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Fibrinogen and Immune Inflammatory Indices in Prediction of Activity of Multiple Sclerosis

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Abstract

It's well known that changes in the extra cellular micro-environment is essential for development of neuro inflammatory diseases, such as MS. Variable evidences are supporting this notion, as the breakdown of blood-brain-barrier (BBB) usually precedes MRI visible lesion and clinical symptoms of MS. Thus, recent efforts have been made to further examine the consequences of severe extravasation of inflammatory markers into CNS, as a result of a leaky BBB.

Keywords: Inflammatory Biomarkers, Multiple Sclerosis, MS.

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1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease affecting the central nervous system (CNS). More than 2.5 million people worldwide are having MS. The onset of MS usually occurs between 20 and 40 years of age and considered the most common cause of neurologic disability in young adults, affecting women more than men by twice [1]. Some aspects of its etiology and pathogenesis are still unclear, however it is generally accepted that MS is an inflammatory disease with autoimmune characteristics influenced by environmental or infectious factors in genetically susceptible individuals. These factors are supposed to interplay to varying extent, contributing to the heterogenesity of MS [2]. Multiple sclerosis is characterized by focal infiltration of lymphocytes into CNS leading to myelin destruction and axonal damage, which leads to neurologic syndromes and physical disability. The clinical manifestations of MS depend on the location of lesions in the CNS. Symptoms may include sensory, visual dysfunction, motor, coordination disorder, spasticity, fatigue, pain, emotional manifestations and cognitive deficits [3]. Anatomo-pathological studies have shown early glial activation in the pre-demyelinating stage of MS lesions and Blood Brain Barrier (BBB) disruption with extravasation of fibrinogen in normal appearing white matter regions [4,5].

Fibrinogen is a 340 kilodalton glycoprotein synthesized by the hepato- cytes. Its primary function is controlling platelet aggregation leading to blood clot formation and thus maintaining hemostasis**.** Plasma fibrinogen increases during inflammatory response, which causes cell aggregation and increase both blood viscosity and endothelin-1 [4-5]

Neutrophils, platelets and lymphocytes constitute the first line of defense mechanism within the body against foreign invaders. Neutrophils, platelets and lymphocytes are the first inflammatory and regulatory markers, respectively, found in injured areas. They activate major cell types involved in acute and chronic inflammation [6].

Fibrinogen

Fibrinogen was initially discovered by Paul Morawitz in 1905. Fibrinogen also called factor I is a 340 kDa glycoprotein produced by hepatocytes and circulates in the blood, The liver is the primary source of plasma fibrinogen, with a steady state rate of synthesis of 1.7–5 g per day and a large intracellular reserve [6]. The normal circulating fibrinogen level is 3 g/L which increases to 4.5 g/L during pregnancy and its levels can exceed 7 mg/ml during inflammatory responses, and its half-life is about 3-4 days .Fibrinogen is not present in the healthy brain owing to an intact BBB. Fibrinogen is a pleiotropic protein with essential roles in coagulation and inflammation [7]. The main function of fibrinogen is controlling of blood homeostasis during vascular injury and tissue damage as the major protein component of blood clots, fibrin binds to activated platelets and acts as a molecular bridge to enable platelet aggregation and haemostasis [8-9].

Fibrinogen is unique among plasma proteins owing to its molecular structure, which contains binding sites for a number of both integrin and non-integrin receptors expressed on a wide variety of cells of the hematopoietic, immune,

nervous systems and for proteins that regulate key nervous system functions [10].

The Blood Brain Barrier is a key component of the neurovascular unit, the dynamic structural and functional unit of the CNS composed of highly regulated interactions between vascular cells, glial cells and neurons. With an intact BBB, endothelial cells are connected by tight-junction and adherens-junction proteins and contain specialized transport systems to regulate paracellular and transcellular movements of molecules and fluid between the brain and blood [11].

