

Review Article

Targeting Inflammatory T Cells in Multiple Sclerosis: Current Therapies and Future Challenges

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Multiple Sclerosis (MS) is an autoimmune inflammatory disorder of the Central Nervous System (CNS), affecting more than one million people worldwide. The pathogenesis of MS involves several genetic and environmental factors, which ultimately lead to the activation of autoreactive T cells in the periphery, their migration into the CNS, where they trigger an acute inflammatory response, thus mediating primary demyelination and axonal damage. Most information on MS derives from studies in animal models of experimental autoimmune encephalomyelitis (EAE), which exhibit many similarities to the pathology of MS. Two distinct subsets of autoreactive T cells have been primarily involved in the pathogenesis of both EAE and MS: the interferon (IFN)- γ producing CD4⁺ T helper (Th) 1 and interleukin (IL)-17 producing Th17 cells. The activity of these cells is controlled by specific regulatory T cells (Treg), which by secreting anti-inflammatory cytokines such as IL-4, IL-10 and tumour growth factor (TGF)- β efficiently inhibit Th1 and Th17 cells.

In this review, we summarize current knowledge on the role and function of pro-inflammatory and Treg subsets in MS. We also discuss the action of current and novel therapies aimed to dampen inflammatory T cells.

Keywords: Multiple sclerosis; Inflammatory T cells; Regulatory T cells; Therapy; Costimulation

Abbreviations

MS: Multiple Sclerosis; CNS: Central Nervous System; EAE: Experimental Autoimmune Encephalomyelitis; IFN: Interferon; Th: T helper; IL: Interleukin; TGF: Tumour Growth Factor; RR: Relapse-remitting; SP: Secondary Progressive; PP: Primary Progressive; BBB: Blood-Brain Barrier; APC: Antigen Presenting Cell; DC: Dendritic Cell; MBP: Myelin Basic Protein; PLP: Proteolipid Protein; MOG: Myelin Oligodendrocyte Glycoprotein; Tregs: Regulatory T Cells; GA: Glatiramer Acetate; GITRL: Glucocorticoid-induced TNF Receptor Ligand; AEs: Adverse Side-Effects; NK: Natural Killer; TCR: T Cell Receptor; PI3K: Phosphatidylinositol-3 Kinase; PIP2: Phosphatidylinositol 3,4-bisphosphate; PIP3: Phosphatidylinositol 3,4,5-trisphosphate; Ab: Antibody; GTR: Glucocorticoid-Induced TNF Receptor-related Protein

Introduction

MS is an autoimmune chronic inflammatory disorder characterized by demyelination and remyelination events and by the loss of sensory and motor functions. Two third of MS patients present the relapsing-remitting (RR) course, which is characterized by relapses usually followed by periods of recovery or remission, but one third of patients progresses to chronic secondary progressive (SP) disease [1,2]. A minority of patients (10-20%) experiences a primary progressive disease (PP), which is characterized by a gradual and constant decline in their neurological functions from the onset of disease [3]. Inflammation is present at all stages of MS [4] and pro-inflammatory cytokines/chemokines play a critical role in the pathophysiology of MS by compromising the blood-

brain barrier (BBB) integrity, recruiting immune cells from the periphery, and activating resident microglia. Conversion of MS from RR to progressive phases has been related to prolonged chronic inflammation in the CNS. Moreover, both SPMS and PPMS patients have generalized inflammation in the whole brain accompanied by cortical demyelination and diffuse white matter injury [4].

Although several cell types within the CNS may contribute to the production of pro-inflammatory cytokines and chemokines, activated autoreactive T cells have a key role in inflammatory demyelination [5]. Indeed, the cytokine and chemokine-producing phenotype of self-reactive T cells in MS patients determines the ability of these cells to cross BBB and cause inflammation in the CNS, thus contributing to disease progression.

This article reviews the current knowledge on the contribution of different T cell subsets in the pathogenesis of MS and discusses the current and novel therapeutic strategies, which aim to dampen the pathogenic inflammatory T-cell response.

