



# Role of CD4<sup>+</sup>T Helper Cells as Mediators of Inflammation in the Pathophysiology of Multiple Sclerosis- A Systematic Review

Kaur KK<sup>1\*</sup>, Allahbadia G<sup>2</sup> and Singh M<sup>3</sup>

<sup>1</sup>Kulvinder Kaur Centre For Human Reproduction, India

<sup>2</sup>Scientific Director, Rotunda-A Centre for Human Reproduction, India

<sup>3</sup>Consultant Neurologist, Swami Satyanand Hospital, India

\*Corresponding author: Dr Kulvinder Kochar Kaur, Scientific Director, Dr Kulvinder Kaur Centre for Human Reproduction 721, GTB. Nagar, Jalandhar-144001, Punjab, India; Tel: +91-181-9501358180/ 91-181-4613422; Email: [kulvinder.dr\[at\]gmail\[dot\]com](mailto:kulvinder.dr[at]gmail[dot]com)

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## Abstract

Multiple Sclerosis (MS) represents a chronic inflammatory autoimmune condition like other autoimmune ones such as rheumatoid arthritis (RA), Psoriasis, Endometriosis, etc. MS results in continuous depletion of axonal myelin in various regions of the central nervous System (CNS) & influences the occurrence of a variety of clinical symptoms like muscle spasms, optic neuritis as well as paralysis. Over a decade advances made in research conducted in animal models of MS to get better insight of pathophysiology of MS disease corroborated that MS is an autoimmune inflammatory disorder secondary to recruitment of self-reactive lymphocytes, basically CD4<sup>+</sup>T cells in the CNS. Actually high concentrations of T helper cells (Th) cell subsets as well as associated cytokines as well as chemokines have been observed in the CNS lesions as well as cerebrospinal; fluid (CSF) of MS patients ,along with breakdown of blood brain barrier (BBB), as well as consequent activation of resident astrocytes as well as microglia and ultimately neuroinflammation. Thus we decided to do a systematic review utilizing the Pubmed Engine, Google Scholar Web of Science utilizing the MeSH terms like Th cell kinds like Th1; Th 17; Th22; Th9 and others from 1980 till date on 31st July 2020. We found over 25,000 articles out of which we selected 154 articles for this review to study the influence of the variety of Th like cells involved in the aetiopathogenesis. No meta-analysis was conducted. Till date various kinds of Th cells have been discovered and named as per the liberated lineage defining cytokines. Significantly Th 1, Th17, Th22, Th9 as well as Th1 like Th17 have been correlated with MS. Thus we have detailed the crosstalk of various Th cell subpopulations as well as their lineage defining cytokines in manipulating the inflammatory responses in MS along with how the innovative drugs that have received FDA approval interact which target T lymphocytes for the therapy of the disease.

**Keywords:** Multiple sclerosis; T helper cells; Neuroinflammation; Immunotherapies

## Introduction

Multiple Sclerosis (MS) represents a chronic inflammatory autoimmune condition of the central nervous System (CNS) that influences roughly 2-3 million people worldwide which gets initiated by both environmental as well as genetic factors [1]. Roughly 15-30% of cases with MS present with relapse-remitting (RR) clinical course, that has the properties of acute episodes of neurological abnormalities like optic neuritis, sensory alterations

or motor dysfunction, that is mostly followed by times of remission or recovery [2,3]. Following different time courses, roughly 50% of RRMS patients propagate to a chronic secondary progressive (SP) clinical stage, having the properties of gradually declining condition. In approximately 15% of patients, MS is propagating right from its initiation, known as primary progressive (PP) MS, a clinical course that has the properties of a

slow and continuous deterioration in the neurological function's [4].

Basic etiopathological typical property is the breaking of the blood brain barrier (BBB), loss of oligodendrocytes, demyelination, and astrocytes gliosis along with axonal degeneration [5]. Inflammation exists in all stages, with key role of pro inflammatory cytokines as well as chemokines in the etiopathology of MS by compromise of blood brain barrier (BBB), immune cell recruitment via the periphery as well as activation of resident microglia. Microglial activation is believed to be one of the initial processes in the formation of MS lesions. Activated Microglial, actually is believed to add to the disease propagation by liberating inflammatory cytokines as well as chemokines along with reactive oxygen species (ROS) as well as glutamate [6]. MS getting converted from RR to the progressive phase has also been associated with continued chronic Inflammation in the CNS. Furthermore both SPMS as well as PPMS patients present with generalized Inflammation in the total brain that is associated with cortical demyelination as well as diffuse damage of the white matter [7]. Despite every cell kind of the innate as well as adaptive immune system might bring about the inflammatory response among the CNS, an essential aid is through the autoreactive CD4<sup>+</sup>T cells. Autoreactive T cells most probably activated in the peripheral lymph node migrate into the CNS [8], where they get reactivated as well as liberate cytokines as well as chemokines which manipulate the inflammatory lesions that are classical of MS [9]. Like, the robust genetic risk factor for MS is the human leukocyte antigen(HLA)-DRB\*15:01, a major histocompatibility complex (MHC)class II allele that is implicated in the presentation of self-peptides to CD4<sup>+</sup>T cells [10]. This review has the objective of giving a detailed part of various CD4<sup>+</sup>T helper cells (Th) cell subsets in the etiopathophysiology of MS along with present treatment methods which target T cell-mediated responses. The part of regulatory T (Treg) cells in repressing the function of autoreactive Th cell in MS is further detailed.

