International Journal of Drug Development & Research
|July-September 2012 | Vol. 4 | Issue 3 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
SJR Impact Value 0.03,& H index 2

Review Paper
Covered in Index Copernicus with IC Value 4.68 for 2010

Gene and Cytokines expression of Multiple Sclerosis and its Therapeutic Regimen: A Systemic Review

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Abstract

Multiple sclerosis (MS) is a polygenic disease driven by dysregulation of the immune system leading to an autoimmune response against antigens of cerebral white matter tissue. Experimental autoimmune encephalomyelitis (EAE) is a widely accepted model *in vivo* for the development of new therapy for the treatment of MS. There are different types of rodent EAE model and immunized with different epitopes for the analysis of gene expression and cytokines response in inflammation, demyelination and degeneration of neurons. The present review aimed to effect of gene and cytokines expression and therapeutic regimen in MS.

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Key words:
Multiple sclerosis, Experimental autoimmune encephalomyelitis, Gene expression, Cytokines

***How to Cite this Paper*:**
Parul Srivastava, Md. Ali Mujtaba*, Manmohan Singhal "Gene and Cytokines expression of Multiple Sclerosis and its Therapeutic Regimen: A Systemic Review", Int. J. Drug Dev. & Res., July-September 2012, 4(3): 55-66

Article History:----------
Date of Submission: 16-06-2012
Date of Acceptance: 27-06-2012
Conflict of Interest: NIL
Source of Support: NONE

1. Introduction

Multiple sclerosis (MS) is a debilitating neurological disease with unknown etiology that has a strong autoimmune component and affecting about two million people worldwide. The inflammatory phase of MS likely involves activation and migration of myelin-reactive T cells across the blood–brain barrier (BBB), resulting in damage to myelin and axons in the central nervous system (CNS) (Wang C *et al*; 2010)[1] and in MS clinical signs and symptoms are very variable and depend on the parts of the CNS it affects, that is, the brain and spinal cord, and include motor, sensory, autonomic and cognitive disabilities (Vosoughi R *et al*; 2010). [2]

$CD4^+$ T helper cells reactive to myelin, which produce proinflammatory cytokines, such as interferon-γ (IFN-γ), osteopontin (OPN) and tumour necrosis factor-α (TNF-α), are known to have a leading role. In the complex of Th1/Th2 paradigm, MS and EAE, several experiments support a role for Th 1 responses in promoting of diseases and Th 2 responses in suppressing of disease and the adoptive transfer of myelin-reactive Th 2 cells to immune-deficient mice to induce EAE and eosinophils regarded as Th 2-associated cells also contribute in disease development (Friese MA *et al*; 2003). [3]

Classification of Multiple Sclerosis

1. Relapsing–remitting (RRMS)
2. Secondary progressive (SPMS)
3. Primary progressive (PPMS)
4. Progressive relapsing (PRMS)

Relapsing–remitting (RRMS):

Almost 80% of MS patients suffered from RRMS disease course, It is the most common phenotype, starts with a single mono- or multi-focal demyelinating episode (clinically isolated syndrome, CIS) with partial or full recovery and about 80% of MS patients suffered from RRMS disease course. In this, similar or varied types of relapses occur in time after initial attack. It is dominated by overt inflammation, demyelination, clinical attacks and the formation of new lesions which appears on MRI; though sub-clinically damage to neurons and axons, gradually diminishing the ability to sustain further events without acquiring disability.

Secondary progressive (SPMS):

It involves slowly progressing phase with or without superimposed relapses (SPMS) and about 50% of patient developed SPMS after 10–15 years disease duration. At this stage of the disease inflammation appears less prominent, and instead the accumulated damage to neurons and axons starts to show itself clinically as neurological progression.

Primary progressive (PPMS):

It is recognized by its progressive course from the outset, with a paucity of inflammation detected in the CNS compared with RRMS and about approximately 15% of MS patients develops PPMS.

Progressive relapsing (PRMS):

It involves relapse-like activity (PRMS) developed over time in about 5% of patients and patients have clinical and radiological evidence of inflammation, but no proven effective treatment (Pedotti R *et al*; 2003). [4]

Common factor of MS

The cause of this disorder is not known, but there are several factors linked to higher risk of developing the disease:

1) Genetics may play an important role and several alleles have been identified as predisposing factors for the disease, most of them related to the human leukocyte antigen (HLA) system;
2) MS is more prevalent in women than in men, suggesting importance of hormones in its pathophysiology.
3) Psychological stress has been implicated in triggering exacerbations, and some meta-analyses suggest a positive relationship between stressful events and higher risk for relapse and increased risk of new gadolinium-enhancing (Gd^+) brain lesions (Perez-Nievas BG *et al*; 2010). [5]
4) Viral factor causes development or progression of MS, such as human herpes virus-6 (HHV-6) or Epstein Barr Virus (EBV). The bacterium Bordetella pertussis causes whopping cough in humans and produces pertussis toxin (PTx) as

its main virulence factor (Weber MS *et al*; 2010). [6]