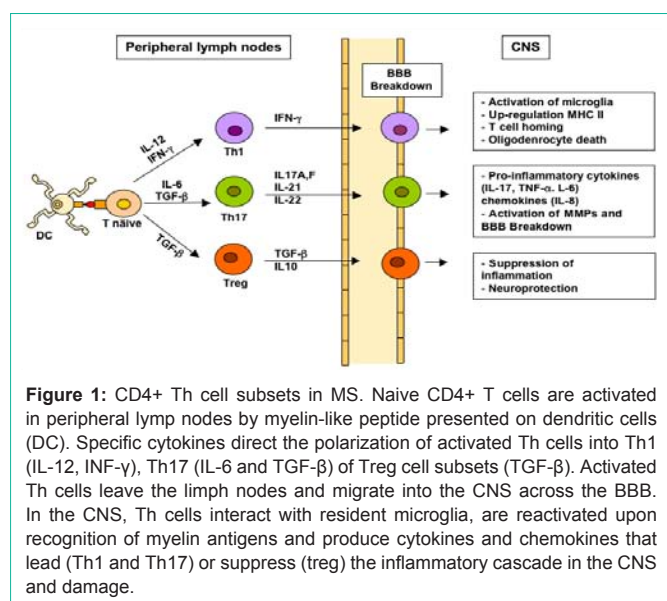
Inflammatory T helper cell subsets in MS: Th1 and Th17 cells

The more accepted pathogenic model for both EAE and MS is that autoreactive myelin-specific CD4⁺ T cells are activated in the periphery, entered the CNS by crossing the BBB and are reactivated by resident antigen-presenting cells (APC), mainly of which is microglial cells [5,6]. The priming and activation of autoreactive myelin-specific CD4⁺ T cells likely occurs in peripheral lymph nodes, where the dendritic cells (DC) may present myelin epitopes to naive T cells, thus inducing the activation and differentiation of autoreactive

effector/memory T helper cells, which in turn migrate to the CNS and cause tissue damage and demyelination. Several evidences support a function for myelin proteins, such as MBP (myelin basic protein), PLP (proteolipid protein) and MOG (myelin oligodendrocyte glycoprotein), as relevant antigens in both EAE and MS [7-11]. In EAE, myelin-specific T cell responses seem to initiate in the CNS-draining cervical lymph nodes, thus suggesting that myelin proteins are constitutively presents in some lymph nodes [12]. Moreover, high avidity myelin-specific CD4⁺ T cells have been isolated from the periphery of MS patients [13,14].

It has been thought for a long time that the pathogenetic cells mediating MS were CD4⁺ Th1 cells, producing large quantity of IFN- γ driven by IL-12 [15,16]. Indeed, IFN- γ -deficient mice as well as knockout mice for IL-12p40, the large subunit of IL-12, were resistant to EAE [17-20]. However, further observations that mice deficient in IL-12p35, the smaller subunit of IL-12, and IL-12R β 2 were susceptible to EAE [21,22] as well as data showing that the administration of IL-12 during the early phases of EAE suppressed EAE in an IFN- γ dependent manner [23], suggested that Th1 cells could not be responsible for the pathogenesis of MS. The discovery that IL-23 shares the p40 subunit with IL-12 clarifies these contradictory data [24]. Cua et al. demonstrated that IL-23 rather than IL-12 is crucial for EAE development by showing that IL-23p19, the smaller subunit of IL-23, deficient mice were resistant to EAE [25,26]. Data from Langrish et al. clearly defined the role of IL-23 in favouring EAE by driving and inducing the expansion of a novel Th subset producing IL-17 [26], designated Th17 cells [27,28].

The hallmark of Th17 cells is the production of the pro-inflammatory cytokine IL-17 (A and F) that affects the functions of a wide range of cells, enhances the secretion of other pro-inflammatory cytokines and chemokines and increases the activation of matrix metalloproteinases, thus contributing to the breakdown of BBB [29]. In the human system, Th17 differentiation can be mediated by IL-6, TGF- β and IL-21, while IL-23 is critical in maintaining the Th17 phenotype [30-35]. Several data demonstrated that CD4⁺ Th17 subset exerts a central role in the pathogenesis of both EAE and



MS. Increased numbers of Th17 cells have been found in the both inflamed CNS [36] and in the periphery of acute EAE [5] as well as in the CSF of MS patients during relapses [37]. High levels of IL-17A have been also detected in circulating leukocytes of MS patients with active disease [38], and higher IL-17A production has been correlated with the number of active plaques on magnetic resonance imaging [39]. All these data strongly support a pivotal contribution of Th17 cells in the pathology of MS [40].