Role of fibrinogen in Multiple Sclerosis pahogenesis:

Breakdown of the BBB is a classic hallmark of white matter lesions. MRI has been used clinically for decades as the gold standard for identifying BBB disruption in active MS lesions by visualizing the leakage of intravenously administered contrast agents such as gadolinium. MRI studies show that BBB breakdown in MS patients is an early event in active lesion formation and actually occurs before the onset of clinical symptoms [12]. Fibrinogen deposition is a prominent feature of MS pathology and is present throughout the course of the disease. The pattern of fibrinogen deposition in MS coincides with the areas occupied by demyelinating lesions, and with the areas characterized by axonal damage. Fibrinogen is found not only in active lesions but also in chronic plaques, where it persists despite a lack of contrast enhancement on standard MRI .Fibrinogen is also deposited in the cortex in patients with progressive MS and correlates with neuronal loss [13-14]. Interestingly, fibrinogen was detected not only in the parenchyma but also intracellularly as also documented in prior studies [15-16]. Dysregulation of the coagulation and fibrinolytic pathways likely contributes to the excessive and prolonged deposition of fibrinogen observed in MS [17]. Of note, fibrinogen deposition and microglial activation in MS lesions precede T cell infiltration, suggesting that fibrinogen serves as a critical molecular signal in MS pathology and plays a role in the initiation and/or progression of inflammation in MS [15].

Fibrinogen has pleiotropic effects and plays a critical role in the pathogenesis of MS by induction of inflammatory process and neuroinflammation. Deposition of fibrinogen in the CNS precedes neuroinflammation in MS [17-18]. In vivo study using photon microscopy demonstrated that the clustering of microglia in the perivascular space induced by fibrinogen is developed before the progression of demyelination in MS, the deposition of fibrinogen in the perivascular space activates microglia and releases pro-inflammatory cytokines causing BBB dysfunction and the progression of MS [19]. Ghorbani and Yong et al. observed in their study that the extracellular matrix acts as a possible modifier of remyelination and neuroinflammation [20]. Fibrinogen deposition in MS lesions correlates with early lesions and areas of demyelination and, importantly, is found in close association with inflammation and damaged axons. Fibrinogen is not merely the indicator of BBB injury in MS but acts as transduction increases microglia activation via triggering expression of αvβ3 integrin on CD11b/CD18 [21].

Interestingly, brain fibrinogen impairs the remyelination process, Many different cellular targets and new molecular mechanisms by which fibrinogen inhibits regeneration and repair mechanisms after CNS injury have been reported [22]. Fibrinogen has been reported to be an important exogenous factor that inhibits neuronal regeneration and remyelination by regulating growth factor receptor signaling and inflammatory responses after CNS injury, disruption of the BBB and fibrinogen deposition in the CNS affects the ability of damaged tissues to regenerate. Indeed, Fibrinogen could inhibit microglia and macrophage polarization, and the differentiation of oligodendrocyte precursor cells. which promotes inflammation, and inhibits regeneration [19].

Lymphocytes:

T cells are a major component of the adaptive immune system. During maturation in the thymus, each T cell expresses a specific T-cell receptor (TCR) that arises by random recombination of gene segments enabling expression of a large repertoire of different TCR specificities [24]. During their thymic maturation, early TCR^+ T cells expressing both CD4 and CD8 major histocompatibility complex (MHC) binding coreceptors are positively selected if they express TCRs that recognize self-MHC proteins resulting in the cell becoming single positive for CD4 or CD8, depending on whether they are restricted to MHC class II or MHC class I, respectively [25].

Thymic maturation also result in the peripheral release of a small number of self-reactive T cells. One self-reactive T-cell population is the unique population of CD4 $FoxP3^+$ regulatory T cells (Tregs**) [26]**. These self-reactive $FoxP3^+$ Tregs play a fundamental role in maintaining immune homeostasis and inhibiting autoimmunity, as they suppress the activation of other immune cell types [27]. In contrast to these regulatory cells, there are infrequent nonregulatory T cells that can recognize self-antigens and can be found in the peripheral pool of T cells, even in healthy individuals [28]. These potentially autoreactive T cells are believed to only recognize the self-antigens, These cells escape suppression causing autoimmune reactions [29]. The entry of T-lymphocytes into the parenchyma of the central nervous system is a critical early feature in the pathogenesis of many experimental and spontaneously occurring immune-mediated illnesses [30]. When T-lymphoblasts are introduced into the circulation they rapidly appear in the CNS tissue. Their concentration in the CNS reaches a peak between 9 and 12 hr, and lymphocytes which have entered, exit within 1 to 2 days [31]. Cells capable of reacting with a CNS antigen remain in the tissue or cyclically reenter to initiate inflammation if they are able to recognize their antigen in the correct MHC context [30].

