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REVIEW

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 patients with multiple sclerosis (MS), 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

Abbreviations

ADAM	A disintegrin and metalloproteinase
BBB	Blood brain barrier
CTF	C-terminal transmembrane fragment
DC	Dendritic cell
mDCs	Myeloid DCs
EAE	Experimental autoimmune encephalomyelitis
Tregs	T-regulatory cells
MMP	Matrix metalloproteinase
MS	Multiple sclerosis
RRMS	Relapsing-remitting MS
MV	Measles virus
PγS	Presenilin-γ-secretase
VLA-4	Very late activation antigen 4
VCAM-1	Vascular cell adhesion molecule-1
HHV-6	Herpes virus 6
TCR	T cell receptor
CTLs	Cytotoxic T lymphocytes
NK	Natural killer
MTOC	Microtubule organization centre
APP	Amyloid precursor protein
LPS	Lipopolysaccharide

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Introduction

Multiple sclerosis (MS) 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: $CD4^+CD25^+$ Tregs, which are either thymic derived (natural Treg or nTreg) or induced in the periphery

(inducible Tregs) and exert their suppressive effects primarily through cell to cell contact. Other inducible Tregs, exert their effects through the secretion of the anti-inflammatory cytokine IL-10 (Tr1) (Battaglia et al. 2006) or TGFβ (Th3) (Faria and Weiner 2005). CD46 is a costimulatory 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 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 I 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 an 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 and a member of the regulators of complement gene cluster 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 pathogen 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 57–67 kDa (Dorig et al. 1993; Naniche 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 adenoviruses (Gaggar et al. 2003; Segerman et al. 2003) and *Streptococcus pyogenes* (Giannakis et al. 2002; Okada et al. 1995). 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 *N. 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. 999). 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 in Cyt2 tyrosine phosphorylation by the Src kinase Lck (Wang et al. 2000). 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. Downregulation of IL-12 as a result of infection is thought to play a role in immunosuppression. 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 Kohno 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 IFNγ, CD46 tails induce signaling that increases IFNα/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 pro-inflammatory responses upon MV infection. Astrocytes produce IL-6 after treatment with MV proteins and CD46 cross-linking (Ghali and Schneider-Schaulies 1998). In the presence of IL-4, infection with MV or CD46 cross-linking 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 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 influenzae* (Croxford et al. 2002; Markovic-Plese et al. 2005; Miller and Eagar 2001; 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 neurotrophism, 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 IFNγ or lipopolysaccharide (LPS), which 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 expanded disability status scale score in relapsing-remitting MS (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 (Berti et al. 2002; Cermelli et al. 2003). As CD46 T cell costimulation 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 activation.

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 costimulatory 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 costimulation induced strong levels of proliferation (Astier et al. 2000). Further studies identified the activation of the signaling molecules Vav, Rac, Erk and MAPK upon CD46 costimulation. The authors also demonstrated that CD3/CD46 activation induced dramatic morphological changes and actin relocalization in T cells (Zaffran et al. 2001). Later, it was demonstrated that ZAP70 and T cell receptor (TCR)/CD3ζ 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 costimulatory molecule for murine T cells, highlighting the role of these costimulatory 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 a CD46 transgenic mouse model demonstrated that the cytoplasmic isoforms Cyt1 and Cyt2 induce antagonistic roles on inflammation. 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 result in a Tr1-like phenotype, with the production of large amounts of IL-10 and inhibition of 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. (2004) confirmed that upon CD46 costimulation $CD4^+$ T cells enhance production of IL-10. However, in contrast to Kemper et al. (2003) $CD4^+$ T cells stimulated with anti-CD28/CD46/CD3 resulted in a Th1 response characterized by a large production of IL-2 and IFNγ. The exact reason for this inconsistency is unclear, but differences in antibody clones and their concentrations may be responsible. Sanchez et al. (2004) also observed that, upon CD46 costimulation, $CD4^+$ T

cell blasts (obtained by activation of peripheral blood mononuclear cells with phytohemagglutinin) produced IFNγ and IL-5 but failed to increase IL-10 production compared to CD3 stimulation alone. Addition of phorbol 12-myristate 13-acetate increased IFNγ, IL-2 and IL-5 with no change in IL-10 production. Thus, Sanchez et al. (2004) conclude that both T cell blasts and $CD4^+$ T cells costimulated with CD28 are predisposed to a Th1 phenotype, but that $CD4^+$ T cells costimulated with CD46 in the absence of CD28 stimulation differentiate into Tr1-like cells. Importantly, the authors do not preclude the induction of Tr1-like 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 granulocyte–macrophage colony-stimulating factor, which promote DC maturation (Barchet et al. 2006).