Common symptom of MS

A) Visual disturbances, which include eye pain, distortion or loss of vision in one eye, or impairment of color perception.
B) Difficulty in walking or performing tasks that require coordination.
C) Loss of sensation.
D) Fatigue and/or weakness.
E) Loss of bowel or bladder control (Ringold S *et al*; 2006). [7]

Experimental autoimmune encephalomyelitis (EAE) is currently the most commonly used animal model for the study of MS. This model causes inflammation and demyelination that is similar to the MS and to simulate different disease progressions where clinical symptoms (monophasic fashion), either acute or chronic, or in a relapsing form (Peiris M *et al*; 2007). [8] It is an inducible model of $CD4^+$ T cell driven demyelination and axon damage. Myelin antigen reactive $CD4^+$ T cells dominate the CNS infiltrate and initiate disease upon adoptive transfer into naive animals (Bettini M *et al*; 2009). [9]

Cytokines have a key role in the EAE process providing the necessary signals to activate T cell specificity against self-antigens and suggest that the T cells mediating EAE are of the Th1 phenotype, producing IL-2, IFN-γ, and TNF-α is related to disease-promoting role, while TGF-β and IL-10 are related to the s recovery of EAE (Castro GM *et al*; 2004). [10]

In early phase of MS, autoreactive T cell crosses the blood brain barrier (BBB) and are estimated by local antigen presenting cell (APC). This results in release of cytokines such as IFN-γ, TNF-α and IL-17 which initiates and perpetuates an inflammatory response by activating microglia and recruiting macrophages and BBB is breached and allow for the influx of more T lymphocytes, additional immune cells and the deposition of humoral components. Subsequently, this process leads to myelin destruction, induction of oligodendrocyte death, axonal degeneration and eventually to the development of severe functional deficits (Wiist S et al; 2008). [11]

2. Various EAE model for MS

A large number of EAE model exist, each parameter reflecting pathologies observed in CNS of MS patients. With different strains of mice, rats or other mammals and immunizing with different epitopes (peptides, whole proteins or complex mixtures like spinal cord homogenate) to achieve a range of pathological and temporal outcomes (Jones MV *et al*; 2008). [12]

2.1. Different types of EAE

Importantly, by stepwise reduction of the complexity of the antigenic material from crude brain tissue and protein extracts through various central myelin protein such as

i. Myelin basic protein (MBP) such as MBP_{1-37}, MBP_{1-11}, MBP_{1-9}, MBP_{83-99}.
ii. Myelin oligodendrocyte glycoprotein (MOG) such as MOG_{55-75}.
iii. Proteolipid protein (PLP) such as $PLP_{139-151}$.
iv. Myelin-associated oligodendrocytic (MOG) basic protein and 2´,3´-cyclic nucleotide 3´-phosphodiesterase.

Some of other myelin constituents such as neurofascin NF 155, other are expressed on myelin and axons such as the neuronal membrane protein neurofascin NF 186, the neuronal cytoskeletal protein neurofilament-M and the astrocyte-typical Ca^{+2}binding protein S100β.

Neurofascin NF 186 caused axonal injury without enhancing inflammation and demyelination in MOG-EAE. In contrast to MOG-EAE, contactin-2/TAG-1-specific T cells induced inflammatory lesions prefentially in the cerebral cortex and the spinal cord white and gray matter.

Tabel 1: Putative protein and lipid auto-antigens in EAE and MS

Antigen	Result in EAE/MS
MBP	T and B cell response in EAE and MS
MOG	T and B cell response in EAE and MS
PLP	T and B cell response in EAE and MS
2´,3´-CNP	T and B cell response in EAE and MS
NF 155	Antibodies recognize the extracelluar domain in MS and cause axonal injury in EAE, but only in pre-existing demyelinated lesions
NF 186	Antibodies recognize the extracellular domain in MS, inhibit axonal conduction in a complement-dependent manner and cause axonal injury in EAE
Neurofilament-M	Neurofilament-M-specific T cells induced severe clinical EAE with confluent demyelination and massive axonal loss
Contactin-2/TAG-1	Contactin-2/TAG-1-specific T cells induce inflammatory lesion in the cortex and white and gray matter thereby opening locally the BBB and causing occasionally clinical EAE
S100β	Strong T cell response in EAE
Phosphatidylserine	Promotion of demyelination in marmoset EAE
Sulfatides	T and B cell response in EAE
Oxidized phosphatidylcholine	Strong antibody reactivity in MS brain and EAE spinal cord
Ganglioside GM1, sulfatide and galactosylceramide	Increased reactivity of pro-inflammatory cytokine secreting $CD8^+$T cells in MS patients
Gangliosides GM3 and GQ1b	Increased T cell response in primary progressive MS patients
Ganglioside GD1a	Increased antibodies in serum and cerebrospinal fluid of patients with MS and optic neuritis
Lactosylceramide and L-α-lysophosphatidylserine	Strong antibody reactivity in serum and cerebrospinal fluid of MS patients