Neutrophils:

Neutrophils are bone-marrow-derived cells that represent the most abundant peripheral blood leucocyte and have a half-life of hours to days. They are able to provide a potential source of autoantigens, triggering autoimmune reactions [32]. Neutrophils are a key population of granulocytes that are frequently involved in the initiation of an inflammatory response. Their characteristic multi-lobular nucleus, as well as the presence of granules arranged into organelles within their cytoplasm, explains their commonly used name polymorphonuclear leukocytes (PMN) [33].

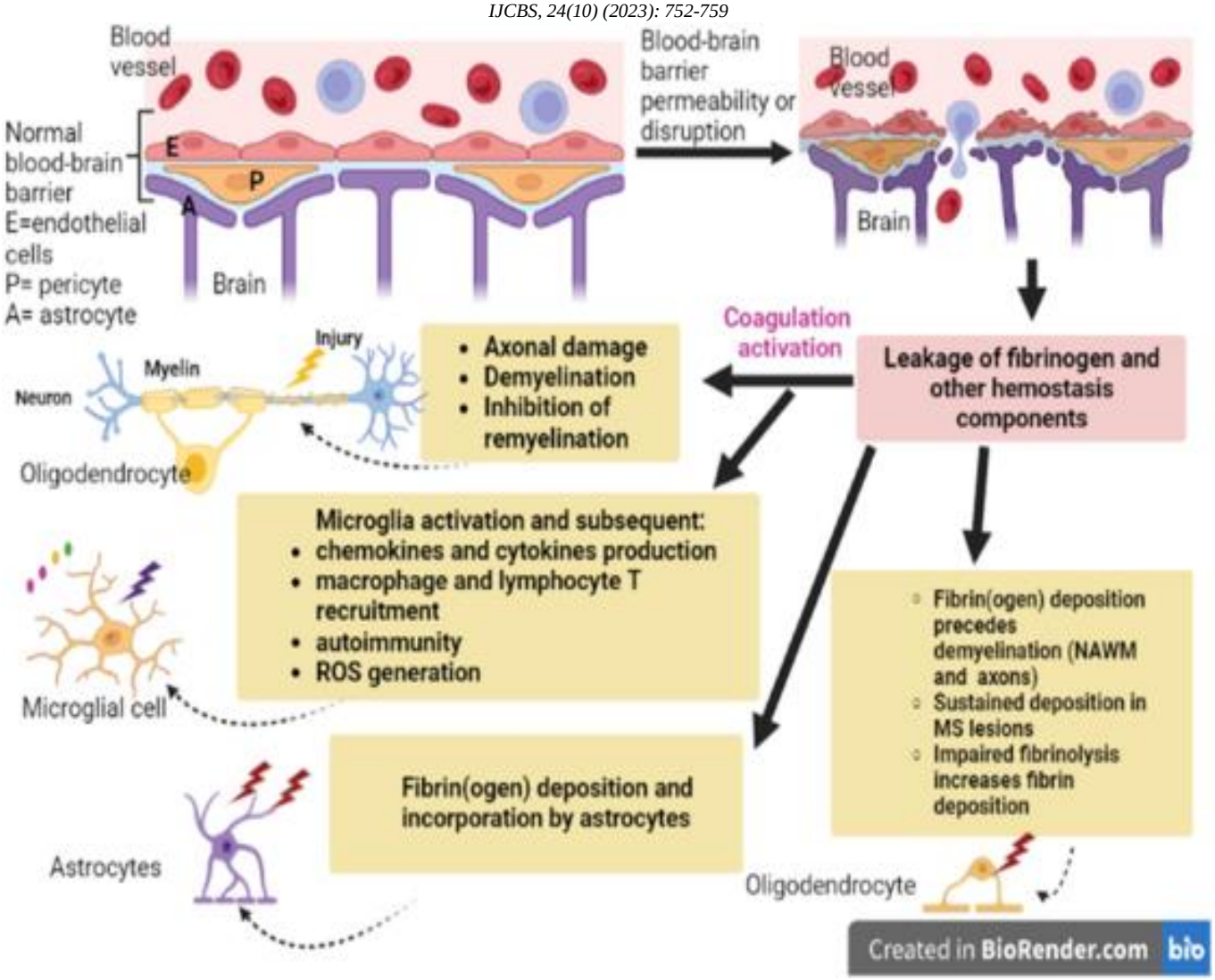

Figure (1): Role of fibrinogen in MS: disruption of the blood–brain barrier (BBB) enhances leakage of fibrinogen from blood vessels. Deposited fibrinogen in the multiple sclerotic lesions leads to the activation of microglia and the release of pro-inflammatory cytokines. As well, fibrinogen triggers demyelination and inhibits remyelination [23].

Upon infection or injury, neutrophils begin a process of migration via chemotaxis, where they move along a chemical gradient in response to inflammatory signals. Once neutrophils have entered the target site and been activated, they may carry out various effector functions to neutralize invading pathogens. The effector functions of neutrophils include phagocytosis, degranulation, and release of reactive oxygen species (ROS) [34]**.** Neutrophils also assists the interaction between the innate and adaptive branches of the immune system, as they could activate antigen presenting cells and then promot the differentiation of the inflammatory T helper 17 (Th17) cell population [35-36]. Of note, their signature cytokine interleukin (IL)-17 is a key mediator of neutrophil recruitment. Many studies identify a relevant contribution of neutrophils to neuroinflammatory processes in MS and showed their role in blood–brain and blood–spinal cord barrier disruption [37-38].

Platelets:

Platelets are anuclear cells circulating in blood, They are derived by budding of megakaryocytes in the bone marrow and are unique in terms of their abundance (with a normal range of 150–400 × 109/L in humans), and rapid turnover (with a lifespan of 8–9 days). Historically associated with a role in hemostasis and more recently, with vascular inflammatory disorders and cancer [39]. Platelets express immunologically relevant ligands and receptors, that have adhesive interactions with endothelial cells, monocytes and neutrophils, and toll-like receptor (TLR) mediated responses. These properties make platelets central to innate and adaptive immunity and potential candidate key mediators of autoimmune disorders [40].

Under the neuroinflammatory conditions caused by the disturbance of BBB, blood platelets quickly adhere to endothelium at the sites of vascular injury and became activated [41]. Multiple interactions between activated blood platelets and inflammatory cells in the endothelium contribute to neurovascular inflammation [42]. It is believed that endothelial and platelet dysfunction contribute significantly to the development of neurodegeneration and inflammation in MS patients [43].

C-reactive protein:

C-Reactive Protein is a multifunctional component of the innate immune system, which is primarily synthesized in the liver and can be reliably assessed in a routine blood test [44]. While it is well known that CRP levels increase as a result of infection, inflammation, and tissue damage, CRP is also increased in people with high body mass index (BMI) and smokers. CRP serves as a biomarker of disease activity in other immune-mediated disorders such as MS, rheumatoid arthritis and Crohn's disease [45].

Role of Inflammatory Biomarkers in Multiple Sclerosis pathogenesis:

Neuroinflammation:

It has been reported by different studies that neuroinflammation relates to the progression of diverse neurodegenerative disorders. Lymphocytes in the CNS activate inflammatory disorders and the progress of neuroinflammation [46-47].