Oliaro et al. (2006) 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 natural killer (NK) cells leads to cell polarization at the site of ligation. Similarly, culture of T cells with L cells expressing surface measles hemagglutinin, a CD46 ligand, resulted in CD46, CD3 and microtubule organization centre (MTOC) localization at the point of contact. However, if CTLs were pre-incubated with soluble CD46 prior to anti-CD3/CD28 stimulation, there was a 55% reduction in polarization, alongside a decrease in IFNγ production. Similarly, incubation of T cells with allogenic DCs after CD46 ligation leads to a reduction of IFNγ 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 (Bettelli et al. 1998; Cohen et al. 2010; Cua et al. 1999). Functional defects in $CD4^+CD25^{high}$ Tregs have been identified in MS (Haas et al. 2005; Viglietta et al. 2004). These defects and the crucial regulatory role of IL-10 led to similar investigations in Tr1 function (Astier et al. 2006; Astier 2008). 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). IFNβ treatment had no effect on IL-10 production, suggesting that 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. (2006), Martinez-Forero and collaborators (2008) observed a significant reduction in IL-10 production upon CD46 activation in MS patients compared to healthy controls. 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 ninefold decrease in IL-10 production compared to controls. TGFβ production was also diminished, especially in active MS. Consistent with Astier et al. (2006), reduced IL-10 production was specific to CD46 stimulation and there was no difference in IFNγ production in MS models and healthy controls (Ma et al. 2009). Genomic studies have demonstrated that MS patients have alterations in the α 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-activated 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-costimulated 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 the presence of LPS leads to increased amounts of CCL3 and CCL5 being 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 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 MV 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 costimulation in RRMS patients (unpublished data). In order for T cells to cross the BBB, first they must attach to the BBB endothelium. This is facilitated by integrins, such as $\alpha4\beta1$ (very late activation antigen 4; VLA-4) and $\alpha4\beta7$, which bind to counter receptors on endothelial cells. Natalizumab, used as a treatment for MS, is a monoclonal antibody that binds to the $\alpha4$ chain of $\alpha4\beta1$ and $\alpha4\beta7$ (Davenport and Munday 2007). VLA-4 binds to the epithelial receptor vascular cell adhesion molecule-1 (VCAM-1). By disrupting the interaction between $\alpha4\beta1$/$\alpha4\beta7$ 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 $\alpha4\beta7$ and CCR9, which is indicative of a gut-homing phenotype (Alford et al. 2008). However, T cells isolated from the cerebrospinal fluid also express $\alpha4\beta7$ 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), *N. gonorrhoeae* (Gill et al. 2003), MV (Naniche et al. 1993), group A streptococcus (Lovkvist et al. 2008) and gingivalis (Mahtout et al. 2009). The 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 downregulation 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 (Elward et al. 2005; Hakulinen et al. 2004). Finally, CD46 undergoes proteolytic cleavage in necrotic cells (Elward et al. 2005) and apoptotic cells (Cole et al. 2006; Hakulinen and Keski-Oja 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 *Loxosceles* 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 MMP-3, -8, -9 (Cole et al. 2006). Our data demonstrate 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 are tightly regulated (Ni-Choileain et al. in press).

The role of CD46 downregulation 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 (Cole et al. 2006; Elward et al. 2005). Importantly, CD46 also contributes to the adaptive immune response and surface regulation likely supports this functionality. The 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 GM6001, a broad-spectrum inhibitor, IL-10 production upon CD46 activation is decreased. This suggests that surface downregulation plays an active role in T cell function (Ni-Choileain et al. in press).

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 MMP-3, -8, -9 and ADAM10 (Cole et al. 2006; Hakulinen and Keski-Oja 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 increased risk in MS (Mirowska-Guzel et al. 2009). MMP-9 increases T cell migration across the BBB, and both IFNβ and steroid treatment can reduce MMP-9 expression (Garcia-Montojo et al. 2010; Trojano et al. 1999). More recently, IFNβ treatment was directly linked to decreased MMP-9 expression in mature DCs and a reduction in their migratory capacity (Yen et al. 2010). In addition to the MMPs, ADAM10 also cleaves CD46. Interestingly, ADAM10 has been implicated in myelin degradation, and 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 *S. pyogenes*, which binds to CD46, results in an increase in ADAM10 production (Lemjabbar and Basbaum 2002) and *S. 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), but 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). *S. pyogenes* binds soluble CD46, perhaps as a mechanism to evade complement attack (Lovkvist et al. 2008). Increased levels of soluble CD46 have also been documented in autoimmune diseases, systematic lupus erythematosus and MS (Kawano et al. 1999; Soldan et al. 2001). In MS, soluble forms of CD46 have been found bound to HHV-6 in the serum of MS patients (Fogdell-Hahn et al. 2005; Soldan et al. 2001). 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. (2010) have recently reported that CD46 is subsequently processed, after extracellular proteolysis, by presenilin-γ-secretase (PγS) upon *N. gonorrhoeae* or *Neisseria meningitides*

infection of epithelial cells. Our data demonstrate that CD46 also undergoes presenilin proteolysis in CD46-activated Tr1 cells (Ni Choileain et al. in press). Other PγS substrates such as amyloid precursor protein (APP), Notch, and ERBB4 release intracellular domains 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. 2000), suggesting an ability to translocate to the nucleus and propagate cell signaling.

Is Regulated Internal Processing 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 regulated internal processing 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. 2000). 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, which may relate to CD46 function, and subsequent signaling, recycling and/or degradation are 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 (Miyake et al. 1998; Yokouchi et al. 1999). 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, Toll-like receptor pathogen recognition (Maiuri et al. 2007), MHC class I and class II antigen presentation (Dengjel et al. 2005; English et al. 2009; 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 Alirezaei et al. (2009) demonstrates increased RNA levels of Atg5 in RRMS patients' peripheral T cells compared to healthy controls. 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 (Alirezaei et al. 2009).

Joubert et al. (2009) 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 MV 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 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 have 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 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 PγS, it seems inevitable that CD46 will follow suit. In MS, cell responses upon CD46 activation are skewed towards a pro-inflammatory response, 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 perhaps 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

Table 1 CD46 dysregulation in multiple sclerosis (MS)

Cell type	Known defects in MS upon CD46 activation	References
Cytokine production		
$CD4^+$ T cell	⇓ IL-10 production in MS	Astier et al. (2006), Martinez-Forero et al. (2008), Ma et al. (2009)
	⇓ TGFß (particularly in active MS) in the monkey model	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 and Astier, unpublished data)
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)

to return the balance of immune responses in patients with MS.

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