Table 2: Comparison of immunological, clinical and therapeutic features of EAE and MS

	EAE	MS
Genetics	Susceptible and resistant strains and colonies, e.g. C57BL/10.S mice and different colonies of Lewis rat	Weak evidence of association, risk alleles: IL-2RA, IL-7RA and EV15
Pathology		
A) Inflammation	Dominant (CD^+ T cells and macrophages)	Rare (Type I/II, CD^{+4}/CD^{+8}T cells, CD^{20+} B cells, macrophages)
B) Demyelination	Rare (Anti-MOG-EAE)	Strong
C) Degeneration	Late (Murine EAE)	Early (type III/IV)
D) Cortical lesions	Rare (MOG-EAE in marmosets)	Rare
Clinical Course		
A) Acute	Frequent (Active EAE)	Rare (Marburg type)
B) Primary Chronic-progressive	Rare (MOG-EAE, AT-EAE)	Rare (<10%)
C) Relapsing-remitting	Rare ($PLP_{139-151}$-EAE, Pertussis toxin-EAE)	Frequent (>90%)

Timelines of milestones of the development of increasingly specific EAE models were:

i. Induction of EAE by transfer of total lymph node cells, isolated MBP-specific T cell-line cells or IL-23-dependent PLP-specific $CD4^+$T helper IL-17 cells into naïve rats or mice, respectively, establishing distinct forms of adaptive transfer EAE

ii. Generation of transgenic mice with deletion or over-expression of pathogenetically relevant genes. For examples genes are those encoding T cell receptors (TCRs), major histocompatibility complex (MHC) molecules, cytokines as well as neurotrophic factors and their receptors.

2.2. Induction of EAE models in different species

The most common mode of EAE induction is based on the injection of an encephalitogenic peptide, mostly MOG_{35-55} or $PLP_{139-151,}$ which is emulsified in CFA containing mineral oil and *Mycobacterium tuberculosis* strains H37RA, followed by intraperitoneal injections of pertussis toxin. The resulting phenotype depends mainly on the antigen source and the genetic background of the animal species and the strains used. For example, $PLP_{139-151}$ induces a relapsing-remitting EAE in SJL mice, whereas MOG_{35-55} triggers chronic-progressive EAE in C57BL/6 mice. Crossing of C57BL/6 mice, which over-express MOG-TCR and MOG specific B cells, resulted in a severe form of EAE with inflammatory lesions of optic nerves and spinal cord. MOG-TCR transgenic mice backcrossed to SJL/J background develop a relapsing-remitting form of EAE with episodes altering between optic nerve, cerebellum and spinal cord. All EAE models are directly accessible to investigation of the immune and nervous system, which interact during the pathogenesis of the disease and which are both targeted by established and experimental therapies (Mix E *et al*; 2010). [13]

Various form of EAE has been induced depending on the immunizing neuroantigen and the redent strain used.

Lewis rat EAE showed a monophasic and self-remitting neuroinflammation in the CNS. Swiss-Jackson Laboratories (SJL) mice and Dark Agouti (DA) rats EAE showed a relapsing and remitting course of the disease.

MOG induced EAE in the C57BL/6 mice showed a chronic progressive course and other EAE model induced in NOD and DBA/1 mice showed a chronic progressive and severe course of secondary progressive/primary progressive form of MS (Donia M *et al*; 2010). [14]

Table 3: Commonly used rodent EAE models (Gold R *et al*; 2006) [15]

Model	Similarities to human disease	Differences from human disease	Further comments
Lewis rat Active EAE (CNS myelin, MBP, MOG, PLP)	T-cell inflammation and weak antibody response	Monophasic, little demyelination	Reliable model, commonly used for therapy studies. With guinea-pig MBP little Demyelination
Adoptive-transfer EAE (MBP, S-100, MOG, GFAP)	Marked T-cell inflammation. Topography of lesions	Monophasic, little demyelination	Homogeneous course, rapid onset. Differential recruitment of T cells or macrophages depending on autoantigen
Active EAE or AT-EAE co-transfer of anti-MOG antibodies	T-cell inflammation and demyelination	Only transient demyelination	Basic evidence for role of antibodies in demyelination
Congenic Lewis, DA, BN strains Active EAE (recombinant MOG (1-125)	Relapsing–remitting disorders, may completely mimic histopathology of multiple sclerosis and subtypes	No spontaneous disease	Chronic disease course, affection of the optic nerve, also axonal damage similar to multiple sclerosis
Murine EAE (SJL, C57BL/6, PL/J, Biozzi ABH) Active EAE (MBP, MOG, PLP and peptides)	Relapsing–remitting (SJL, Biozzi) and chronic-progressive (C57BL/6) disease courses with demyelination and axonal damage	No spontaneous disease	Pertussis toxin required for many strains, whilst it is often not needed for SJL and some Biozzi EAE models. Higher variability of disease incidence and course, often cytotoxic demyelination in C57BL/6. With rat MBP inflammatory vasculitis with little demyelination
Murine EAE in transgenic mice or knockout mice (mostly C57BL/6 background)	Specifically addresses role of defined immune molecules neurotrophic or Cytokines or neuroanatomical Tracts	Most results obtained with artificial permanent transgenic or knockouts	Extensive backcrossing (>10 times) on C57BL/6 background required. Future work with conditional (cre/loxP) or inducible (e.g. Tet-on) mutants