Lymphocytes and Multiple Sclerosis pathogenesis:

The characteristic active demyelinating lesions of the brain and spinal cord in patients with MS are associated with inflammation around blood vessels and brain parenchyma cell infiltration, which is composed of T and B cells [48]. In MS patients, T cells are activated in the peripheral, and then they penetrate the CNS, trigger a central immune response, and further self-maintenance, leading to the myelin sheath and axon damage [49]. T cells in MS lesions express cytotoxic effector phenotypes, mainly CD8+ effector memory T cells (TEM), indicating local antigen stimulation [50]. Like T cells, B cells also have proinflammatory and anti-inflammatory subsets. In relapsing MS, T cells are the pathogenic cells, while B cells are the main antigen-presenting cells [51]. On the other hand, in progressive MS, B cells can enhance the conditioned response of the CNS through lymphoid follicles and secretory factors. The intrathecal synthesis of immunoglobulin reflects the clonal expansion of B lymphocytes and plasma cells [52]. The interaction between B cells and T cells is the central feature of MS pathogenesis [53]. CD8+ T cells and CD20+ B cells dominate in the pathogenesis of all disease stages in MS. CD8+ T cells recognize the endogenous antigenic peptides presented by MHC class I and differentiate into cytotoxic T cells after activation [54]. In active MS lesions, the activation of astrocytes, oligodendrocytes, and axons gradually upregulated the expression of MHC class I, making these cells potential targets for CD8+ T cells in the disease course [55]. Next to T cells, B cell contributes to adaptive immune inflammation of MS patients [56]. This can be confirmed by the discovery of clonally amplified B cells in cerebrospinal fluid, meninges, and brain parenchyma of MS patients. B cells can pass through the BBB and form ectopic germinal centers in the CNS. This has coincided with the observation of immunoglobulin synthesis in the CNS of MS patients [55].

Though T cells are widely considered to be major contributors to inflammatory demyelination in MS, growing evidence suggests a significant role for B cells in disease pathogenesis [57]. Both antibody-dependent and independent mechanisms are thought to underlie B-cell mediated central nervous system (CNS) injury in MS. In addition to antibody secretion by plasmablasts and plasma cells, B-cell functions implicated in pathogenesis include (i) antigen presentation to T cells and driving autoproliferation of brain-homing T cells (presumably by memory B cells), (ii) production of pro-inflammatory cytokines and chemokines that propagate inflammation, (iii) production of soluble toxic factors contributing to oligodendrocyte and neuronal injury, (iv) contribution to the formation of ectopic lymphoid aggregates in the meninges, and (v) providing a reservoir for Epstein-Barr (EBV) virus infection [56]. These B cell actions may contribute to both MS relapses and disease progression [58]. The importance of B cells in MS is underscored through clinical trials revealing that anti-CD20 monoclonal antibodies are highly effective in limiting new relapsing disease activity [59]. Of note, this therapy does not directly target plasma cells, nor does it appear to significantly impact the abnormal cerebrospinal fluid (CSF) antibody profile [60]. Peripheral B cells of MS patients exhibit aberrant pro-inflammatory cytokine responses, including exaggerated lymphotoxin-α, tumour necrosis factor (TNF)-alpha, interleukin (IL)-6 and granulocyte macrophage-colony stimulating factor (GM-CSF) secretion [61].

Cytokine production, costimulation, and antigen presentation may contribute to the development of pathogenic B and T cells entering the CNS [62]. This mechanism may be affected by the interaction between genetic and environmental risk factors. A number of identified genetic risk locis, including HLA-DRB1#1501, seem to enhance the B and Th cells [63]. In addition, infectious factors may change the function and reactivity of MS such as EBV as mentioned previously [64].

However, the lymphocyte count in the peripheral blood of MS patients may not be significantly increased [65]. High T cells counts are markers of the disease process and damage activity. The higher the disease activity is, the more these cells are in the tissue. However, there are regional differences in the distribution of these cells. In the late stage of progressive MS, inflammation composed of T and B cells may drop to the level of the age-matched control group [66]. Some studies have also shown that MS patients may have decreased lymphocytes in peripheral blood before treatment [67]. A part of the reason may be the high migration rate of lymphocytes to the CNS, which leads to the increase in the NLR ratio [68]. There is also a theory that psychological stress caused by nervous system disorders in MS patients may change the balance between innate immunity and adaptive immunity, resulting in an increase in NLR [65].

