Published in final edited form as:
J Neuroimmunol. 2007 November ; 191(1-2): 70–78.

Abnormal Tr1 differentiation in multiple sclerosis

Anne L. Astier[1,*] and **David A. Hafler**
Laboratory of Molecular Immunology, Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.

Abstract

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). In the recent years, accumulating evidence has supported an immunosuppressive role for regulatory T cells (Tregs). Most studies in the context of autoimmunity have focused on the defects of the $CD4^{+}CD25^{high}$ Tregs. However, we recently demonstrated an altered function of Tr1 Treg cells in MS, characterized by a lack of IL-10 secretion. Therefore, several major regulatory T cell defects are involved in human autoimmune disease. Hence, the induction of Tregs or the stimulation of Treg activity may be beneficial for the treatment of such diseases.

Keywords

Multiple sclerosis; Tr1 regulatory T cells; IL-10; CD46

1- Introduction

Despite advances in the understanding of the mechanisms regulating T cell activation, T cell-mediated autoimmune diseases are still not well understood. Among them, multiple sclerosis (MS) is a complex genetic disease with inflammation in the central nervous system (CNS) white matter mediated by activated autoreactive lymphocytes (Feldmann and Steinman, 2005; Hafler and De Jager, 2005; Hafler et al., 2005; Hohlfeld and Wekerle, 2004). Once in the CNS, these autoreactive T cells target the myelin basic protein on the myelin sheath, and recruit more inflammatory immune cells to the site of attack (Bruck, 2005; Liu et al., 2006; McQualter and Bernard, 2007). The pathology of the inflammatory reaction is consistent with a T-cell mediated immune response, leading to tissue damage through activated macrophages and microglia. This repeated inflammation and subsequent demyelination will then instigate nerve impulses to be slowed or stopped, causing the symptoms of MS. Therefore, the understanding of the factors controlling T cell activation, inflammation and migration within the brain is of crucial importance (Adorini, 2004; Hohlfeld and Wekerle, 2004). Worldwide, MS may affect 2.5 million individuals including 400,000 subjects in the US, and 80,000 individuals in the UK and it is the most common disease affecting young adults. Hence, new approaches need to be developed in treating this disease.

In the recent years, the characterization of regulatory T cells and of their role in controlling the immune response has been highlighted. Indeed, the loss of Treg function seems to be a critical

1 Address reprint requests and correspondence to ALA, phone: +44 131 650 7289 Fax: +44 131 651 3619, email a.astier@ed.ac.uk.
* Present address: University of Edinburgh, Institute of Immunology and Infection Research, Ashworth Laboratories, King's Buildings, West Mains Road, Edinburgh, UK.

factor in the pathogenesis of human autoimmune diseases (Kretschmer et al., 2006; Paust and Cantor, 2005; Wraith et al., 2004). Several classes of Tregs have now been identified, including the naturally occurring CD4+CD25^{high} Tregs, as well as induced Tregs such as Tr1 and Th3 cells. Most studies of these cells in the context of autoimmunity have focused on the defects of the CD4^{+}CD25^{high} Tregs. However, we also recently demonstrated an altered function of Tr1 regulatory T cells in MS, characterized by a lack of IL-10 secretion. Therefore, several major regulatory T cell defects that encompass the various sorts of Tregs are involved in human autoimmune diseases. This suggests that therapies aiming at enhancing or inducing Treg responses might be beneficial for such diseases.

2- Regulatory T cells and Multiple Sclerosis (MS)

In the past years, a resurgence of interest in regulatory T cells (Tregs) has emerged. Such T cells have been shown to regulate the immune response by turning off the signals initiated during the immune response. A variety of lymphocyte populations with suppressive capabilities have been reported in both animals and humans. Shimon Sakaguchi first described Tregs as the major contributors in controlling autoreactive T cells and maintaining a state of peripheral tolerance to a range of self-antigens (Sakaguchi et al., 1985; Sakaguchi et al., 1995). An early observation on suppressive activity that was lessened in patients with MS was published in 1986 (Antel et al., 1986). The absence or depletion of Tregs cells leads to autoimmune destruction of a wide range of target organs (Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003).