2.3. Behavioural analysis of animals after EAE induction

2.3.1. EAE scoring (Clinical scoring)

Immunized animals are observe daily by measuring their body weight, feed weight and assessing clinical signs of EAE, starting at 7 days post immunization.

Table 4: EAE scoring system of immunized animals

0	No deficit
0.5	Partial loss of tail tone or slightly abnormal gait
1	Complete tail paralysis or both partial loss of tail tone and mild hind limb weakness
1.5	Complete tail paralysis and mild hind limb weakness
2	Tail paralysis with moderate hind limb weakness
2.5	No weight bearing on hind limbs but with some leg movement
3	Complete hind limb paralysis with no residual movement
3.5	Hind limb paralysis with mild weakness in forelimbs
4	Complete quadriplegia but with some movement of head
4.5	Moribund
5	Dead

2.3.2. Grip strength test

Strength of forelimb and hindlimb are tested for grip strength by using Grip Strength Meter. In Grip strength, grip power of hindlimb primarily and majorly affected and forelimb less affected in EAE induced animals.

2.3.3. Rotarod performance

Rotarod test is used to assess motor co-ordination and balance of animals. Each mouse is placed on stationary rod and start the rotation of rod at 4 rpm and accelerated 4 rpm every 30s. The time and speed at which the mouse falls down from rod is recorded in EAE induced animals.

2.3.4. Open field activity test

Open field activity test is determined by using Open Field Photobeam Activity System. It is used to assess total activity and rearing activity of animals. Mice are placed into the clear plastic chamber for the assessment of interruption of infrared beams. In EAE induced mice, the total activity and rearing activity both are decreased consistently (Jones MV *et al*; 2008). [12]

3. Gene Expression Profile of EAE model

Microarray analysis technique is usually used for expression of gene in MS and EAE. Oligonucleotide microarray represents 11000 genes (6000 full-length gene and 5000 expressed sequence tags) which are expressed in inflamed spinal cord of EAE mice at the time of onset and the peak of disease determined by the clinical evaluation of the disease. The oligonucleotide microarray data are generally validated by the use of quantitative RT-PCR method to analyse a subsets of gene either down-regulated or up-regulated. The microarray technique enables to identify differentially express gene with a high degree of validity. Increased expression of genes for extracellular matrix, cell adhesion molecules and molecules involved in cell division, death and transcription, differential regulation of molecule involved in signal transduction, protein synthesis and cell metabolism in inflammation.

Genes are group according to their presumed biological functions and to the fold change in gene expression using normal spinal cord as a baseline (Ibrahim SM *et al*; 2001). [16]

4. Neural dysfunction in Spinal Cord during EAE

The protein levels of plasma membrane Ca^{2+} ATPase 2 (PMCA2), an essential ion pump expressed exclusively in grey matter and involved in Ca^{2+} extrusion, synapsin IIa and syntaxin 1B, important regulators of vesicular exocytosis decreased coincident with the onset of clinical symptoms and changes in the expression of several other ion pumps, vesicular proteins, mitochondrial enzymes and sodium channels also occurred in disease. Glutamate and Kainic acid significantly reduced PMCA2 mRNA levels. In EAE pathogenesis, suppresses neuronal PMCA2 expression leading to Ca^{2+} dyshomeostasis at initial clinical phases.