Regulatory T cells (Tregs)

Tregs are negative regulators of T helper cell responses and contribute to T cell “anergy” and to the maintenance of self-tolerance, thus protecting against autoimmunity [41,42]. Tregs can be identified through their surface phenotypes and cytokine producing profiles. Tregs may be divided into two main subsets, natural Tregs (nTregs) and inducible Tregs (iTregs). The CD4⁺CD25⁺ FOXP3⁺ nTregs develop in the thymus and their TCR repertoire is skewed towards the recognition of self-antigens. In contrast, iTregs, originate from naive T cells in the peripheral lymph nodes, are either CD4⁺ or CD8⁺ and may or not express FOXP3 [43].

Dysfunctions or impairment maturation of Tregs have been observed in animal models of MS [44]. The presence of myelin-specific Tregs within the CNS during EAE highlights their role in the control of disease. Indeed, the accumulation and frequency of Tregs in the CNS has consistently been shown to correlate with recovery from EAE. However, myelin-specific Tregs were not sufficient to reduce the function of encephalitogenic effector T cells during the peak of EAE [45].

In MS, several reports have shown that human Tregs are functionally impaired, have decreased FOXP3 expression compared to healthy individuals, or have deficit in their maturation, or in their thymic emigration [44]. Reduced number or impaired suppressive functions of Tregs have been also found in the peripheral blood of MS patients [46-49]. Therefore, dysfunction in the number and/or functions of Tregs may concur to the immunopathogenesis of MS by decreasing the suppression of activated pathogenic immune cells (Figure 1).

T-cell based therapeutic strategies

Our understanding of the key role of T cells in MS has led to a vast research during the last two decades in an attempt to develop novel therapeutic strategies for T-cell immunotherapy. Below are summarized some that are currently used and others that required further studies.

First-line treatments: IFN- β and glatiramer acetate (GA)

The most common disease modifying drugs used in the first-line treatment of RRMS include IFN- β or GA. Although the mechanisms of action of these compounds are still poorly understood, they exert some effects on T lymphocytes.

IFN- β belongs to a family of cytokines, which interfere with virus replication and exhibit immunomodulatory activities. In 1981, the administration of IFN- β to MS patients resulted in a significant reduction of the relapse rate [50], thus encouraging further clinical trials [51]. IFN- β is actually approved as first-line therapy for RRMS, SPMS with additional relapses in the European Union (EU) [52],

but not for PPMS patients, who showed no significant reduction of disability after treatment [53].

The immunomodulatory activities of IFN- β in ameliorating RRMS include its ability to interfere with T cell activation by inhibiting the up-regulation of MHC class II molecules induced by IFN- γ on APC [54] as well as to inhibit costimulatory molecule functions (i.e. CD40/CD40L and CD28/B7) [55,56]. More recently, the immunosuppressive effects of IFN- β were related to immune deviation. Distinct reports showed that IFN- β treatment impairs Th17 cells by reducing IL-23 levels and up-regulating IL-10, which has general suppressive effects on Th cells or by inducing IL-27, which inhibits Th17 cell differentiation [57-59]. A role of IFN- β in inducing Tregs has also been evidenced [60,61]. In a longitudinal follow-up stadium performed on stable RRMS patients before and after 6-months of IFN- β therapy, Namdar et al. evidenced a significant increase of both frequency and suppressive functions of Treg cells of IFN- β - treated patients [61]. Increased frequency of Treg cells was also observed in EAE mice after IFN- β treatment. This event seems to be correlated to enhanced proliferation of Treg cells mediated by the up-regulation of glucocorticoid- induced TNF receptor ligand (GITRL) on the surface of dendritic cells [60].

Besides the beneficial effects of IFN- β in MS, common side effects have been reported, which include flu-like symptoms, injection-side reactions (i.e. pain, swelling, lipoatrophy), lymphocytopenia and elevated liver enzymes. Moreover, a significant proportion of MS patients (between 7 % to 49%) do not respond to IFN- β [62]. One of the main causes of IFN- β treatment failure is related to the development of neutralizing anti-IFN- β antibodies [63].

GA is a copolymer of alanine, lysine glutamic acid and tyrosine that exerts multiple immunomodulatory activities. Its immunosuppressive effects on Th cells has been related to the inhibition of IFN- γ and stimulation of IL-4 secretions, thus favouring a shift of the immune response from pro-inflammatory Th1 to anti-inflammatory Th2 [64,65]. Another mechanism of the anti-inflammatory effects of GA is the expansion of Tregs through the up-regulation of FOXP3 [66,67]. However, beneficial effects of GA administration were demonstrated only in RRMS [68,69].