### Subsets of Th cell

CD4<sup>+</sup>T cells represent the central controllers of the adaptive immune response that works opposite to a lot of microbes utilizing the aiding B lymphocytes to generate antibodies (Ab) by liberating particular cytokines which offer efficacious protection from pathogens. Separate Th cell Subsets, generating 1 or greater lineage defining cytokines as well as expressing master transcription factors as well as homing receptors, differentiate from naive CD4<sup>+</sup>T cells after provocation by a particular class of pathogenic microorganisms along with to the cytokines surroundings. Naive CD4<sup>+</sup>T cells get activated inside the peripheral Lymph Nodes through mature dendritic cells (DC's) which present pathogen derived peptides correlated with MHC as

well as costimulatory molecules facilitate T cells proliferation along with forming polarising cytokines that then stimulate T cells differentiation into separate Th cell Subsets, like Th 1, Th2, Th17, Th22 as well as Th9 [11]. Additionally to this protective aspect from pathogens, Separate Th cell Subsets display a key part in the pathogenesis as described.

### Th 1 cells

Th 1 Cells got isolated in the late 80's [12]. Being a subset of CD4<sup>+</sup>T cells which bring about effective adaptive immune response from intracellular pathogens by liberating Interferon (IFN $\gamma$ ) which activates macrophages for killing intracellular microbes along with facilitating the generation of opsonising Ab [11]. Th 1 can get isolated through surface expression of the CXC chemokine Receptor Type 3(CXCR3) as well as interleukin (IL)-12 Receptor (IL-12 R) chains  $\beta$ 1/ $\beta$ 2, in addition to the intracytoplasmic expression of the master transcription factor T-bet [13]. IL-12 as well as IFN $\gamma$  work together for the induction of the expression of T-bet, hence escalating the Th 1 polarization [14]. Earlier studies conducted in an experimental autoimmune encephalomyelitis (EAE) animal model of MS showed a key part of Th 1 Cells in the pathogenesis of MS. Actually IFN $\gamma$  liberating Th 1 Cells were identified as the most common Th 1 Cells subset in the central nervous system (CNS) as well as EAE animals [15]. Plenty of IFN $\gamma$  was identified in the CNS lesions of EAE [16] along with the active lesions of MS patients [17], as well as the adoptive transfer of Th 1 Cells was enough to generate EAE in the mice receiving it [18], These results along with initial observations that delivery of IFN $\gamma$  to MS patients augmented the disease [19], that is further corroborated by the significant part of IL-12/ IFN $\gamma$  axis as well as Th 1 Cells in both EAE as well as MS pathogenesis. Despite the neuropathological part of infiltrated Th 1 Cells into the CNS is not known properly, there are different proofs pointing that microglia is the main cell target of Th 1 Cells. Microglia represent the resident macrophages of CNS, based on the type of external stimuli, might differentiate into inflammatory M1 like or anti-inflammatory M2 like phenotype [20]. Th 1 Cells generate various effector molecules which activate resident microglia along with initiating their differentiation into the inflammatory as well as neurotoxic M1 like phenotype [21]. Further Th 1 Cells promote the upregulation of class II MHC as well as costimulatory molecules present on microglia, hence facilitating the reactivation of the infiltrated Th Cells along with their differentiation further [22]. Nevertheless, more research regarding mice deficient in IL-12p35 subunits [23] or IL-12R $\beta$ 2 chain [24] or IFN $\gamma$  [25] were prone to EAE in addition to the results demonstrating that delivery of IL-12 at the time of initial phases, the disease repressed EAE in an IFN $\gamma$  dependent way [23], reduced the significance of this concept that Th 1 Cells were the major

pathogenic Cells subset in EAE as well as MS. Ultimately the invention that IL-23 shares the p40 subunits with IL-12 [26] as well as IL-23R comprises or IL-12R $\beta$ 1 chain [27], explained these conflicting results as well as explained the part of IL-23 in facilitating EAE by initiating as well as stimulating the expansion of Th 17 Cells, that is a subset of Th Cells that generates IL-17 [28,29].