Table 5: EAE related changes in gene expression in spinal cord

Genes	Changes from Day 16 (onset) to Day 22 (peak)
Antigen processing and presentation	
Ia-associated invariant chain, I-E (β-b), I-A (β), H2, Ia, MHC class II region, MECL1, HAM1, LMP2, α-D-Mannosidase (MAN2B1), H2-IA (α-d and α-q haplotype), Ma, MHC Class II, H2-TL-T10-129, β-Glucuronidase	Increased
Qu-Tia, LMP-7, Q8-9d, PA28, H2-D(k), H2-M(ab), H2-M3	Decreased
Immune related	
Mast cell high-affinity IgE receptor, MAMA, Endothelial monocyte- activating polypeptide I, C1q C-chain, IL-1rn antagonist chain, TNF-2 α receptor, Interferon-induced 15 kDa protein, Interferon-inducible protein 1-8D, C10 like chemokines (SCYA9), B- cell translocation gene-1 protein, c-fms proto-oncogene, Fc-γ RI, Immune-responsive gene 1 (IRG1), Macrosialin, Immunoglobulin κ, FC-γ (RIIB), FGF-binding protein 2, NMC-4κ chain, Ig light chain, Anaphylatoxin C3a receptor,	Increased
Phasphatidylinositol-linked antigen (pB7=CD52), Secretory protein YM-1, T-cell-specific protein, Factor B, 24p3-protein, β-interferon (type 1), RANTES (SCYA5), Macrophages interferon-inducible protein IP-10, IgG1/IgG2b Fc-receptor, γ interferon-induced Mg11, T-cell receptor β chain, p6-5, GMCSF receptor low affinity subunit, 65κDa macrophages cytosolic protein, PDGF-inducible protein, C1q B chain, C3 component, Lymphocyte-specific transcript, Monokine induced by γ-interferon, Interluekin-3 receptor (CSE2R-β2), Common cytokine receptor γ chain, FK506-binding protein 25 homologue, Fractalcine	Decreased
Extracellular matrix, cell adhesion and Matrix degradation	
Galectin 3, Fibrolectin	Increased
Intergin β 7 subunit, β Ig-h3, Mastocytoma proteogylcan core protein serglycin, CD53, CD18 β subunit (LFA1), Cytohesin binding protein, Ly-6C.2, Ly-6C variant, Ly-6.2, Ly-6A.2, Membrane-type matrix metalloproteinase 1	Decreased
Signal transduction	
Protein tyrosine phosphatase, Calcium binding protein, GDP dissociation inhibitor, Parvalbumin, Protein tyrosine kinase (JAK3), Vasodilator-stimulated phosphoprotein (VASP), PNG protein	Increased
GBP-2, II GP protein, I GTP, IRG47, Ion channel homologue RIC, EN-7, SOCS3, G protein β 36 subunit, Keratinocyte growth factor, flk2, GTP binding protein, cAMP-dependent protin kinase C β subunit, Calcineurin catalytic subunit, A-raf proto-oncogene, Phosphatidylinositol transfer protein α (PITPN)	Decreased
Transcription	
Replication-dependent histone H2A.1, Histone H2A.X, Eucaryotic intiation factor eIF-2B β subunit, Transcription factor, Ribonuclease inhibitor, Interferon regulatory factor 5, Signal transducer and activator of transcription (STAT1)	Increased
Putative purine nucleotide binding protein, Cysteine-rich intestinal protein, Interferon consensus sequence binding protein, Interferon regulatory factor 1 and 7, Poly (A) binding protein, Ring finger protein	Decreased
Protein synthesis and Cell metabolism	
Arginase, 5-LOX activating protein, Glutathione peroxidise-related protein 1, Argininosuccinate synthetase, Ribosomal phosphoprotein P2, Heat shock protein 25, Protein-tyrosine sulfotransferase, Ribosomal protein S6 kinase polypeptide 1, Plasma glutathione peroxidise,	Increased
Nitric oxide synthase, Ribosomal protein S26, UDP-galactose transporter related isozyme 1, Carboxypeptidase E, Sterol carrier protein X, Pyruvate dehydrogenase, Calcium-transporting ATPase sarcoplasmic reticulum type slow Heat shock protein 86, Arginine-tRNA protein transferase 1, Muscle creatine kinase	Decreased
Cell structure, movement and secretion	
Coronin-like protein, SM-22-α homologue, Actin and cytoplasmic 1, Erythroid ankyrin, Tropoelastin, α-actinin 4, Profilin, γ-Actin, Capping protein, Moesin	Increased
Endothelial actin-binding protein, β-Adaptin, Skeletal α-chain, Chromogranin B	Decreased
CNS related	
Aminopeptidase P, Brain neurotensin receptor, Preprosomatostatin, Synaptosomal-assosiated protein 25, Glycine receptor β subunit	Increased
Glutamate decarboxylase, X11 protein, Glutamate dehydrogenase, Brevican, Lissencephaly 1 protein, Neuroendocrine protein 7B2, Quaking type 1, PLP, Neural visinin-like Ca^{+2}-binding protein type 1	Decreased
Cell division and death	
Spi2 proteinase inhibitor, Cathepsin H prepropeptide, Tumour-induced 32κDa protein, Transglutaminase, Heamatopoietic-specific protein 1, Cathespin B-like protease, Annexin V, Lactate dehydrogenase-A	Increased
MPS 1, Preprodipeptidyl, peptidase I, Cystatin B, Cathespin S precursor, CTLA-2α, Bcl-w, KAP3A	Decreased
Miscellaneous	
Major vault protein, Acrogranin, Retinoic acid-inducible E3 protein, Renin-binding protein, Chloride channel (CLCN4), Round spermatid protein 29	Increased
Apolipoprotein C2, Ferritin L subunit, Membrane protein TMS-1, Lymphocyte antigen 86, Breast-cancer associated gene 1 protein (BCG1), GLVR1, Intracisternal A particle (IAPIL3)	Decreased

