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Multiple sclerosis: Enigmatic factors and new controversies

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Introduction

Multiple sclerosis (MS) is a chronic, inflammatory disorder of the central nervous system (CNS). It is consider an autoimmune disease which is triggered by an environmental agent in a genetically susceptible person and to be principally mediated by thymus-derived lymphocytes (T cells) [1].

Multiples factors had been proposed since the first definition of this disease. With a concordance rate of only 25–30% in monozygotic twins, however, it is clear that nongenetic factors must also influence risk of MS as well. Migration studies have also tended to support an environmental role [2-5]. Rapid and apparent changes in risk over a single generation strongly suggest an environmental factor or factors in MS etiology. Because it seems very probable that MS etiology is going to involve multiple disease-susceptibility genes and multiple environmental events (with a long latency period between exposure and symptom onset), elucidation of the different factors that determine MS is likely to be difficult. Nevertheless, certain possible factors are suggested by the epidemiology of MS. For example, the apparent latitude effect might suggests that sunlight exposure plays a role [6] and, in fact, the presence of vitamin D receptors on activated T cells and the ability of vitamin metabolites to inhibit interleukin-2 (IL-2) suggest a potential mechanism whereby sunlight might exert an effect [7]. Also, MS affects women twice as often as men, suggesting sex hormones as potential factors in MS etiology. Other potential causal agents include a variety of infectious organisms or vaccinations, although, again, no consistent association has yet been found to support these.

Pathology

MS is a disease that damages the myelin and causes axonal loss in the CNS. Early in the course of an attack there is perivascular inflammation, dominated by T cells and macrophages containing intracytoplasmic granules of myelin debris. Subsequently, astrocytosis and glial scarring occur. These pathologic processes are generally most conspicuous in the CNS white matter. Nevertheless, cortical and spinal cord gray-matter involvement can also be seen, although usually with less lymphocytic infiltration than in white-matter plaques [8]. Recent work suggests that MS is pathologically heterogeneous and that, perhaps, some cases of MS are due to primary oligodendrocyte pathology rather than to a chronic inflammatory process. For example, one study described four distinct patterns of demyelination, two of which were highly suggestive of a primary oligodendrocyte injury [9]. It is even possible that oligodendrocyte apoptosis is the initial event in the formation of an MS plaque.

One noteworthy aspect of the MS success story is that, despite progress, we still do not have a coherent model of pathogenesis. We do not know the primary trigger or triggers, the specificity of the culprit pathogenic immune cells, or the mechanism underlying progressive disability in longstanding disease. MS is likely to deliver 1 or 2 more big surprises before the final story is fully played out, and a final understanding may well prove to be simpler than suggested by our current complex models of pathogenesis [10].

Conclusion #1:" *we still do not have a coherent model of pathogenesis, but it is clear that the pathogenesis of the MS is multifactorial*".

Genetics

With the advancement in technology and research methods, the genetic associations with Multiple Sclerosis have gone further beyond the longstanding human leukocyte antigen (HLA) association in MS which was first identified 40 years ago and as of now there are more than 50 non- HLA genetic risk factors [11,12].

Sawcer *et al.* [13] noted that the initial attempts to point out the susceptible genes in MS were successful by pin pointing the relevance of Major Histocompatibility Complex (MHC) on chromosome 6p21 to multiple sclerosis. In the early 1970s, investigators were able to show association of MS with particular HLA genes (HLA-A3, HLA-B7, and DR2), however it was realized that the association of MS with these genes was not independent but a reflection of Linkage Disequilibrium [13].

Genome-wide association studies (GWAS) have contributed greatly to the understanding of MS pathogenesis through the identification of close to 110 non-MHC associations [14], these interact within related canonical gene-gene interaction pathways. MS-associated variants appear to influence the function of the adoptive and innate immune system rather than the nervous system, but probably this is because our incomplete knowledge about the CNS role in many genes initially identified as having some immune function [14].

New HLA related genes, like IL-7 receptor, was also found to be a gene with susceptibility to MS, IL-7 receptor αγc heterodimer is responsible for mediating IL-7 signaling, which is key in mediating the differentiation and survival of T cell and B cell [15-17]. Few allelic variants responsible for MS may be involved in various other autoimmune conditions, pointing towards a common underlying factor, for instance, IL2RA mediated susceptibility effects are not only seen in multiple sclerosis but also in other autoimmune conditions like graves diseases, type 1 diabetes mellitus and rheumatoid arthritis. Analysis of epidemiologic data confirms genetic variation as a key

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determinant of susceptibility to multiple sclerosis and these variations may play an important role in the timing of onset of symptom, course of the disease and response to treatment.