2a- CD4+CD25^{high} regulatory T cells

Characterization—CD4+CD25^{high} regulatory T cells contribute to the maintenance of peripheral tolerance by active suppression and require cell contact *in vitro* to exert their negative regulation. These cells were initially characterized in mice by expression of CD25 on CD4+ T cells (Sakaguchi et al., 1995), and the expression of FoxP3 transcription factor is crucial to their development and function (Hori et al., 2003; Khattri et al., 2003). Several groups demonstrated that Tregs also exist in humans and that they are very similar in phenotype and function to their murine counterparts (Baecher-Allan et al., 2001; Dieckmann et al., 2001; Jonuleit et al., 2001; Stephens et al., 2001; Taams et al., 2001; Taylor et al., 2001). However, while CD25 is a useful marker to identify murine Tregs, only high CD25 expression should be considered as Tregs in human, as intermediate CD25 expressing T cells do not exhibit suppressive activity (Baecher-Allan et al., 2001; Baecher-Allan et al., 2003; Roncador et al., 2005). They also expressed FoxP3, although again activated human T cells also express low amounts of FoxP3 albeit in lower amounts than Tregs (Roncador et al., 2005; Walker et al., 2003). They are anergic when stimulated by T cell receptor (TCR) cross-linking *in vitro*, and suppress T cell activation in a non-HLA-restricted, contact-dependent manner. IL-2 signaling is required for maintaining the homeostasis of Treg cells *in vivo* (Fontenot et al., 2005; Maloy and Powrie, 2005). Additional phenotypic characterizations include CD62L expression that is downregulated on effector T cells, no expression of CCR7 (Hoffmann et al., 2004; Noma et al., 2005), the exclusion of cells expressing the early activation marker CD69 (Gray et al., 2003; McNeill et al., 2007), and high-level expression of glucocorticoid-induced TNFR family-related gene/protein (GITR) (Ono et al., 2006). Finally, the expression of E3 ubiquitin ligase, GRAIL, is upregulated in CD4+CD25+ Tregs, and its forced expression induces a regulatory phenotype (Mackenzie et al., 2007).

CD4+CD25+ T cells in MS—A role of these CD4+CD25^{high} regulatory T cells has first been shown in regulating autoimmune diseases in animal models, including EAE (Kohm et al., 2002; Nishibori et al., 2004). In MS patients, the levels of circulating CD4+CD25+ T cells and CD4+CD25^{high} Treg cells are not altered (Putheti et al., 2004). However, we and others

have reported a decrease of $CD4^{+}CD25^{high}$ regulatory T cell function in patients with MS (Haas et al., 2005; Huan et al., 2005; Viglietta et al., 2004). Indeed, a significant decrease in the suppressive activity of $CD4+CD25^{high}$ regulatory T cells from peripheral blood of patients with MS as compared with healthy donors was observed (Viglietta et al., 2004). Interestingly, only patients in the relapsing-remitting phase exhibit impaired Treg function, characterized by a reduction in proliferation and interferon-gamma production of CD4+CD25− responder T cells (Venken et al., 2006). Secondary progressive patients have normal CD4+CD25+ Tregs. Furthermore, consistently with their suppressive capacity, CD4+CD25+ Tregs from secondary progressive MS patients have normal levels of FoxP3 expression while FoxP3 expression was decreased in relapsing remitting MS patients.

2b. Tr1 regulatory T cells and IL-10 production

Characterization and induction—Two other populations of regulatory T cells have been described. Type 1 regulatory (Tr1) T cells mainly exert their suppressive activity through the secretion of IL-10 (Roncarolo et al., 2001), a potent immunosuppressive cytokine (Moore et al., 2001), while Th3 cells suppress cell activation through TGFβ release (Bach, 2001; Chen et al., 1994). Both *in vitro* and *in vivo* studies with recombinant IL-10 and neutralizing antibodies revealed pleiotropic activities of IL-10 on B, T, and mast cells (Moore et al., 2001). The anti-inflammatory role of IL-10 was demonstrated by the development of inflammatory responses in IL-10 deficient (IL-10−/−) mice (Berg et al., 1995; Kuhn et al., 1993). Indeed, IL-10-deficient mice spontaneously develop inflammatory bowel disease (Kuhn et al., 1993) due to a defect in Tr1 cells that attenuates sensitivity to intestinal flora (Asseman et al., 1999). Hence, IL-10 and IL-10 secreting cells appear to play a role in peripheral tolerance and in protection against autoimmunity.