In summary, the mechanisms of action of either IFN- β or GA partly target T-cell mediated inflammatory response in MS. However, both drugs often fail to exhibit sufficient clinical effectiveness and have very little effects in progressive phases of the disease that is currently treated with mitoxantrone, an immunosuppressive cytotoxic drug with immunomodulatory activity, but with serious side effects, such as myelosuppression and cardiac toxicity (Table 1) [70-72].

Second-line therapeutic drugs

Second-line drugs, commonly used in case of failure or intolerance to the first-line therapeutic agents, include natalizumab, fingolimod and cladribine. Generally, these agents are more potent than those used in the first-line therapy, but exhibit potential adverse side effects (ASEs) [73,74].

Natalizumab is a humanized recombinant monoclonal antibody directed against the $\alpha 4$ subunit of $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrins expressed on lymphocytes. In particular, the integrin $\alpha 4\beta 1$ binds to the vascular-cell adhesion molecule (VCAM)-1 on endothelial cells of brain and spinal

cord vessels, thus allowing immune cells to cross BBB. Natalizumab prevents the migration of lymphocytes into the CNS [75]. In humans, natalizumab strongly reduces the numbers of CD4⁺ T lymphocytes in the CNS [76]. The results obtained by both AFFIRM and SENTINEL clinical trials demonstrated a strong reduction of both the annualized relapse rate and disability progression in natalizumab-treated RRMS patients [77,78]. However, it may induce severe secondary effects [74,77,79] and clinical complications such as progressive multifocal leukoencephalopathy by polioma JC virus [80].

Fingolimod (FTY720) binds to sphingosine-1-phosphate receptors (S1PR) and blocks the egress of both T and B cells from secondary lymphoid organs into circulation. Moreover, due to its lipophilic properties, fingolimod may cross the BBB and by binding the S1PR expressed on astrocytes, oligodendrocytes and microglia, may promote their survival and favour remyelination [81,82]. The results obtained by both TRANSFORMS [83] and FREEDOMS [84] clinical trials showed a strong reduction in both relapses and accumulation of new or enlarging T2 lesions. Moreover, the availability of oral active forms of fingolimod may also circumvent the ASEs (e.g. skin indurations and lipoatrophy) related to infusion or injection and may exhibit higher adherence and long-term persistence [85]. However, important ASEs, including infections, arrhythmias, hypertension, macula edema as well as serious ASEs, such as asystole, bradycardia and malignant neoplasm have been reported [83,84,86,87]. More recently, an observational study by Carruthers et al. aimed to compare the effectiveness of natalizumab and fingolimod in clinical practice evidenced the superior effectiveness of natalizumab [88]. Thus, a deeper assessment of the effectiveness, safety and adherence during a long-term period of treatment should be required.

Cladribine is a synthetic deoxyadenosine analogue used as an oral immunomodulatory drug in the treatment of chronic progressive MS patients [89-91]. Cladribine targets lymphocytes resulting in sustained reduction of peripheral T and B lymphocytes. The administration of oral cladribine resulted in reducing relapse-rates and disability progression in patients with RRMS [92-95]. Despite the strong lymphopenia observed in patients treated with oral cladribine, no significant differences in the overall incidence of infections were observed. However, herpes zoster infections as well as neoplasm occurred in cladribine-treated patients, thus suggesting further monitoring to weigh the benefits against potential risks.

Other anti-inflammatory drugs are approved as second line-line therapy (Table 1). However, most of these non-specific therapeutic strategies compromise the ability of T cells to fight infections or to control cancer development and progression, thus more studies are required to determine the most efficacious, safe and tolerable treatment.

Targeting specific surface molecules or soluble cytokines for dampening inflammatory T cells in MS

Other therapies in clinical trials include the use of specific antibodies (Abs) targeting T cells or pro-inflammatory cytokines.

Daclizumab is a humanized monoclonal Ab directed against CD25. CD25 is the alpha chain of the high affinity IL-2 receptor. CD25 is up-regulated on activated T cells and is necessary for IL-2-driven survival and proliferation of activated T cells, including autoreactive

Table 1: Currently approved therapies in MS.