### Th 17 cells

Th 17 Cells got isolated by 2005 [28], being a significant subset of CD4<sup>+</sup>T cells which bring about effective immune response against extracellular bacteria as well as fungi [30]. Th 17 Cells might be isolated depending on the particular surface markers like CD161. The chemokine receptor CCR6 as well as CCR4, the cytokine receptors IL-23R as well as IL-1R [31], the intracytoplasmic expression of retinoic acid as well as receptor-associated orphan nuclear receptor  $\gamma$ (ROR  $\gamma$ ) as well as the generation of particular cytokines like IL-17A-, IL-21 as well as IL-22 [32]. Especially IL-17A as well as IL-17F work on various cell kinds, like macrophages, epithelial as well as endothelial cells by stimulating the expression of inflammatory cytokines as well as chemokines as well as facilitating the recruitment of neutrophils in inflammatory zones [33]. IL-21 gives a positive feedback loop for good expansion of the Th 17 Cells subsets [34], while IL-22 works on epithelial cells facilitating the generation of anti-microbial peptides along with mucus [35]. Besides these roles of facilitating the host defense against pathogens, tissue damage might also result ending in chronic inflammatory diseases [36]. Noticeably Th 17 Cells are implicated in the pathogenesis of various autoimmune diseases that includes MS [37], where they have a key part in disrupting the blood brain barrier (BBB) [38] Along with targeting resident astrocytes as well as microglia within the CNS & hence facilitate their activation as well as neuroinflammation in EAE [21,39,40] (Figure 1). Especially Th 17 Cells are markedly efficacious in controlling astrocytes instead of microglia. Astrocytes represent resident CNS cells having particular anatomical positions along with morphological as well as functional properties. Their location is at the border between the BBB as well as neurons and control the motion of molecules as well as cells among circulation as well as CNS. Furthermore by generating neurotrophic factors (NF's) they also control neurogenesis along with tissue repair. Secondary to CNS damage, astrocytes go through significant morphological as well as functional alterations, a process known as astrogliosis [41]. Astrocytes express a functional IL-17 receptor A which is upregulated more in EAE [42]. IL-17 is known to upregulate the generation of inflammatory cytokines as well as chemokines [43] along with IL-17 signaling once dysfunctional in astrocytes abrogates EAE [44]. Th 17 Cells along with aid of Th 1 have been demonstrated to control the function of astrocytes via stimulating

the downregulation of NF's as well as the upregulation of inflammatory cytokines as well as chemokines [39,45]. Other posited pathological function of Th 17 as well as IL-17 is the inhibition of maturation along with survival of oligodendrocytes (OL's) [46], besides their apoptosis [47]. OL's represent myelinating giant cells of the CNS at the time of formation and right through adulthood. In case of MS constant demyelination as well as neurodegeneration get correlated with impairment as well as apoptosis of OL's resulting from direct cytotoxicity from Antigen specific T cells along with autoantibodies along with T cells modulated pro inflammatory cytokines which activate resident microglia [48].

In the last decade, various studies conducted in EAE mice [9,28,49], in addition to the immunological examination of MS cases proved a key part of Th 17 Cells in the etiopathogenesis of MS [29,50]. High amounts of IL-17 generating CD4<sup>+</sup>T cells have been observed in the peripheral blood or CSF of RRMS patients at the time of relapses [50] as well as in MS patients having active disease [51]. Further the preferential expansion of Th 17 Cells has been associated with the total amount of active plaques on magnetic resonance imaging (MRI) [52], along with disease propagation [53]. IL-17A, together with IL-6, has further been illustrated to facilitate the breakdown of BBB in RRMS patients [54], by changing the expression of adhesion molecules on endothelial cells [55] as well as promoting the depolymerisation of the actin cytoskeleton near tight junctions [56]. Like human Th 17 Cells display an enhanced migratory ability in RRMS patients with greater disease severity as well as inflammatory lesions [57]. Various transcription factors, of which most dependable ROR $\gamma$  [58] as well as STAT3 [59], along with cytokines like IL-6, transforming growth factor beta (TGF- $\beta$ ), interleukin 21(IL-21), IL-1- $\beta$  as well as IL-23 have been detailed to facilitate IL-17 expression as well as Th 17 Cells differentiation in both human as well as mouse [60,61]. In case of mouse system, the differentiation of Th 17 Cells is facilitated by TGF- $\beta$ , along with IL-6, IL-21, IL-1- $\beta$  [62]. In the human system, certain GRPS pointed that IL-1- $\beta$  as well as IL-23 can provide for TGF- $\beta$  functions by stimulating human Th cell differentiation [63,64]. Rest of researchers saw a key role of TGF- $\beta$  along with IL-6, IL-21, IL-1- $\beta$ , IL-23 for stimulating the expression of ROR $\gamma$  as well as Th 17 Cells differentiation [65]. In human but not in mouse [66], CD28, a significant costimulatory molecule expressed on T cells [67], as well as implicated in the control of both tolerance as well as autoimmunity [68], has further been implicated to stimulate CD4<sup>+</sup>T cells to generate inflammatory cytokines as well as chemokines associated with Th 17 Cells phenotype [69] as well as to escalate the inflammatory response in MS by reprogramming T cells metabolism as well as promoting the expression of Th 17 Cells-related cytokines [70].