4.1. Expression of Glutamate-mediated changes in neuronal dysfunction in EAE symptoms

Intracellular Ca^{2+} level are often increases often and causes cell dysfunction, injury and death in CNS, including Multiple sclerosis, Ischemia, Alzheimer and Parkinson disease. In EAE, loss of voltage-dependent channel-mediated Ca^{+2} balances depicts defect in mechanism that regulate the Ca^{+2} entry, extrusion or intracellular sequestration. PMCAs are major pumps modulating Ca^{2+} extrusion and sarcoplasmic and endoplasmic reticulum Ca^{2+}-activated (SERCA) ATPase isoforms causes sequestration of Ca^{2+} into the endoplasmic reticulum. PMCA2 expression decreases in early phase, which is followed by a reduction in SERCA2 transcript levels clinical phases. The PMCA gene family encodes four isoforms, causes maintenance of basal Ca^{2+} levels. PMCA2 is expressed primarily in brain and heart, in a region- and cell-type specific manner and in pathological condition of EAE, glutamate receptors are over stimulated and overload of intracellular Ca^{2+} level.

4.2. Role of Aberrant neurotransmitter exocytosis in EAE

The level of Synapsin and Syntaxins are decreases in EAE. Synapsin and Syntaxins are vesicle protein and exhibit important role for exocytosis in presynaptic terminals in clinical symptom of EAE. Synapsins are required for the tethering of vesicles to each other and to cytoskeletal proteins (actin). It is also essential for the formation, maintenance and regulation of the reserve synaptic pool in the vicinity of the active zone and the decreased level of synapsin causes reduced number of synaptic vesicles distal to the active zone, exhibiting synaptic fatigue. Syntaxins belongs to SNAREs class (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) and essential for the docking and fusion of synaptic vesicles. Vesicle-associated SNAREs (synaptobrevin) and target membrane-associated SNAREs (syntaxins and SNAP-25) induces membrane fusion. The level of syntaxin 1B and SNAP-25 are reduced but reduction in syntaxin 1B occurred at onset of symptoms whereas SNAP-25 at a later clinical stage. The expression of synapsin and SNARE proteins interfere with normal neuronal communication during EAE.

4.3. Role of Mitochondria in cellular injury at EAE

Mitochondrial function plays important role in cellular injury due to the increased level of mitochondrial cytochrome c oxidase subunits IV and Vb. Cytochrome C oxidase used as a marker for neuronal metabolic activity, especially in oxidative stress. Elevated level of cytochrome c oxidase express in Alzheimer's disease, following traumatic brain injury and preceding apoptotic death of spinal cord motor neurons after sciatic nerve avulsion and also reflect aberrant energy metabolism and oxidative damage in the spinal cord during EAE.

4.4. Role of sodium channel in impulse conduction during EAE

Na^+ channel also played an important role in EAE and MS. The expression of Na^+ channels decreases that cause impairment of nerve conduction at advanced stages of EAE. Expression of sensory neuron-specific sodium channels in Purkinje cells changes during EAE symptoms (Nicot A *et al*; 2003). [17]

5. Role of Vitamin D

Vitamin D play important role in the immune system particular on T cell-medicated immunity. Receptor of Vitamin D is found on the surface of T lymphocyte and macrophage cells and highest concentration is found in the immature immune cells of the thymus and the mature CD^{+8} T lymphocytes (Deluca HF *et al*; 2001). [18]

5.1. Animal model of Autoimmunity

In autoimmune disease, T cells attacks on self tissue. T cells are thymus-derived cells and subsets of T cells called the T-helper (Th) cells that express CD^{+4} markers on their surface and also played an important role in EAE and diabetic model to mice. In these models, level of CD^{+4} T cells are decreased. EAE is mediated by CD^{+4} T specially Th1 type (IL-2, TNF-α, IFN-γ) cells that recognize protein in CNS but suppress the Th2 type (IL-4, IL-10) cells.

