelial cells and there is also direct cleavage at the cell surface (Hakulinen and Keski-Oja, 2006). The venom from *Loxocles* induces cleavage of CD46 in epithelial cells and neutrophils via the activation of metalloproteinase of the adamalysin family (Van Den Berg et al., 2002). CD46 on apoptotic neuronal cell lines was shown to be cleaved by MMP3, -8, -9 (Cole et al., 2006). Our data demonstrates that CD46 is also cleaved extracellularly by (a) metalloproteinase(s) in CD46-activated T cells. Therefore, it is becoming apparent that CD46 expression and processing is tightly regulated (Ni-Choileain, Astier, submitted).

The role of CD46 down-regulation at the surface has not been completely elucidated. However, it is known that CD46 downregulation facilitates pathogen entry and increases cell susceptibility to complement attack (Schnorr et al., 1995). Moreover, CD46 is released from apoptotic cells in a caspase-dependent manner (Elward et al., 2005; Hakulinen and Keski-Oja, 2006) and also appears to regulate complement activation during cell death (Elward et al., 2005; Cole et al., 2006). Importantly, CD46 also contributes to the adaptive immune response and surface regulation likely supports this functionality. CD46 endosomal/lysosomal pathway is known to support the major histocompatibility complex (MHC II) presentation of MV antigens (Gerlier et al., 1994). Thus, constitutive CD46 recycling by DCs may play a role in environment sampling and antigen presentation (Crimeen-Irwin et al., 2003). CD46 is shed during T cell activation and this downregulation is time dependent. Furthermore, upon the addition of a GM6001, a broad-spectrum inhibitor, IL-10 production upon CD46 activation is decreased. This suggests that surface down-regulation plays an active role in T cell function (Ni-Choileain, submitted).

Metalloproteinases and CD46 Regulation: Implications in MS?

As yet, signaling events occurring after proteolytic cleavage of CD46 are unknown. However, it is unlikely to be a trivial event considering the array of functions associated with CD46. CD46 can be cleaved by MMP3, -8, -9 and ADAM10 (Hakulinen and Keski-Oja, 2006; Cole et al., 2006). If proteolysis of CD46 can induce signaling cascades, it is likely that any imbalance of associated metalloproteinases will disrupt CD46 induced inflammatory responses. In MS, MMP-9 is upregulated in the plaques and serum of patients compared to healthy controls (Waubant et al., 1999). Polymorphisms in MMP-9 have been linked to an increased risk factor in MS (Mirowska-Guzel et al., 2009). MMP-9 increases T cell migration across the BBB, and both IFN β and steroids treatment can reduce MMP-9 expression (Trojano et al., 1999; Garcia-Montojo et al.). More recently, IFN β treatment was directly linked to decreased MMP-9 expression in mature dendritic cells and a reduction in their migratory capacity (Yen et al., 2010). In addition to the matrix metalloproteinases, ADAM10 also cleaves CD46. Interestingly, ADAM10 has been implicated in myelin degradation and an altered ADAM10 expression in MS patients has been reported (Kieseier et al., 2003). Increased levels of ADAM10 are also associated with familial hemolytic uremic syndrome (Hakulinen and Keski-Oja, 2006). However, metalloproteinases are not solely pro-inflammatory and are known to have beneficial properties in axonal growth and remyelination (Larsen et al., 2003). Interestingly, infection by *streptococcus pyogenes*, which binds to CD46, results in an increase in ADAM10 production (Lemjabbar and

Basbaum, 2002) and *streptococcus pyogenes* induces a Tr1-like phenotype characterized by IL-10 and granzyme B production (Price et al., 2005). Thus, alterations in the milieu of metalloproteinases and their inhibitors could potentially alter cell fates by utilizing the plasticity of CD46 to enhance or dampen inflammation.