Familial recurrence risk studies show an increased association of developing the condition with a positive family history [18]. Studies in twins show a higher risk of multiple sclerosis among monozygotic twins when compared to dizygotic twins [19,20]. Another key fact to note is the correlation of multiple sclerosis prevalence in monozygotic twins and latitude. Variations in MZ concordance by latitude is influenced by environmental and genetic factors, supporting multifactorial risk factors for multiple sclerosis [21]. With these observation of inheritance pattern, the familial recurrence rate nor the twin concordance supports a Mendelian inheritance pattern. Instead that the MS-prone genotype results from multiple interacting polymorphic genes [22]. Strong correlation between HLA and phenotype in patients with MS has been observed and despite clinical heterogenecity between Primary Progressive MS (PPMS) and Relapsing Remitting (RR)/ Secondary Progressive MS (SPMS), studies suggest shared HLA patho-aetiology [23]. HLA genetic risk burden explains almost 15% of MS risk [24], in contrast, greater genetic link has been observed in twin studies, with tetrachoric correlation of 71% in monozygotic and 46% in dizygotic pairs [25]. Exerting strongest effect on risk are class II alleles: DQA1*01:01-DRB1*15:01 and DQB1*03:01-DQB1*03:02 [24,26] and odds ratios of * 3 and * 1.26 have been found for and DRB1*03:01, respectively [27]; whereas HLA A*02 and HLA B*44 independently reduce susceptibility to MS, but only HLA B*44 has shown to influence disease [28]. In addition, DRB1*15:01is thought to be linked with earlier disease onset and is more frequently found in females [24,29].

Conclusion # 2: "*HLA genes, non HLA genes like IL7, allelic variations, and a positive family history all play an important role in the pathogenesis of MS*".

Environmental

The major environmental factor is often attributed to a latitudinal gradient, with the prevalence of the condition being more common in temperate areas compared to tropical areas [18]. One possible explanation for this distribution could be due to the migration pattern of Northern Europeans, with the parts of the world having higher prevalence where Northern Europeans settled [30]. Studies suggest that the risk changes with migration, when considering individuals migrating from regions of higher prevalence to regions of lower prevalence and vice versa [31]. However, while taking into account the global distribution of multiple sclerosis one can point out that there are exceptions to the rule of latitudinal gradient, as some populations have been seen to have higher rates of multiple sclerosis compared to their geographic neighbors [32]. Around 15–20% of the affected individuals living in temperate regions, report to have a family history of multiple sclerosis with a significantly higher rate than the expected prevalence in these regions.

With a concordance rate of only 25–30% in monozygotic twins, however, it is clear that non genetic factors must also influence risk of MS as well. Migration studies have also tended to support such an environmental role [2,4,5]. Such apparently rapid changes in risk over a single generation strongly suggest an environmental factor or factors in MS etiology. Because it seems very probable that MS etiology is going to involve multiple disease-susceptibility genes and multiple environmental events (with a long latency period between exposure and symptom onset), elucidation of the different factors that determine MS is likely to be difficult. Nevertheless, certain possible factors are suggested by the epidemiology of MS. For example, the apparent latitude effect might suggest that sunlight exposure plays a role [6] and, in fact, the presence of vitamin D receptors on activated T cells and the ability of vitamin metabolites to inhibit interleukin-2 (IL-2) suggest a potential mechanism whereby sunlight might exert an effect [7]. Also, MS affects women twice as often as men, suggesting sex hormones as potential factors in MS etiology. Other potential causal agents include a variety of infectious organisms or vaccinations, although, again, no consistent association has yet been found to support these.

In addition, other environmental factors, such as Vitamin D deficiency, EBV, smoking, Western diet, and commensal microbes, might contribute as risk factors into the development of multiple sclerosis. Furthermore they may epigenetically interact with genetic risk loci associated [33,34]. Different risk factors such as geographic location, month of birth as well as individual and maternal UV exposition during pregnancy ultimately determine increased disease risk as well. Precise mechanism of action of vitamin D in modulating disease onset and course remains to be determined. Vitamin D receptor binding can potentially modify HLADR15 haplotype gene expression [34], which is considered as the main genetic risk factor [35].