Drug	Mode of action	Approved in Europe	Major side-effects
IFN- β	Immunomodulation: - Reduction of IL-23 and upregulation of IL-10 levels - Expansion of Tregs	- RRMS (first-line) - SPMS with relapses	- Flu-like symptoms - Injection-side reactions - Hepatotoxicity
Glatiramer acetate (GA)	Immunodulation: - Reduction of IFN- γ (Th1) and up-regulation of IL-4 (Th2) - Expansion of Tregs	- RRMS (first-line)	- Flu-like symptoms - Injection-side reactions (lipoatrophy)
Dimethyl-fumarate	Immunomodulation: - Depletion of peripheral blood T cells - Reduction of IFN- γ (Th1) and up-regulation of IL-4 (Th2)	- RRMS (first-line)	- Flushing - Gastrointestinal side effects - Hepatotoxicity - Leukopenia
Teriflunomide	Inhibition of proliferation of active dividing cells	- RRMS (first-line)	- Gastrointestinal side effects - Hair loss - Hepatotoxicity - Leukopenia - Teratogenic potential
Natalizumab	Blocking migration of immune cells to the CNS	- RRMS (second-line)	- Allergic infusion reactions - Infections (PML) - Hepatotoxicity
Fingolimod	Blocking egress of lymphocytes from lymph nodes	- RRMS (second-line)	- Infections (HSV, VZV) - Bradycardia and arrhythmias - Macular edema
Alemtuzumab	Depletion of immune cells expressing CD52 (i.e. T cells)	- RRMS (highly active)	- Allergic infusion reactions - Infections (HSV) - Autoimmune disorders (thyroid and Good-pasture syndrome)
Mitoxantrone	Inhibition of proliferation of active dividing cells	- RRMS (highly active) - SPMS	- Cardiotoxicity - Gastrointestinal side effects - Acute myeloid leukemia

cells. Binding of daclizumab to CD25 blocks the survival and proliferation of activated T cells through the preferential expansion of regulatory CD16-CD56^{bright} natural killer (NK) cells with anti-viral and anti-tumour activities [96,97]. In phase III trials, daclizumab resulted in a significant reduction of the relapse rate and disability progression by more than 50% [98]. However, the AEs reported in both phase II and III clinical trials were higher rate of infections, skin disorders, diffuse lymphadenopathy, and elevated liver enzymes [98-100].

Alemtuzumab is a humanized monoclonal Ab against CD52, a surface glycoprotein expressed on several immune cells including T and B cells, eosinophils and monocytes. Its selective expression on mature cells but not on lymphoid progenitors made it a good target for immunotherapy [101]. The interaction of alemtuzumab with CD52 leads to the depletion of CD52⁺ cells [102], through complement and/or cell mediated lysis [103]. Data from clinical trials evidenced a strong reduction of relapses and neurological disability in RRMS patients [104,105]. However, given the prolonged immunosuppression after treatment with alemtuzumab, its impressive effectiveness was associated with a high risk of thyroid autoimmune diseases [106]. Thus, the drug was not approved by the FDA due the negative risk/benefit ratio but is currently approved in Europe (Table 1).

Despite the encouraging data obtained by the administration of neutralizing anti-IL-12 or anti-IL23p19 monoclonal Abs in EAE [107-109], treatment of RRMS patients with IL-12/23p40 neutralizing monoclonal Ab (ustekinumab) did not result in any significant therapeutic benefits in a phase II trial [110].

CD28/B7 costimulatory molecules as therapeutic targets

Optimal priming and activation of CD4⁺ T cells requires T cell

receptor (TCR)-recognition of peptide-MHC class II complexes together with signals delivered by costimulatory molecules expressed on the surface of professional APCs. CD28 is constitutively expressed on 90% of naive and activated CD4⁺ T cells and on 50% of CD8⁺ T cells. By binding its cognate ligands B7.1/CD80 or B7.2/CD86 on the surface of professional APCs, CD28 lowers TCR activation threshold, thus leading to the augmentation of early signalling events necessary for efficient cytokine production, cell cycle progression and survival. Furthermore, CD28 ligation enhances the expression of CD40L and adhesion molecules necessary for trafficking, such as VLA-4. Moreover, CD28 is able to emanate TCR-independent autonomous signals, which account for its critical role in the regulation of pro-inflammatory cytokine/chemokine [111,112] and Th17 amplification in MS [113].

Another function of CD28 is its involvement in the development and homeostasis of Tregs. CD28 is required for both efficient generation of Tregs in the thymus [114] and for limiting T cell activation by sustaining the survival of Tregs in the periphery [115]. These evidences support the hypothesis that the presence of B7, even on cells that are not displaying the cognate antigen, may control adaptive immune responses [116]. Therefore, CD28 deficiency can lead to a reduced disease potential as well as to an enhanced susceptibility to autoimmune diseases by altering T cell effector and Tregs compartments, thus representing an interesting target to ameliorate cell dysfunctions in autoimmune diseases.