Soluble CD46 is the product of proteolytic cleavage of membrane CD46. Soluble CD46 is found in plasma, tears and seminal fluid (Hara et al., 1992), however its role is largely unknown. Increased levels are found in cancer patients' sera (Seya et al., 1995). Soluble and vesicular CD46 released from cancer cells retain C3b binding capacity and may support metastasis of cancer by protecting the microenvironment and smaller tumors from complement attack (Hakulinen et al., 2004). *Streptococcus pyogenes* binds soluble CD46, perhaps as a mechanism to evade complement attack (Lovkvist et al., 2008). Increased levels of sCD46 have also been documented in autoimmune diseases, systematic lupus erythematosus (SLE) and multiple sclerosis (Kawano et al., 1999; Soldan et al., 2001). In MS, soluble levels of CD46 have been found bound to HHV-6 in the serum of MS patients (Soldan et al., 2001; Fogdell-Hahn et al., 2005). Therefore, it is clear that soluble CD46 maintains its binding functions. However whether it can also act as a regulator of T cell activation remains unknown.

CD46 is a novel substrate of presenilin- γ -secretase

Following extracellular cleavage of CD46, a C-terminal transmembrane fragment (CTF) remains. Although, some CTFs can retain signaling activity (Parks and Curtis, 2007), no signaling capacities of the CD46 CTF have been published to date. However, Weyand et al have recently reported that CD46 is subsequently processed, after extracellular proteolysis, by presenilin- γ -secretase (P γ S) upon *Neisseria gonorrhoeae* or *Neisseria meningitidis* infection of epithelial cell (Weyand et al.). Our data demonstrate that CD46 also undergoes presenilin proteolysis in CD46 activated Tr1 cells (Ni Choileain et al, in press). Other P γ S substrates such as amyloid precursor protein (APP), Notch and ERBB4 release intracellular domains (ICDs) with transcriptional activity upon proteolytic cleavage (Parks and Curtis, 2007). Both CD46 cytoplasmic domains contain nuclear localization motifs (Kawano et al., 1999; Wang et al., 2000a) suggesting an ability to translocate to the nucleus and propagate cell signaling.

Is regulated internal processing (RIP) involved in T cell signaling?

CD46 signaling cascades in T cells are largely unknown, however, general T cell activation events that have been identified in the CD46 pathway could accommodate RIP of CD46. Phosphorylation of the APP CTF increases γ -secretase processing (Vingtdeux et al., 2005) and CD46 Cyt2 cytoplasmic domain, but not Cyt1, undergoes tyrosine phosphorylation in T cells upon CD46 ligation (Wang et al., 2000a). Interestingly, our unpublished results show that broad-spectrum γ -secretase inhibitors have greater effects on the expression of Cyt2 than of Cyt1. Endocytosis of Notch receptors and subsequent signaling, recycling and/or degradation is closely related to ubiquitination (Fortini, 2009). CD46 co-activation induces p120^{CBL} a negative regulator of T cell signaling (Murphy et al., 1998) that is implicated in ligand-induced ubiquitination, degradation and downregulation of receptors (Yokouchi et

al., 1999; Miyake et al., 1998). Lipid rafts play a key role in spatially organizing TCR signaling molecules (Dykstra et al., 2003) and are also associated with the localization of γ -secretase (Vetrivel et al., 2005). Thus, CD46 T cell stimulation induces a fertile environment for γ -secretase regulation and may play an important role in CD46 signaling.

The role of CD46 in Autophagy and MS

Autophagy plays numerous roles in the innate and adaptive immune response; degradation of pathogens, TLR pathogen recognition (Maiuri et al., 2007), MHC class I and class II antigen presentation (English et al., 2009; Dengjel et al., 2005; Paludan et al., 2005) and T cell survival and proliferation (Li et al., 2006). Autophagy also plays a key role in T cell selection in the thymus and is required for the generation of tolerant T cells (Nedjic et al., 2008). There is growing evidence that autophagy plays a role in MS. A recent paper by Alirezai et al. demonstrates increased RNA levels of Atg5 in RRMS patients' peripheral T cells compared to healthy controls (Alirezai et al., 2009). Atg5 is also found in encephalitogenic T cells from RRMS brain samples, and EAE studies demonstrate a strong correlation between disease severity and Atg5 RNA expression levels. Thus, increased levels of Atg5 may facilitate prolonged survival and/or proliferation of T cells and promote immune mediated demyelination (Alirezai et al., 2009).