Tr1 cells were first identified by Roncarolo and colleagues (Groux et al., 1997). Unlike CD4 +CD25+ Tregs, no cell surface marker uniquely identifies Tr1 cells. They are not characterized by CD25 expression, although they may express low levels following activation. They don't express FoxP3 (Vieira et al., 2004) and are functionally defined by their secretion of large amounts of IL-10, modest amounts of IFN-γ, and no IL-2 or IL-4 (Bacchetta et al., 1994; Groux et al., 1997). *In vitro* induction of Tr1 might be achieved by stimulation of naïve human $CD4^{+}$ T cells with anti-CD3 mAb in the presence of exogenous IL-10 and IFNα (Levings et al., 2001). Similarly, the combination of the two immunosuppressive drugs, vitamin D3 and dexamethasone, induces human and mouse naive CD4+ T cells to differentiate *in vitro* into regulatory T cells secreting large amounts of IL-10 (Barrat et al., 2002; Cantorna et al., 1996). Finally, CD46 activation of T cells in the presence of IL-2 leads to Tr1 differentiation characterized by a massive secretion of IL-10 and bystander CD4+ T cell suppression (Kemper et al., 2003), and will be further discussed in the following section. On the other hand, activation of OX40L pathway will inhibit Tr1 differentiation (Ito et al., 2006).

Tr1, IL-10 and MS—Numerous data have revealed the importance of IL-10 in regulating EAE (Anderson et al., 2004; Bettelli et al., 1998; Burkhart et al., 1999; Cua et al., 1999; Rott et al., 1994; Zhang et al., 2004). The neutralization of endogenous IL-10 increased the severity and incidence of SEB- or TNF-induced EAE relapse (Crisi et al., 1995) and the severity of the disease is more severe in IL-10 deficient mice than in wild-type (Bettelli et al., 1998; Samoilova et al., 1998). Mice transgenic for human IL-10 expressed under the control of the MHC class-II promoter were completely protected from induced EAE (Cua et al., 1999). In humans, while a preferential up-modulation of TNFα and lymphotoxin α is observed in active MS, an increased IL-10 production is associated with stable disease (Navikas et al., 1995), and by IFNβ treatment (Chabot and Yong, 2000). Alternatively, a decreased production of IL-10 associated with a significant increased production of IL-12p40 is detected in patients with secondary progressive MS (Balashov et al., 2000; Soldan et al., 2004; van Boxel-Dezaire et

al., 1999). Dendritic cells from patients with MS produce more IL-23 than healthy controls, which affects IL-10 production (Vaknin-Dembinsky et al., 2006). Low amounts of IL-10 production are associated with higher disability and MRI lesion load in secondary progressive multiple sclerosis (Petereit et al., 2003). Patients with MS have also diminished frequencies of IL-10 secreting innate TCR-reactive T cells (Vandenbark et al., 2001). Altogether, these data suggest a likely defect in T cell activation leading to generation of regulatory T cells and regulatory cytokines such as IL-10 in MS (Beebe et al., 2002). As mentioned above, vitamin D3 induces Tr1 cells secreting IL-10, and inhibits Th1-mediated autoimmune diseases including EAE (Cantorna et al., 1996). A definitive proof of the role of IL-10 in controlling EAE was recently shown by Spach and colleagues (Spach et al., 2006). The authors demonstrated that the strong inhibition of myelin oligodendrocyte peptide (MOG(35–55))-induced EAE development by vitamin D3 and 1,25-(OH)(2)D(3) was dependent on the functional expression of both IL-10 and IL-10R (Spach et al., 2006). These data also suggest that 1,25-(OH)(2)D(3) may be enhancing an anti-inflammatory loop involving IL-10 secreting Tr1 cells This is further supported by a study in severe asthma patients that describes the *in vitro* inhibitory potential of human Tr1 cells induced by vitamin D3 and dexamethasone, to inhibit cytokine production by allergen-specific Th2 cells (Xystrakis et al., 2006). Dexamethasone is ineffective in the induction of IL-10 in CD4+ T cells from glucocorticoid resistant asthma patients as compared with their glucocorticoid-sensitive counterparts. The authors now show that the addition of vitamin D3 with dexamethasone could potentially increase the therapeutic response to glucocorticoids in glucocorticoid resistant asthma patients, via the induction of IL-10 producing cells. Hence, a definitive role of IL-10 and IL-10 secreting cells has been demonstrated in human pathologies.