5.2. Vitamin D and Immune system

Vitamin D regulated Ca^{+2} homeostasis and causes bone formation and resorption. The active form of vitamin D ($1,25\text{-}(OH)_2D_3$) affected on the immune system. In EAE, level of vitamin D is decreases and upregulated the Th1 cells by increasing proliferation and cytokine (IL-2, TNF-α, IFN-γ) secretion. Vitamin D receptors are found in the nuclease of cells and bound to the ($1,25\text{-}(OH)_2D_3$) and regulated the transcription of targeted genes. Vitamin D act as transcriptional regulator of Th cell cytokine synthesis as a regulator of STAT 1, 4 or 6, as a regulator of GATA-3 or regulate Th cells differentiation (Cantorna MT; 2000). [19]

6. Cytokines signaling pathways in EAE and Multiple sclerosis

$CD4^+$ T helper (Th) cells play a critical role in MS and EAE (Experimental Autoimmune encephalomyelitis

is an animal model for MS). The major types of Th cell are type 1 helper T (Th 1) cells that produce interleukin (IL-2), tumor necrosis factor-α (TNF-α), IFN-γ and type 2 helper T (Th 2) cells that produce IL-4, IL-5, IL-10 and IL-13 and type 3 helper T (Th 3) cells that primarily secrete transforming growth factor-β (TGF-β).

Intracellular signaling mechanisms provide the link between the bindings of the cytokine with its receptor and the effect of the cytokine on cellular function. The Janus kinase and signal transducer and activator of transcription (Jas/STAT) family of transducer/transcription-activating factors play a critical role in the signaling of many cytokine receptors. Cytokine binding to the specific receptors activates the Jas molecule associated with the receptor causing phosphorylation of tyrosine residues. This facilitates binding of STAT proteins to the phosphorylated receptor, which dissociates from the receptor and activates transcription of genes containing specific *cis*-regulatory STAT-binding sequences.

Role of individual cytokines during demyelinating process in MS and EAE

Cytokines play an important role in the pathogenesis of MS because of altered level of cytokines in the CNS and peripheral mononuclear cells of MS patients.

6.1. Interferon-γ (IFN-γ)

It is produced by T cells and Natural killer cells and causes activation of mononuclear cells, differentiation of T cells to Th1 type, induction of MHC I and MHC II expression and apoyptosis of T and other cells types. IFN-γ is expressed in the CNS at the onset of EAE and its expression increases during the peak of disease and decreases during disease remission. Over-expression of IFN-γ in the CNS causes progressive demyelinating disease. INF-γ has an activating role in inflammation in EAE and MS.

6.2. Tumor Necrosis Factor-α (TNF-α)

It is produced by activated mononuclear phagocytic cells, natural killer cells, B cell, activated T cells, astrocytes and microglia in the CNS. TNF-α express Th1 type responses and induced activation of variety of cell types and expression of adhesion molecules, chemokines and cytokine. Role of TNF-α is characterized by significantly increases inflammation and demyelination in EAE and MS. TNF-α has a direct effect on the induction of oligodendrocyte apoptosis and demyelination and also promoted the proliferation and induced cell death of oligodendrocyte progenitor cells.

6.3. Interleukin-12 (IL-2)

IL-2 is produced by monocytes and the dendritic cells and express Th1 type responses. Over-expression of IL-12 in the CNS resulted in increased inflammation and cellular infiltrates.

6.4. Osteopontin (OPN)

Osteopontin is a pleotrophic cytokine that activates macrophage chemotaxis, promotes Th1 responses and activate B-cells. OPN increases plaque formation in the brain of MS patients.

6.5. Interleukin-18 (IL-18)

IL-18 is synthesized as an inactive precursor protein and its level increases in the serum of MS patients.

6.6. Interleukin-1 (IL-1)

It is produced by monocytes, macrophages, endothelial cells, B cells and activated T cells and upregulated in the CNS and causes increased neuronal cell death and edema. Neuronal apoptosis and neurotoxic effect appear due to expression of iNOS.

6.7. Interleukin-3 (IL-3)

IL-3 is a cytokine growth factor produced by CD^{+4} T cells and microglia. IL-3 mRNA is up-regulated in the CNS of MS patients. Systemic over-expression of IL-3 in the CNS causes degeneration and vacuolation of neurons.

6.8. Interleukin-6 (IL-6)

IL-6 is produced by mononuclear phagocytes, vascular endothelial cells and fibroblast and

promoted Th1 cytokines. It is important for the growth and differentiation of B-cell and also up-regulated in the CNS during the induction phases of disease.

6.9. Interleukin-4 (IL-4)

IL-4 is produced by CD^{+4} Th2 cells and involved in differentiation and growth of B-cells. IL-4 inhibits the activation of Th1 cells and decreases the production of IL-1 and TNF-α. IL-4 amplifies the Th2 response through activation of the intracellular signaling factor STAT6, which induces transcription of Th2 related genes.

6.10. Interleukin-10 (IL-10)

IL-10 is produced by monocytes, macrophages, B cells and Th2 cells. It inhibits the production of IL-1 and TNF-α cytokines by macrophages and also reduces MHC II and co-stimulatory molecules expression.