Th 17 Cells show tremendous plasticity along with utilize various functional profiles, based on the inflammatory or anti-inflammatory surroundings [71]. Th 17 Cells have the ability to differentiate into markedly pathogenic Th 1 like Th 17 as well as Treg Cells, and actually have certain similar features, like the expression of CD49b as well as the transcription factor aryl hydrocarbon receptor (AhR) possibly in view of a shared TGF- $\beta$  need for their differentiation [72]. Regarding this Gigliani et al. demonstrated in mice that at the time of resolution of inflammation, Th 17 Cells go through a reprogramming of transcription, resulting in their differentiation into T reg Cells in TGF- $\beta$  presence that is AhR dependent [73]. Recently Capone et al. encountered the great plasticity of Th 17 Cells in cases of MS, who demonstrated that human Th 17 Cells polarized from the naive CD4<sup>+</sup>T cells along with from peripheral blood Th 17 Cells of RRMS patients upregulated the expression of IL-1R as well as generated greater amounts of IL-21, IL-2 as well as tumor necrosis factor (TNF)- $\beta$  hence resulting in acquiring a greater pathogenic profile [74]. Constantly escalated expression of IFN $\gamma$  as well as CXCR3 along with decreased expression of anti-inflammatory IL-10 was observed in Th 17 Cells from clinically active MS patients [75]. Th 17 Cells further generate GM-CSF, that is another cytokine which has been pointed to have a key part in the pathogenicity of Th 17 Cells in EAE models [37]. Further the percentage of IL-17 as well as granulocyte-macrophages colony stimulating factor (GM-CSF) cogenerating cells were enriched with the cerebro spinal fluid (CSF) of RRMS [76] and they escalated at the time of relapse [77]. Th 17 Cells have been implicated to generate IL-22 [24], a cytokine which enhances pro inflammatory innate defense modes in epithelial cells [78], which along with other cytokines having the Th 17 signature have been implicated in various inflammatory as well as autoimmune diseases [79].

### Th 1 like Th 17 cells

A novel Th 1 subset known as Th 1 like Th 17 cells which generate both IFN $\gamma$  as well as IL-17 have been isolated in both human as well as mice recently [80]. They express IL-23 as well as coexpress CXCR3 as well as T-bet along with CCR6 as well as ROR $\gamma$ , generate lesser quantities of IL-17 A as compared to classical Th 17 cells but greater amount of IFN $\gamma$  [32], The origin of Th 1 like Th 17 cells is not fully clear but various studies corroborate the belief that these cells originate from Th 17 cells in IL-12. TNF- $\alpha$  as well as /or IL-1 $\beta$  presence [81,82]. In mice the greater pathogenicity of Th 1 like Th 17 cells as compared to Th 17 cells correlated with the generation of various inflammatory cytokines as well as chemokines like GM-CSF, IL-22, CC chemokine Ligand 4(CCL4), and CXCR3 [83]. These results got corroborated in human system by the evaluation of the transcriptional profile of peripheral blood Th 17 cells as well as

Th 1 like Th 17 cells in MS [75]. These data got further extended utilizing data from single cell transcriptome evaluation conducted in experimental autoimmune encephalitis (EAE), by demonstrating that the differentiation of Th 17 cells in the CNS from precursors in the Lymph Nodes was determined by four particular genes, Gpr65, Toso, Plzp, as well as Cd51 that were also implicated in disease proneness [84].

This Th 1 like Th 17 cells participating in neuroinflammation has been examined in studies in both EAE as well as MS patients [85]. Like Th 1 like Th 17 cells had the capacity to cross the BBB as well as collect in the CNS of acute EAE as well as were observed in brain tissues from MS patients [86], as well as upregulated in RRMS patients during relapse [87]. Myelin-particular Th 1 like Th 17 cells escalated in both peripheral blood CSF of patients with MS [88], as well as Th17.1 cells, a Th 1 like Th 17 subpopulation expressing raised amounts of IFN $\gamma$ , GM-CSF, very late antigen 4(VLA4), as well as low amounts of IL-17, have been correlated with the disease activity in case of RRMS patients [89]. The neuropathic actions of Th 1 like Th 17 cells within CNS are still getting studied but have a chance to overlap with those observed in Th 1 as well as Th 17 cells (Figure 1).