3- CD46, T cell activation and IL-10 production

3a. CD46

CD46 (previously called Membrane Cofactor Protein, MCP) is a ubiquitously expressed protein, first identified as a member of the regulators of complement activation family (Seya et al., 1999). It is a type I membrane protein which is a regulatory part of the complement system. It has cofactor activity for inactivation of complement components C3b and C4b by serum factor I, which protects the host cell from autolysis by complement (Kemper and Atkinson, 2007). In addition, CD46 can act as a receptor for many pathogens (Cattaneo, 2004; Riley-Vargas et al., 2004), including the Edmonston strain of measles virus, human herpesvirus-6, adenoviruses A and B, type IV pili of Neisseria gonorrhoeae and Neisseria meningitidis as well as group A streptococcus, and has been called a “pathogens’ magnet” (Cattaneo, 2004). The basic structure of CD46 is composed of four “short consensus repeats” and a region rich in serine, threonine and proline (STP region) followed by a transmembrane segment, an intracytoplasmic anchor of 12 amino acids and a short cytoplasmic tail. So far, eighteen isoforms are produced due to the alternative splicing of various exons (Dhiman et al., 2004). In particular, four major isoforms are produced (BC1, BC2, C1, and C2), depending on the alternative splicing of an exon in the STP region (B) and of the exon 13 that results in two distinct intracytoplasmic tails of 16 (Cyt-1) or 23 (Cyt-2) amino acids (Russell et al., 1992). These isoforms are usually co-expressed in any given tissue, except for brain and kidney where a preferential expression of Cyt-2 is observed (Johnstone et al., 1993).

3b. CD46 and the CNS

The blood-brain barrier (BBB) is composed of tight junctions, which prevent the entry of large proteins into the CNS, and crossing this barrier is precisely regulated and crucial for the immune surveillance of the brain. Interestingly, CD46 is highly expressed at the BBB (Shusta et al., 2002). This has been shown by a subtractive expression cloning methodology, identifying

proteins with enriched expression at the BBB in comparison to liver and kidney tissues. Johansson *et al*. analyzed the infection of human CD46-expressing transgenic mice by Neisseria meningitides, the causative agent of meningococcal meningitis (Johansson et al., 2003), which binds to CD46 (Kallstrom et al., 2001). They show that transgenic mice expressing human CD46 were susceptible to meningococcal disease, because bacteria crossed their BBB. Therefore, CD46 mediates access to the meninges by promoting passage of the BBB. As Cyt-2 is predominantly expressed in the human brain, it might enhance inflammatory responses, and explain the lethal effect of Neisserial infection in CD46 transgenic mice. Therefore, one can hypothesize that CD46 plays a role in the activation and/or migration of T cells in the brain of patients with MS.

3c. CD46 is a costimulatory molecule for human T cells

T cell activation occurs upon TCR engagement. However, efficient T cell activation needs a concomitant stimulation with a costimulatory molecule. The major costimulatory molecule described so far is CD28, a member of the B7 family (Sharpe and Freeman, 2002). However, CD3/CD46 costimulation promotes T cell proliferation with a potency comparable to CD28 (Astier et al., 2000). Enhanced proliferation was accompanied by drastic morphological changes of primary human T cells and actin relocalization (Zaffran et al., 2001), along with activation of Vav, critical for TCR activation and T cell activation-induced actin cytoskeleton rearrangements, as well as Rac activation, a GTPase of the Rho family. Such findings were reinforced by a recent report showing that CD46 modifies T cell and NK cell polarization (Oliaro et al., 2006). Of note, the functional orthologue of CD46 (that is not expressed in rodents) in rat or in mice (Crry) is also a costimulatory molecule for murine T cells (Fernandez-Centeno et al., 2000; Jimenez-Perianez et al., 2005), suggesting a new biological function for these complement regulatory molecules (Morgan et al., 2005).