All CNS-infiltrating mononuclear cells in EAE, including CD4⁺ T cells, express CD80/B7.1 [117,118]. However, conflicting and opposing results have been obtained by using CTLA4-Ig or anti-B7 antibodies on EAE mice. Although, CTLA-Ig efficiently inhibits TCR-induced activation of autoreactive T cells by blocking CD28/

B7 interaction, it cross-links CD80 expressed on either APCs or activated autoreactive T cells, thus inducing IFN- γ production and contributing to the disease exacerbation [119]. Moreover, several studies evidenced that peripheral myelin-reactive T cells from MS patients are primarily effector/memory T cells in a higher activation state, which express high avidity TCRs and are less dependent from CD28/B7 costimulation in terms of TCR-activation [120].

Blockade of CD28/B7 interaction by recombinant CTLA4-Ig (adaptacept), which binds B7.1/B7.2 with high affinity, is being evaluated in RRMS in both phase I [121] and phase II clinical trials (ClinicalTrials.gov, identifier: NCT01116427).

More recently, anti-CD28 superagonistic Abs have been successfully used to inhibit the onset, progression and clinical course of EAE, by preferentially activating and expanding the immunosuppressive Tregs [122]. However, when administered to volunteers in phase I clinical trial, a humanized CD28 superagonistic Ab (TGN1412) induced a rapid and massive production of pro-inflammatory cytokines (i.e. IFN- γ and TNF- α), thus causing a severe systemic inflammatory response syndrome [123]. Moreover, our group has recently observed that the human superagonistic CD28 ANC28.1 Ab strongly up-regulated CD4⁺CD25^{high}FOXP3⁺ Tregs at a similar extent in RRMS patients and healthy donors [113].

These data arise, at least, two important considerations: 1) CD28 signals can mediate different functions depending on the presence or not of TCR co-engagement and 2) the translation of experimental results from mice to men could determine dramatic effects, supporting differences in CD28 functions and signalling capability between humans and mice. Thus, the blockage of CD28 signalling, rather than its stimulation, may have heuristic implication for the development of more efficient therapies in MS.

CD28-associated PI3K signalling pathway as a potential therapeutic target

Phosphatidylinositol-3 kinase (PI3K) is a critical mediator of CD28 signalling [124]. The intracytoplasmic tails of CD28 binds and activates class 1 PI3K that phosphorylates inositol phospholipids on carbon atom 3, thus generating the phosphatidylinositol 3,4-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-triphosphate (PIP3) [112,125]. PIP2 and PIP3 lipids bind the pleckstrin domains of several molecules involved in CD28 signals, in particular the protein kinase B/Akt [126,127], thus affecting both the fate and movement through the body of T cells [128,129].

The PI3K/Akt pathway plays a central role in regulating inflammation and abnormalities in this pathway could be linked to the development of autoimmunity [130]. The activity of the PI3K network can be manipulated by pharmacological compounds, several of which are approved for clinical use in some inflammatory and autoimmune diseases. Blockage of class I PI3K γ subunit has resulted effective in reducing both disease severity and incidence in a mouse model of systemic lupus erythematosus [131] and the delivery of oral active form of PI3K γ inhibitor AS-605240 reduces joint inflammation and cartilage erosion in mouse models of rheumatoid arthritis [132,133].

Our recent results on the ability of AS-605240 to inhibit the pro-inflammatory responses induced by CD28 stimulation in RRMS, suggest PI3K as a valuable therapeutic candidate for MS. Moreover,

the availability of oral active forms of PI3K inhibitors [134] may also circumvent the ASEs (e.g. skin indurations and lipoatrophy) related to infusion or injection and may exhibit higher adherence and long-term persistence, as observed for other oral compounds (e.g. fingolimod) [85].

Conclusion

Inflammatory T cells have a well recognized role in the pathogenesis of MS. Most of the current therapeutic strategies focus on blocking the pro-inflammatory responses in a non-specific manner, thus compromising the ability of T cells to fight infections or to control cancer. From basic researches and pre-clinical findings in animal model, a growing number of therapeutic agents are upcoming. A future challenge will be to identify the most efficacious, safe and tolerable anti-inflammatory drugs that used in combination with treatments that block neurodegeneration may also favour remyelination and tissue repair.

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