Obesity and smoke exposure have been related to presence of HLA-DR15 and absence of the protective HLA-A02 allele expression [34,36,37]. Yadav et al made an accurate gathering of evidence sustaining environmental risk factors. They show how smoking duration, intensity, passive exposure, and prior history each show independent correlations with multiple sclerosis risk; in addition, being an active smoker associates with higher disease activity, severity, and quicker progression. More established risk factor is Western diet and specially fat and animal food intake and in contrast, decreased risk for CNS demyelination has been observed with higher intake of fish omega-3 [38,39]. Chronic low-grade inflammation observed in obesity is likely to affect immune response since pro-inflammatory cytokines and reactive oxygen species cause persistent tissue damage, as well as longer duration of leukocytes at the site of inflammation. However, given high disparity in prevalence and cohort studies relating MS and obesity in adult individuals, Palavra et al hypothesize that obesity contributes a higher risk factor early in life, whereas in adulthood this may be neutralized. Female childhood obesity has been observed to increase risk for MS later in life, whereas male studies show little evidence, although research in a similar direction [34,40-42,]. Evidence suggest that high carbohydrate and fat rich diet influences intestinal permeability, indirectly affecting microbiota derived autoimmunity. Western diet also has been seen to shift the structure of the microbiota in mice, altering the microbiome's gene expression and molecular pathways [46-48].

The study of Germ Free mice have led to relate digestive flora to several aspect of human life, such as behavior, brain development, sleep architecture or immune system [43,46]. Particularly in MS, gut microbiota implication has been studied from different perspectives. Recently Held et al related innate mucosal-associated invariant (MAIT) cells to MS pathogenesis, because these cells development ultimately depends upon microbial flora. Additionally the observation that a small fraction of CD8+ T cells in early MS lesions consist of MAIT or MAIT-related cells, led authors to theorize that these cells seem to be crucial in the "gut-brain axis" with MS [47]. These cells are innate T cells lymphocytes restricted to major histocompatibility complex (MHC) class I B, most of which are CD8+, which are frequently found in gut mucosa, liver and in low proportion in blood and lungs. They have been also related to the pathogenesis of other diseases such as

inflammatory bowel disease or hepatitis C virus infection. However, its role in general immunity and particularly in MS must still be clarified [48]. Although MS microbiota signature remains unclear, the influence of microbiota molecular mimicry has been postulated to also be an explanation for autoimmunity and MS [44].

Disease suppression was observed when altering the composition of gut microbiota in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Investigators deduced that it was actual innate natural killer T (iNKT) cells depletion the causative agent, as they were involved in maintaining reduction of mesenteric Th17 cells, given high implication of Th17 cells in MS pathogenesis [49] and disease improvement observed after induction of the innate natural killer T cells. Thus, these authors propose that gut flora may influence the development of EAE and in extension that of MS, in a way that altered microbiota relates to increased iNKT cells and iTh17 cells [50]. This is also supported by the fact that when Western diet rich in fat and sugar and low in Vitamins B and D was administered to animal models, increased gut CD4+ Th17 and modification in floral structure was observed [51]. Lastly, several authors have proposed that increased intestinal permeability (IP) may allow the passage of macromolecules, toxins, and bacterial species, and triggering of autoimmunity [45,52]. In this line, Buscarinu et al observed significant difference in IP in patients with MS, 73% versus 28% in healthy controls.

Despite lacking well controlled clinical studies evaluating disease impact of salt in MS, sustained pathological evidences suggest high implication in both pathogenesis and progression of disease. Reboldi et al observed Interleukin 17-producing T helper cells (TH-17 cells) to be the first to cross brain blood barrier. Under salt-enriched conditions, T cells exhibit significantly increased potential to differentiate into Th17 cells in vitro [49,53,54]. Other several studies have shown how sodium increase reactivity of endothelial cells to TNFα, NF-kB and reactive oxygen species, by overexpressing adhesions molecules and facilitating migration into brain tissue [53]. In addition, high sodium conditions also promote monocytes, macrophages and CNS-resident microglial cells activation which play an important role in the establishment and perpetuation of inflammation within the CNS. Refer to Huche et al., 2016 for detailed information [53].

EBV is another widely recognized environmental risk factor of multiple sclerosis. Actually, large prospective double matched case control study, found a total absence of incident MS among individuals without detectable serum antibodies to EBV with a mean interval between primary EBV infection and MS onset of 5.6 years, however given study limitations and confounding factors recognized by authors, only increased risk of MS onset in seropositive EBV can be stated. Moreover, an enhanced association between smoking, high anti-EBNA titer and increased multiple sclerosis risk has been observed as well. This association might sustain in shared EBV activation and nicotine metabolism including pathways Jun-c-kinase, MAPK, PKC, and NF-κB. In addition, although less consistent, increased CDT8-cells have been reported in heavy smoking [55].