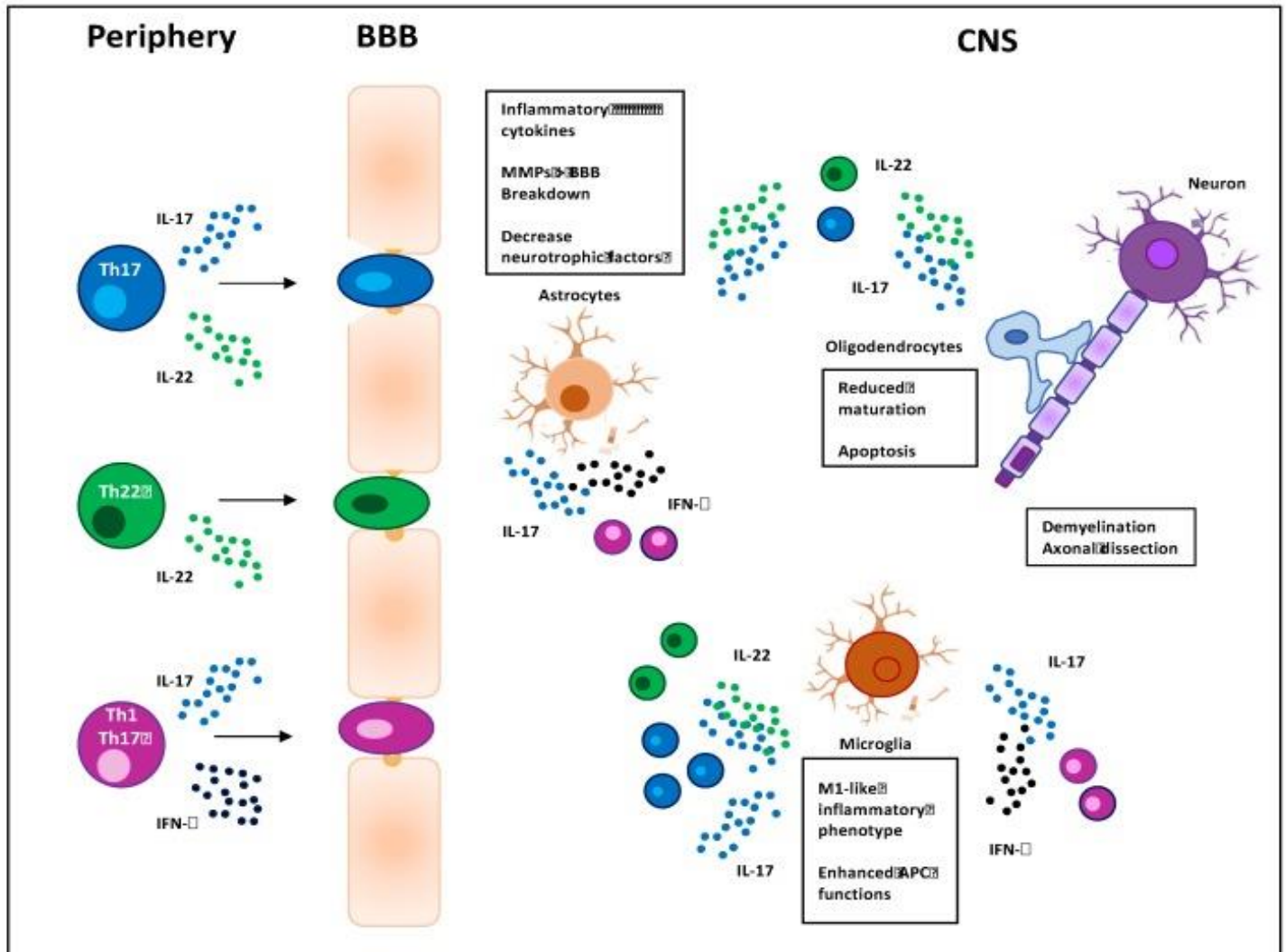
### Th22 Cells

IL-22 is generated by Th 17 cells as well as by a unique CD4<sup>+</sup>T cells subset, that got discovered in 2000[90] known as Th22 [91]. Other cellular resources for IL22 are natural killer (NK) cells [92]. ILC3subset of innate lymphoid cells as well as as well as to a lesser degree, other leukocytes that are macrophages [93]. IL-22 represents being a, member of the IL-10 family with its main actions being exerted on non haemopoietic cells like epithelial cells. IL-22, actually stimulates the regeneration as well as proliferation of epithelial cells as well as facilitates the generation of antimicrobial peptides needed for epithelial barrier functions as well as protection against extra cellular (EC) pathogens [94].

Despite the part of IL-22 in MS has not been fully investigated, proof that is coming out points to the involvement of IL-22 in MS immunopathogenesis. Earlier studies by Kabir et al., observed the upregulation of IL-22R in the brains of MS patients as well as the part of IL-22 in synergism with IL-17A in disruption of the integrity of BBB tight junctions by decreasing the expression of occludin in epithelial cells [94,95]. Data that has been more recent, demonstrated greater amounts of IL-22 in serum of relapsing MS as compared to healthy donors [77,20-22], as well as a decrease of IL-22 was seen during the recovery phase of acute EAE [96]. The number of IL-22 generating cells also escalated in both peripheral blood [97] as well as CSF [76] of RRMS patients that were resistant to IFN $\beta$  therapy [97]. In view of great amounts expression of CCR6, that is needed for the movement into the CNS, myelin particular IL-22 generating cells

can act synergistically with Th 17 cells, and hence result in breaking of the BBB as well as start the autoimmune response against constituents of the CNS myelin [54]. Like myelin particular IL-17 as well as IL-22 generating CD4<sup>+</sup>T cells resistant to corticoids has been correlated with active brain lesions in the MS patients, especially in the plaques and mainly in the astrocytes [95], that might enhance the permeability of the BBB by liberating matrix metallo proteinases (MMPs) [41].

Significantly recent studies point a part of IL-22 as well as IL-22 generating cells in controlling the survival as well as action of both OLs as well as astrocytes in MS [95,98] (Figure 1). Lastly, the isolation of single nucleotide polymorphism (SNP) of IL-22 binding protein (IL-22BP, also known as IL-2RA), an antagonist of IL-22, as MS risk gene [99], agrees with the part of IL-22 generating cells in the immunopathogenesis of MS.