6.11. Transforming growth factor (TGF)

The TGF family of molecules has 2 principal members that is TGF-α and TGF-β. TGF-α is a polypeptide growth factor for epithelial and mesenchymal cells and TGF-β is a growth factor for cell survival. TGF-β is produced by T cells by T cell, activated monocytes, astrocytes and microglia. It inhibits proliferation of T cells, maturation of cytotoxic lymphocytes, natural killer cell and also inhibits the activation of macrophages and activates the pro-inflammatory cytokines (Imitola J *et al*; 2005). [20]

7. Therapies for EAE and MS

The goal of MS is to produce a period of stabilization of symptoms and interrupt the progress of disease. There is no current treatment that can alter neurological damage (Hadaway LC; 1985) [21] but only very few therapeutics that are successful in pre-clinical EAE trials have shown similar efficacy in MS patients. The majority of new treatments are either less effective in MS patients and worsened disease or caused severe adverse events (Friese MA *et al*; 2006). [3]

Table 6: Some immunomodulatory approaches of multiple sclerosis and their development from EAE or *in vitro* studies to clinical application

Treatment approach	Efficacy in EAE	Efficacy in MS	Status of development
Glatiramer acetate	Yes	Yes	Approved
Altered peptide ligand	Yes	No	No
Oral myelin	Yes	No	Not continued after phase III due to lack of efficacy
Anti-α_4 integrin	Yes	Yes	Approved
Anti-CD40L	Yes	No	No
Anti-CD4	Yes	Less efficacy	Halted in phase II
Anti-CD52	Not applicable	Yes, Insufficient data	Approved for other indication
Anti-CD25	Less efficacy	Yes, Insufficient data	Approved for other indication
CTLA-4-Ig	Yes	Not known	In phase III
IFN-β	Yes	Yes	Approved
IFN-γ	Yes	No	Stopped in phase I
Anti-TNF antibodies	Yes	No	Approved for other medication
TNFR-Ig fusion protein	Yes	No	Approved for other medication
TGF-β2	Yes	No	Stopped in phase I
IL-10	Less efficacy	Insufficient data	Stopped in phase I
IGF-1	Yes	Insufficient data	Phase IIa, not continued
PDE4 inhibitors	Yes	Insufficient data	Halted in phase II
PPARγ agonists	Yes	Not tested	Not yet tested in multiple sclerosis
Statins	Yes	Yes but insufficient data	Approved for other medication
Mitoxantrone	Yes	Yes	Approved
Linomide	Yes	Yes	Stopped in Phase III due to cardiotoxicity
Laquinimod	Yes	Yes but insufficient data	In phase II
FTY720/SP-1 agonist	Yes	Yes but insufficient data	In phase II
Deoxyspergualin	Yes	No	Stopped after phase II due to lack of efficacy
Sulphasalazine	Less efficacy	Less efficacy	In phase III
IVIG	Less efficacy	Less efficacy	In phase II
Haematopoietic stem cell transplant	Yes	Yes but insufficient data	In phase II

Conclusion

At present, the exact cause of MS is still unknown in pre-clinical EAE studies, which involve in multiple immune cell types, target cells and pathways but conventional EAE play an important role as first line animal model system for the development of novel treatment for MS. Gene expression and cytokines levels play an important role in MS. Oligodendrocyte microassay is a technique which are used for detection of gene expression contributing to earlier stage of diseases. It represents 11000 genes (6000 full-length gene and 5000 expressed sequence tags) in inflamed spinal cord of EAE induced animals, 213 genes are regulated differentially and 100 genes are showed consistent differential regulation throughout the disease. Gene expressions are upregulated in inflammation because increased expression of immune related molecules, extracellular matrix, cell adhesion molecules and those molecules which are involved in cell division and transcription. Alteration in cytokine balance also involved in autoimmune disease pathogenesis. Th2 cytokines (IL-4, IL-5, IL-10 and IL-13) appears to play a protective role in the survival and differentiation of neurons and oligodendrocyte in the CNS during demyelination injury and Th1 cytokines (IL-2, TNF-α AND IFN-γ) play a degenerative role in MS patient by the activation of mononuclear cells, differentiation of T cells, B cell class switching and apoptosis of T and other cell types. The future of multiple sclerosis therapy looks bright, but increasingly complex. There are number of agents who are used for the treatment of MS through orally and parenterally and some are in different stages of investigation or awaiting approval by federal agencies. All of these medications have demonstrated partial efficacy along with different side effect profiles.

Neurodegeneration occurs through non-inflammatory mechanisms but depend upon prior inflammation. In these case, anti-inflammatory therapies are best prevent the progression of disability in early course of the disease, before the cascade of events that leads to axon degeneration has been irretrievably established. This raises the difficult prospect of exposing well non-disabled young adults to potentially toxic treatments; a decision made all the more complex by the unpredictable nature of multiple sclerosis. Finally, these drugs continue to inform basic science, revealing aspects of the pathogenesis of multiple sclerosis and reminding us of the complexity of the human immune system.

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