3d. CD46 is an inductor of human Tr1 cells

The role of CD46 in human T cell activation has been strongly supported by the fact that CD46/CD3 costimulation of human primary T cells in the presence of IL-2 induced a T regulatory (Tr1) phenotype, characterized by a massive production of IL-10 and granzyme B, and the ability to suppress the proliferation of bystander $CD4^+$ T cells (Grossman et al., 2004; Kemper et al., 2003). Low strength of TCR stimulation leads to a lack of sustained proliferation of CD3/CD46-generated Tr1-like cells that is due, at least partially, to a G0/G1 blockage in their cell cycle progression, with the inability to degrade p27/kip1, and to an increased sensitivity to cell death (Meiffren et al., 2006). However, depending on the costimulatory signals, CD46 activated T cells can also differentiate towards a Th1 response with increased IL-10, IL-2 and IFNγ secretion, but decreased IL-5 production (Sanchez et al., 2004). As CD46 acts as a receptor for many pathogens, Kemper's group has investigated if such pathogens could directly induce Tr1-like cells through their interaction with CD46 (Price et al., 2005). They showed that interaction of the streptococcal ligand for CD46 indeed led to Tr1 differentiation. These data highlight the importance of CD46 in the regulation of the immune response through the induction of Tr1 cells and IL-10 production.

4- Tr1 induction is dysregulated in patients with MS

The importance of regulatory T cells in the pathology of autoimmune diseases has been demonstrated by various groups, who demonstrated a defect in the $CD4^+CD25^{high}$ regulatory T cells in patients with MS (Balandina et al., 2005; Haas et al., 2005; Huan et al., 2005; Viglietta et al., 2004) as well as in other human autoimmune diseases (Bluestone and Tang, 2005; Christen and von Herrath, 2004; Feldmann and Steinman, 2005). Considering the central role of Tr1 cells and IL-10 in regulating immune responses, we postulated that patients with MS would have multiple defects in immunoregulatory T cells, including Tr1 cells. As mentioned

above, CD46-activated T cells acquire a Tr1 phenotype. We therefore determined whether CD46 activation was impaired in patients with MS. A striking difference was observed between healthy donors and patients (Astier et al., 2006). While no significant difference was observed in the proliferation of the cells, little to no IL-10 was secreted by CD46-activated T cells from patients with MS as compared to healthy donors. The lack of IL-10 production was specific to CD46 as it was not affected upon CD28 stimulation. Increasing strength of stimulation by stronger TCR stimulation or enhanced IL-2 concentrations did not restore IL-10 production. The deficit in IL-10 secretion was also specific to this cytokine as the concentrations of IFNγ secreted by CD46-activated T cells were not affected. These data demonstrate that human autoimmune diseases can be associated with multiple defects in regulatory T cell populations.

As mentioned above, an increased IL-10 production is often associated with remissions (Clerici et al., 2001; Correale et al., 1995; Navikas et al., 1995), and induced by IFNβ treatment (Chabot and Yong, 2000; Ozenci et al., 1999). However, when IL-10 secretion by T cells from untreated and IFNβ treated patients was examined, no difference was observed between these two groups of patients. This suggests that while IFNβ has a therapeutic effect, it does not appear to target Tr1 cells, but likely acts on the other cells producing Il-10 such as Th2 cells, B cells or monocytes. It would be interesting to determine what affects IL-10 production by Tr1 cells. Nevertheless, our results suggest that pharmacologic interventions that induce IL-10 secretion by CD4 cells are viable interventions in patients with MS.