Joubert et al have revealed an important role of CD46-Cyt1 (but not Cyt2) in the induction of autophagy. Upon CD46 ligation, Cyt1 binds to the scaffold protein GOPC via its PDZ domain which links to the autophagosome formation complex VPS34/Beclin1. The CD46-Cyt1/ GOPC pathway is induced upon binding of measles virus and Group A streptococcus, initiating pathogen degradation (Joubert et al., 2009). The role of autophagy in the CD46 adaptive immune response has not been investigated. However, autophagy is known to promote T cell homeostasis and is promoted upon TCR ligation and in the presence of IL-2 (Li et al., 2006), both of which are crucial for the induction of the CD46 Tr1 phenotype. Indeed, the increased expression of Cyt2, observed in MS patients at the RNA level (Astier et al., 2006) may alter T cell homeostasis by dysregulating the balance of Cyt1-induced autophagy.

Conclusions

The signaling capabilities of CD46 are diverse and are sensitive to ligand interaction and external stimuli. It is only recently that the extent and complexity of CD46 signaling has become apparent. Distinct CD46 isoforms and regulatory processing are mechanisms that likely support the multitude of cell responses and regulate the timing of any given response. CD46 has the capacity to either promote or suppress an inflammatory response. It is regulated at the surface by metalloproteinases, shedding and internal downregulation. As of yet, distinct signaling cascades have not been identified for cleaved CD46 fragments. However, considering the analogous properties of CD46 with other P γ S, it seems inevitable that CD46 will follow suit. In MS, cell responses upon CD46 activation are skewed towards a pro-inflammatory response while in healthy controls they are anti-inflammatory, as summarized in Table 1. The underlying defects in this response remain to be elucidated. Regulated intracellular processing of CD46 opens the door to increased diversity in the

signaling pathway and identifies new avenues of research that may unravel the cause of CD46 dysfunction in MS, and may be in other human diseases. Indeed, pathogens have long utilized the signaling capacity of CD46 to induce anti-inflammatory signals; perhaps with time these same pathways can be manipulated to return the balance of immune responses in patients with MS.

Acknowledgement

This work was supported by a research grant from the MS society (UK) to ALA (859/7).

Abbreviations

ADAM	A disintegrin and metalloproteinase
BBB	Blood brain barrier
CTF	C-terminal transmembrane fragment
DC	Dendritic Cell
EAE	Experimental autoimmune encephalomyelitis
ICDs	Intracellular domains
iTregs	Inducible T-regulatory cells
MBP	Myelin basic protein
MMP	Matrix metalloproteinase
MS	Multiple Sclerosis
MV	Measles virus
PγS	Presenilin- γ -secretase
ROS	Reactive oxygen species

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Table 1
CD46 dysregulation in Multiple Sclerosis

CD46 Dysregulation in Multiple Sclerosis		
Cell Type	Known Defects in MS upon CD46 activation	References
Cytokine Production		
CD4+ T Cell	↓ IL-10 production in MS ↓ TGF- β (particularly in active MS) in the monkey model	(Astier et al. 2006, Martinez-Forero et al. 2008, Ma et al. 2009) (Ma et al. 2009)
Dendritic Cell	↑ IL-23 production associated with ↑ specific IL-23p19 subunit.	(Vaknin-Dembinsky et al. 2008)
Inflammatory Cell Migration		
CD4+ T cell	↑ CXCR3 in RRMS	(Ni Choileain, Astier, unpublished)
Dendritic Cell	↑ CCL3 and CCL5	(Vaknin-Dembinsky et al. 2008)
CD46 Expression		
CD4+ T cell	Increased expression of Cyt2 at the RNA level	(Astier et al. 2006)