Neutrophils and Multiple Sclerosis pathogenesis:

Previous studies suggested that neutrophils are simple phagocytes of the innate immune system, but the current view is that neutrophils are important effectors and regulatory circuits that control the quantity and quality of immune response [38]. So far, the role of neutrophils in the pathogenesis of neuroinflammation has become more and more attractive [69]. Neutrophil infiltration in the CNS of MS patients may be an early trigger factor of inflammation-causing BBB injury [34]. Neutrophils can secrete a series of cytokines that can influence MS and EAE. These cytokines, such as TNF-α, IL-6, IL-12, IL-1β, and IFN-γ, are considered to have contribution to the cascade of inflammation in the CNS [38]. The concentrations of neutrophil-activated chemokines and neutrophil-derived enzymes in the blood of MS patients were higher than those in control, and these molecules were related to the formation of new inflammatory lesions. These chemokines included CXC chemokine ligand-1 (CXCL1),

CXCL8, myeloperoxidase (MPO), cytokines ROS and matrix metalloproteinase 9(MMP9).

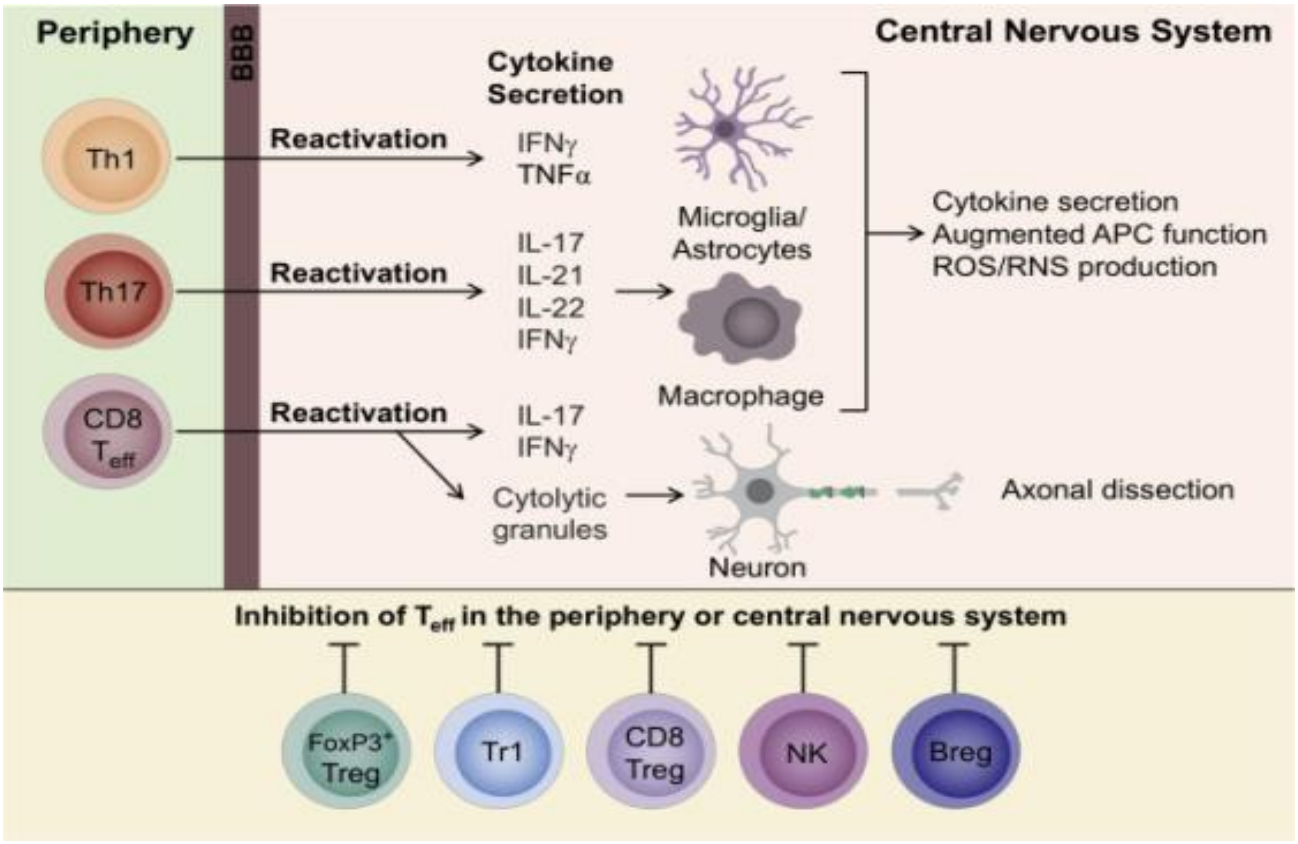

Figure (2): Effector T Cells in Multiple Sclerosis Peripherally activated T cells cross the blood-brain barrier (BBB) into the central nervous system (CNS), where they are re-activated and secrete cytokines to exert their effector functions. T helper (Th)-1 cells produce their lineage-defining cytokine, IFNg, as well as TNFa, while Th17 cells secrete their defining cytokine IL-17, as well as IL-21 and IL-22, and can also express IFNg, which contributes to their pathogenicity. CD8+ effector T cells (Teffs) can also be a source of IL-17 and IFNg. This cytokine secretion leads to the activation of CNS-resident immune cells (such as microglia, astrocytes, and macrophages), as well as to the production of cytokines, increased antigen-presenting cell (APC) function, and the enhanced production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). CD8 T cells can also release cytolytic granules causing axonal dissection. Effector T cells can be regulated in the periphery or in the CNS by FoxP3+ regulatory T cells (Tregs) and by Tr1 cells, CD8 Tregs, natural killer cells (NK cells), and regulatory B (Breg) cells [62].

In addition, in progressive MS patients, BBB leakage is related to the increased abundance and activity of MMP9 in serum and cerebrospinal fluid (CSF). Furthermore, larger numbers of neutrophil extracellular traps (NETs) were found in the blood of some MS patients, supporting the role of neutrophils in MS pathogenesis [70].