**Figure 1:** Courtesy ref no 40-Pathogenic T helper (Th) cell subsets in multiple sclerosis (MS). Self-reactive Th1, Th22 cell, and Th1-like Th17 subsets activated in peripheral lymph nodes cross the blood–brain barrier (BBB) and migrate into the central nervous system (CNS). In the CNS, T cells are reactivated and, by producing their lineage-defining cytokines, regulate the functions of CNS-resident cells (microglia, astrocytes, oligodendrocytes) by enhancing inflammatory cytokine production, antigen-presenting cell (APC) functions, and apoptosis, thus contributing to axonal damage and demyelination.

### Th9 cells

In the last 10yrs a separate subset of effector CD4<sup>+</sup>T cells subset, having the properties of generating IL-9 got identified. Despite

initial correlation of IL-9 in the start with a Th-2 response, studies done more recently gave a newer definition to the IL-9 generating CD4<sup>+</sup>T cells as Th 9 cells [100]. In MS the part of Th 9 Cells have mainly been evaluated in a mouse model of EAE. The initial

study contrasted the encephalitogenic activity of myelin oligodendrocyte glycoprotein (MOG)-particular Th 9, Th 17, as well as Th 1 cells in an EAE model of adoptive transfer. It was shown that Th 9 cells receiving mice had lesser infiltrates of lymphocytes in the meninges as compared to EAE formed by Th 1 as well as Th 17 cells, hence pointing that the mode of EAE induced via Th 9 cells was separate from the mode of Th 1 as well as Th 17 cells [101]. More studies conducted in knockout mice showed contradictory outcomes. Actually mice where IL-9R was absent displayed a course of greater severity, pointing to a controlling part of the cytokine in regulating pathogenic modes of immune responses [102]. Constantly, in EAE, IL-9 was documented to be an anti-inflammatory cytokine generated by non-pathogenic Th 17 cells in conjunction to IL-10 [103]. Conversely, rest of groups observed that IL-9 neutralization as well as IL-9R deficiency ameliorated EAE [104].

Ruocco et al. evaluated the action of IL-9 in MS by associating the amounts of IL-9 in the cerebrospinal fluid (CSF) of 107 RRMS patients on diagnosis along with at the time of course of disease. Intriguingly they observed that IL-9 amounts in the CSF of RRMS patients inversely associated with indices of inflammatory activity, neurodegeneration, as well as propagation of MS –correlated disability [105]. An earlier study in MS patients demonstrated that IL-9 amounts were less at the time of clinical relapses as well as escalated after prednisone therapy, hence pointing the existence of Th-9 cells in the CSF of MS patients, in which IL-9 could have a protective part in MS disease by decreasing the amounts of IL-17 generated by Th 17 cells [106] as well as the expression of the mitochondrial apoptotic pro-apoptotic factor Bax [107], hence escalating neuronal survival in organotypic human brain slice cultures [108]. In view of the contradictory outcomes documented on the aid of Th-9 cells as well as IL-9 in either MS or EAE, more studies are needed for clarification of the part of Th-9 cells in MS pathogenesis.

### Treg cells

The formation of autoimmunity gets finely controlled by particular subsets of T cells which manipulate immune responses by checking pathogenic Th-cells under regulation. Initially isolated in 1995 by Sakaguchi et al, Treg Cells were a subset of CD4<sup>+</sup>CD25<sup>+</sup>T cell subset having the capacity of repressing effector inflammatory T cells as well as sustain self-tolerance [109]. In the past two decades, work has been done regarding further characterizing Treg Cells in relation to autoimmune diseases with a minimum of 2 major subsets of CD4<sup>+</sup> Treg Cells have been isolated –natural n Treg Cells which form in the thymus after the recalling of self-antigens as well as express the transcription factor forkhead box P3 (Fox P3) [110] as well as inducible iTreg cells, which get produced from the naive CD4<sup>+</sup> T Cells under particular situations of antigen stimulation as well as

in the existence of specific cytokine milieu. iTreg cells is made up of various subsets that are Fox P3 Treg cells, type 1 regulatory (Tr1) T cells generating large amounts of IL-10 as well as Th3 generating TGFβ [111]. In humans, circulating iTreg further show large amounts of phenotypic heterogeneity strictly associated with their differentiation status as well as immunosuppressive functions [112]. Changes in both amount as well as repressive functions of separate Treg subsets have been documented in MS, particularly in RRMS patients [113], as well as exert disease actions on Treg cells [112].