5. Divergent roles of the two intracytoplasmic isoforms in a murine model of inflammation

The two intracellular tails of CD46 produced by alternative splicing, Cyt1 and Cyt2, are co-expressed in human cells, although the proportion of Cyt1 to Cyt2 isoforms can slightly vary (Russell et al., 1992), with a predominant expression of Cyt2 in the brain. As suggested by Russell, it is possible that a selective recruitment of each isoform to determined specific signaling complexes might result in a different signaling outcome, and therefore in a different biological response (Russell, 2004). The specific role of each cytoplasmic isoform has been evaluated using a model of transgenic mice expressing either one of the intracytoplasmic isoforms. Mice do not express CD46, except in testes. Furthermore, there is no homology between the sequences of the mouse and human cytoplasmic domains. The two cytoplasmic tails exhibited antagonist effect on T cell-dependent contact hypersensitivity reaction. Cyt1 inhibited the inflammatory reaction, whereas Cyt2 augmented the inflammation (Marie et al., 2002). The two isoforms exerted opposite effects on CD4+ T cell proliferation, as Cyt1 expression enhanced proliferation while Cyt2 inhibited it. Of note, the morphological changes observed in human T cells after CD46 stimulation were only reproduced when CD46-Cyt1 was expressed. In contrast, only the Cyt2 isoform promoted $CD8^+$ T cell cytotoxicity. Finally Cyt1 engagement was shown to inhibit IL-2 secretion, while the Cyt2 isoform inhibited IL-10 secretion (Marie et al., 2002). Thus, CD46 differentially regulates T cell–mediated inflammatory responses and contact hypersensitivity reactions depending on its cytoplasmic tail. This suggests that depending on which cytoplasmic tail is expressed or activated, CD46 stimulated T cells might acquire or not a regulatory phenotype.

6. Altered cytoplasmic isoforms expression in T cells from patients with MS

As discussed above, the analysis of CD46 transgenic mice showed that the two distinct cytoplasmic isoforms of CD46 have distinct functions in terms of T cell activation and cytokine production, and differentially control inflammation. As these two isoforms are co-expressed in any given tissue, except for brain and kidney where a preferential expression of Cyt2 is observed (Johnstone et al., 1993), their role in IL-10 secretion hadn't been elucidated in humans. Expression of both CD46 cytoplasmic isoforms was studied in healthy donors and

patients with MS (Astier et al., 2006). Their relative expression was determined by qRT-PCR using primers specific for each cytoplasmic tail. When patients with MS were compared to healthy donors, no difference was observed in freshly isolated T cells. However, upon activation, an increase in Cyt2 isoform was detected in patients with MS, but not in healthy donors. Hence, the reduced secretion of IL-10 by Tr1 cells from patients with MS was associated with an increased expression of the Cyt2 isoform of CD46. Of note, these data correlated with the results found in the mouse, where Cyt1 inhibited inflammation while Cyt2 augmented it (Marie et al., 2002). These data suggest that CD46 Cyt2 might be the most important isoform in the regulation of inflammation in human, although more data should be collected, and it should be assessed in different autoimmune diseases.

7. CD46 and HHV6 in MS

As mentioned above CD46 is also the receptor for HHV6 (Santoro et al., 1999). This is of much interest in the case of MS, as links between viral infection such as HHV6 infection and development of MS have been demonstrated. HHV-6 is present in active MS plaques (Challoner et al., 1995), and the patients have increased IgM response towards HHV6 antigens during the RR phase of the disease (Soldan et al., 1997). Moreover, a recent study followed the HHV6 viral load and clinical data in a one-year follow-up of a cohort of 63 patients and healthy donors (Alvarez-Lafuente et al., 2006). They show that RR patients in relapse have active HHV6 replication and increased EDSS, suggesting that exacerbations are associated with active HHV6 replication. Hence, one may hypothesize that the increasing viral load will activate the T cell population in the brain through CD46. As CD46 is deficient in patients with MS, this will lead to further damage and inflammation.