Platelets and Multiple Sclerosis pathogenesis:

Historically, there has long been a postulate of a role for platelets in MS [55]. Such evidence first emerged with reports by Putman et al. suggesting a role for venule thrombosis in CNS demyelination [71]. Subsequently, multiple studies demonstrated increased platelet adhesiveness in MS, relative to other neurological disorders [72]. Platelets cross the BBB, become closely associated with production of pro-inflammatory factors to neural cells via lipid rafts [73]. This results in activation of inflammatory cascade which are independent of lymphocytic infiltration [41].

Concurrently, platelets drive the generation of autoreactive T cells in the peripheral circulation [74]. This presumably occurs by degranulation of platelets immediately upon BBB disturbance, resulting in the release of multiple soluble factors serotonin (5HT), Platelet Factor 4 and Platelet Activating Factor, which specifically stimulate differentiation of T cells toward pathogenic Th1, Th17, and IFN-γ/IL-17-producing CD4 T cells [75]. Platelets are also the main source of IL-1α which affect brain endothelium and enhance entry of immune cells into the CNS causing cerebrovascular inflammation [76]. Under inflammatory conditions, platelets bind to other platelets (aggregate) and multiple immune cell types. The interplay between platelets, endothelial cells and leukocytes is the direct cause of BBB damage [77].

Platelet activation results within seconds in promoting endothelial cell permeability, together with recruitment and attachment, on the endothelium, of several classes of leukocytes, including neutrophils, monocytes, dendritic cells and B and T lymphocytes. Monocytes and neutrophils are the

major leukocytes that form complexes with platelets. Platelet-neutrophil interactions also directly modulate dendritic cell maturation leading to dendritic cell antigen presentation to T cells [78]. CD40L expression on platelets also enhance CD8+ T cell responses, as well as isotype switching in B cells from IgM to IgG [79]

Finally, interactions between platelets and their cellular partners are bi-directional, resulting in amplification and expansion of the inflammatory response [80]. Thus, platelets have evolved a range of mechanisms resulting in an extensive functional repertoire enabling signaling to multiple immune cell subsets [81]. These mechanisms underlie the evolution of innate to adaptive immunity, thereby placing platelets in a central position for the development of inflammation in MS disease [82].

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