### T cell targeting treatment

The role of autoreactive inflammatory T cell subsets in the MS pathogenesis gets corroborated by knowing that maximum of the approved disease-manipulating treatments target T cells. Glatiramate acetate (GA), a mixture of synthetic polypeptides, having only 4 amino acids – glutamate, lysine, alanine, as well as tyrosine got approval through FDA for therapy of RRMS in 1996. Despite the mode of action of GA are not clear, various studies point to a key part of GA on various antigen presenting cells (APC), by facilitating the differentiation of microglia, IFN-β, an antiviral cytokine, having immunosuppressive as well as antiproliferative actions, has been the 1<sup>st</sup> line drug approved via FDA for the therapy of RRMS in 1993. The maximum significant action of IFN-β on T cells are the inhibition of T cell activation as well as liberation of cytokines [114], the shift of Th cells from an inflammatory Th1 to an anti-inflammatory Th2 cell phenotype, the decrease of both Th 17 as well as IL-17 amounts, as well as an escalation of suppressive Treg [115]. A massive reduction of IL-22 in the serum of RRMS patients following 6 as well as 12 mths of therapy with IFN-β, has also been recently documented as well as corroborated the reduction of MS severity [116].

macrophages as well as dendritic cells (DCs) into the suppressive M2 phenotype generating anti-inflammatory cytokines like TGF-β as well as IL-10 [117]. The outcomes of these actions on T cells involve the shift of reactive T cells from Th1 to Th2 [118] as well as the enhancement of Treg [119], which repress the action of all autoreactive inflammatory Th cell subsets. Regarding this Spadaro et al., showed that in GA-responder of RRMS patients, anti-inflammatory Treg cells remain for greater than 10 yrs following GA therapy [120]. Akin to IFN-β, GA is not efficacious in decreasing the disability of propagating MS [121], however, due to its long term effectiveness as well as safety it is the commonest 1<sup>st</sup> line therapy registered all over the world for RRMS [122].

Dimethyl fumarate (DMF), a fumaric acid ester having anti-inflammatory action got approval for therapy of RRMS patients in 2013 [123]. It causes both direct as well as indirect actions on T cells [124]. DMF decreases the generation of inflammatory cytokines like IL-1 β, tumor necrosis factor (TNF) as well as IL-6

in microglia [125], as well as inhibits inflammatory cytokines generation in human peripheral blood mononuclear cells (PBMC) [126], possibly by influencing the activation of nuclear factor (NF)- $\kappa$ B family of transcription factors [127]. Besides a total decrease in peripheral T cell numbers seen in RRMS patients given therapy with DMF [128], marked alterations in circulating Th cell subsets were further seen [129], with a special decrease of memory CD4<sup>+</sup> T Cells [128], DMF therapy further stimulates a shift of the balance various Th cell subsets, with a decrease of IFN- $\gamma$  generating Th1 as well as IL-17 generating Th-17 cells as well as an escalation of IL-4 generating Th2 cells [130]. Lastly an ideal response to DMF therapy also correlated with an enhancement of Treg cells in the peripheral blood of RRMS patients [129,131]

Fingolimod, that is a 1<sup>st</sup> oral treatment molecule that got approval for RRMS [132,133], works as an antagonist of sphingosine-1-phosphate receptor (S1PR), which is necessary for the exit of T lymphocytes from Lymph Nodes [134]. Via binding of S1PR as well as stimulating its internalization, Fingolimod modulates the naive as well as effector memory T lymphocytes are set apart from the lymphoid organs [135]. A detailed evaluation of effectiveness of Fingolimod on various T cell subsets in RRMS patients who received therapy with Fingolimod showed a selective decrease in the frequency of both IFN- $\gamma$  as well as IL-17 generating cells [136]. Dominiguez-Villar et al, demonstrated that Fingolimod therapy robustly decreased central memory Th1, Th17, as well as Th 1 like Th 17 cells while effector memory Th1, as well as Th 1 like Th 17 cells subsets were not that much influenced. The evaluation of Fingolimod actions on T cells at the time of 12 mths of also experienced a manipulation of phenotype of Th 17 cells as well as Treg cells. Effector Th Cells displayed a reduction of the exhaustion markers like programmed death1 (PD1) as well as Tcell immunoglobulin as well as mucin domain containing 3 (TIM3) [137]. Further, the repressive functions of Treg in Fingolimod therapy receiving RRMS patients were further upregulated, hence pointing to another part of Fingolimod in restoring peripheral tolerance, apart from the retention of T lymphocytes in Lymph Nodes [137,138].

Natalizumab is a disease manipulating drug which influences the movement of T cells was approved in 2004. It represents a monoclonal Ab (mAb) which targets VLA4 expressed by activated T as well as B lymphocytes [115] as well as inhibits VLA4 interaction with the vascular cellular adhesion molecule1 (VCAM1) expressed on endothelial cells [139], hence blocking T cells penetration of the BBB [91-94], especially the Th 1 like Th 17.1 subset [89]. In spite of the effectiveness of both Fingolimod as well as Natalizumab in peripheral setting apart of inflammatory T cells as well as avoiding their migration across BBB, respectively, aberrant inflammatory actions as well as recurrence along with clinical relapses have been seen following

cessation of Fingolimod [140], as well as Natalizumab therapy in case of MS patients [141]. Furthermore continued therapy with Natalizumab been correlated with the reactivation of latent John Cunningham virus, hence facilitating propagating multifocal leukoencephalopathy [142].