8- Future pathways

MS is a complex disease with genetic predisposition and environmental influences, as well as immunological defects. It has proven heritability, and the association of selective allelic variants likely leads to a higher risk of developing disease (Hafler and De Jager, 2005; Hafler et al., 2005). Ultimately, several immunologic alterations will lead to the profound loss of tolerance associated with CNS white matter inflammation. Hence, future investigations can examine defects in IL-10 secretion and whole genome association scans to determine whether it is related to genetic or environmental influences. Similarly, the use of whole genome RNAi libraries will be of use to determine new genes involved in the regulation of IL-10 (Astier et al, manuscript in preparation). Future studies will then focus on the role of the newly discovered genes in IL-10 production in patients with MS. Ultimately, the precise dissection of the cascade leading to IL-10 production and Tr1 differentiation will be understood. It will open new means to manipulate the immune system in humans, with an impact in autoimmune diseases such as MS in which a deficit in IL-10 production likely participates in the neuroinflammation observed in these patients.

Conclusion

CD46 has been only recently identified as a regulator of T cell activation. However, our recent data as well as others' have demonstrated its crucial role in the fine regulation of the immune response. According to the results found in the CD46 transgenic mouse model, CD46 cytoplasmic isoforms could induce either an anti-inflammatory response through Tr1 differentiation or a pro-inflammatory response. This is further supported by our findings in MS. CD46 is dysregulated in patients with impaired IL-10 production, and an increased Cyt2 isoform expression, as summarized in Figure 1. Hence, the interference with the signal transduction cascade initiated by CD46 on human T cells may be targets of novel strategies to treat autoimmune diseases, such as MS.

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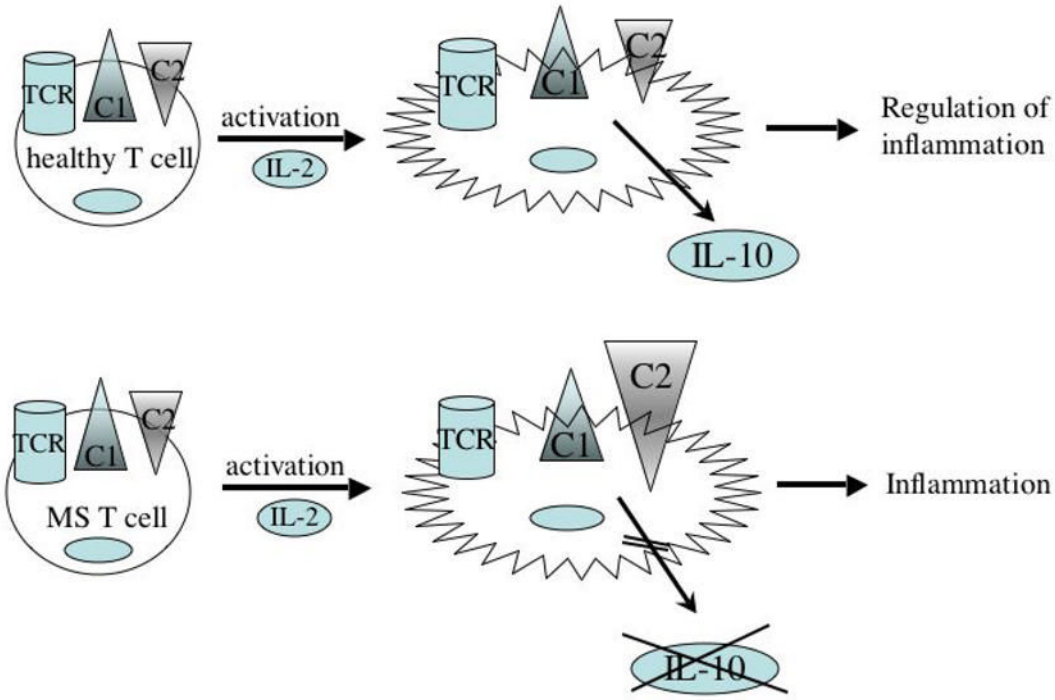

Figure 1. Altered Tr1 differentiation in patients with MS.

CD46 activation of human T cells induces Tr1 differentiation and IL-10 secretion with an equivalent level of both CD46 cytoplasmic isoforms Cyt1 (C1) and Cyt2 (C2). This pathway is dysfunctional in patients with MS, as upon CD46 stimulation, T cells do not produce IL-10. This is also associated with an increased expression of CD46 –Cyt2 (C2) isoform. This likely contributes to the neuro-inflammation observed in these patients.