Two humanized mAb targeting T cells for RRMS got approved by food and drug administration (FDA) as well as EMA. Alemtuzumab, got approval in 2013, which targets CD52 which is expressed at great amounts on B as well as T cells [143], hence resulting in depletion of both CD4<sup>+</sup> T Cells as well as CD8<sup>+</sup> T cells by Ab dependent (ADCC) as well as complement dependent cytotoxicity (CDC). Daclizumab that got approval in 2016, represent a mAb that targets CD25, the high affinity subunit of IL-2 receptor (IL-2R), that gets expressed on activated T Cells as well as Treg [144]. In comparison to Alemtuzumab, Daclizumab does not bring about T cells by ADCC or CDC however, by neutralizing IL2 binding to CD25, as well as the high affinity IL-2R, like NK cells as well as resting T cells [145]. In view of high risk of serious as well as potentially fatal immune responses like encephalitis as well as meningomyelitis, in May 2018, the marketing authority of Daclizumab was stopped by EMA [146]. In November 2019 EMA further recommended a restrictive utilization of Alemtuzumab (Lemtrada) for RRMS patients with markedly active disease in spite of therapy with one of the disease manipulating therapy [147]. Intriguingly, Gingele et al. documented recently that ocrelizumab, a humanized mAb targeting CD20, that got approval in 2017 for relapsing MS as well as PPMS as well as believed to particularly targeting B [148], was further efficacious in removing a markedly activated CD20<sup>+</sup> T cell subset generating inflammatory cytokines [149]. As CD20<sup>+</sup> inflammatory T cells are located in the peripheral blood as well as brain of MS patients [149] their removal might explain the effectiveness of ocrelizumab therapy.

Besides the approved T cells targeting therapies, various therapeutic methods with the objective of inhibition of autoreactive inflammatory T cells are getting evaluated currently in various clinical trials of MS [150]. Of the lot, maximum effectiveness offered by mAb targeting Th17-associated cytokines, especially IL-17A that are undergoing trials for MS. Especially, Sekukinumab, a totally humanized anti IL-17A mAb with the objective of repressing pathogenic Th17, as well as Th 1 like Th 17 cell subset, got approval in 2005 as 1<sup>st</sup> line treatment for psoriasis [151]. The significant decrease (67%) of new magnetic resonance imaging (MRI) lesions seen in 73 treatment naive RRMS patients follow 24 wks of therapy with Sekukinumab [152] resulted in a placebo controlled randomized phase II clinical trial (NCT01874340). Nevertheless, this trial was ended by the sponsor on the basis of formation of another anti IL-17A mAb, Ixekizumab, having better action act Sekukinumab for

psoarthritis [153] as well as better potential for therapy of MS. Clinical trials are still ongoing.

Besides that trials are on regarding utilization of adipose derived stem cells in refractory MS (reviewed by us) [154].

The diagnosis of multiple sclerosis in children, like in adults, needs proof of dissemination of inflammatory activity in > than one region in the CNS (dissemination in space) and recurrent disease over time (dissemination in time). The identification of myelin oligodendrocyte glycoprotein antibodies (MOG-Ab) and aquaporin-4 antibodies (AQP4-Ab), and the consequent discovery of their pathogenic mechanisms, have led to a shift in the classification of relapsing demyelinating syndromes. This is reflected in the 2017 revised criteria for the diagnosis of multiple sclerosis, which emphasizes the exclusion of multiple sclerosis mimics and aims to enable earlier diagnosis and thus treatment initiation. The long-term efficacy of individual therapies initiated in children with multiple sclerosis is hard to evaluate, owing to the small numbers of patients who have the disease, the relatively high number of patients who switch therapy, and the need for long follow-up studies. Nevertheless, an improvement in prognosis with a globally reduced annual relapse rate in children with multiple sclerosis is now observed compared with the pretreatment era, indicating a possible long-term effect of therapies. Given the higher relapse rate in children compared with adults, and the impact MS has on cognition in the developing brain, there is a query whether rapid enhancement or potent agents should be used in children, while the short- and long-term safety profiles of these drugs are being established. With the results of the first randomized controlled trial (RCT) of fingolimod versus interferon- $\beta$ 1a in paediatric MS published in 2018 and several clinical trials underway, there is hope for further progress in the field of paediatric multiple sclerosis. Thus early and precise diagnosis of MS is crucial. The discovery of antibody-mediated demyelination has changed the diagnosis and management of relapsing demyelination syndromes. Traditional escalation therapy is being challenged by induction therapy [155].

## Conclusions

Over the past 2 decades our insight on the immunopathogenetic modes in MS has robustly escalated, hence observing a basic part of encephalitogenic Th cells, especially Th 1 like Th 17, Th 17, Th 22, as well as GM-CSF generating CD4<sup>+</sup> T Cells, in starting as well as continuation of the inflammatory responses as well as the subsequent neurodegeneration in MS. Hence, most of the treatment protocols that have received approval aim to ameliorate the inflammatory T helper cells as well as emerging T cell-based therapies having greater selectivity are the currently ongoing Clinical trials for MS. The differences in adult and paediatric MS has been touched along with slow escalation of drugs with

provisional damage to growing brain and subsequent influence in cognition.

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