Conclusion #3: *"Latitudinal gradient can be attributed as the most important environmental factor but attention should also be given to other factors such as migration, positive family history and sunlight exposure. As women are twice as likely to be affected by MS compared to men, the role of sex hormones cannot be neglected. Other possible environmental factors that could play a role in pathogenesis of MS would be Vitamin D deficiency, EBV infection, smoking and obesity. Western diet, which is rich in fat and sugar, can alter the gut flora and promote the pathogenesis of MS. Lastly, increased intestinal permeability and maternal UV exposition to UV are other possible environmental risk factors that could be associated with MS."*

Demyelination

Marked brain atrophy and spinal cervical and thoracic cord atrophy with dilation of the cerebroventricular and outer cerebrospinal spaces is typically observed in MS patients. In addition, patients with progressive forms of MS have increasing CNS atrophy in the spinal cord, cerebellum, and cerebral cortex [56]. It is accepted that axonal loss, demyelination and iron deposition would result in subcortical grey matter shrinkage of great proportions and general atrophy [57]. It is theorized that blood flow insufficiency in MS patients would explain the above mentioned findings. Gorucu et al. observed higher volume of caudocranial and craniocaudal CSF flow volumes and stroke volume in MS patients with respect to controls [58].

Luchinetti et all defined different patterns of demyelination, where patterns I and II showed close similarities to T-cell-mediated or T-cell plus antibody-mediated autoimmune encephalomyelitis, respectively and where in patterns III and IV were highly suggestive of a primary oligodendrocyte dystrophy or apoptosis, reminiscent of virus - or toxin-induced demyelination rather than autoimmunity; the most common observed pattern is type II which has a typical architecture involving perivenular inflammation and hypoxia-like tissue injury type III [9]. Type III is also thought to be a distinctive early demyelination pattern, characterized partly by preferential loss of myelin-associated glycoprotein, and expression of hypoxia-related antigens, including the prominent nuclear expression of hypoxia-inducible factor-1a (HIF-1a) [59]. The lesions vary in pattern of myelin loss and mechanism of tissue injury, but the pattern of demyelination is uniform within each patient, which suggest several distinct mechanisms in early MS.

In classical white matter lesions axonopathy, gliosis, demyelination and microglial activation are hallmarks in pathology. Low-grade T cell infiltration, axonal injury and vascular leakage due to BBB disruption even in normal appearing white matter (NAWM) have been reported as well [60]. These changes are found to be significantly less pronounced in patients with acute and relapsing multiple sclerosis than in progressive patients [8,61,62]. Initial antigen-specific cytotoxic T cells and autoantibodies directed against neuronal and glial antigens would cause axonal and neuronal injury and oligodendrocytes death in consequence activated macrophages and microglia would lead to tissue destruction. Outside-In model or Wallerian degeneration, usually induced by anti-myelin autoimmune cells generated in the periphery, support this pattern of primary CNS demyelination [63].

Grey matter demyelination can be found in neocortex, thalamus, basal ganglia, hypothalamus, hippocampus, cerebellum, and spinal cord. This pathology would correlate with progression of disease and degrees of disability [64]. Major patterns of inflammation are observed in focal white matter and active lesions, whereas gray matter lesions, results from extension of axonal injury and microglia activation from white matter [65,66]. However, grey matter primary lesions may also occur. Bo et al identified four types of cortical lesions. Type 1 lesions extend across both white matter and grey matter, this lesions would not extend to the surface of the brain and its center may be situated in white matter, type 2 are lesions within the cerebral cortex that do not extend to the surface of the brain or to the subcortical white matter. Type 3 or subpial, the most common, with characteristic expansion of long ribbons of subpial demyelination through adjacent gyri; other type 3 lesions 'wedge-shaped" would have its base over the brain surface, a

combination of these two patterns is frequently observed. Lastly, type 4 lesions extend through the full width of the cerebral cortex without reaching subcortical white matter [66].

In progressive MS preexisting lesions are seen to be expanded throughout normal appearing white and grey matter showing microglia activation causing axonal injury. These findings are almost exclusive to SPMS and PPMS and rare or absent in patients with acute or relapsing disease. Grothe et al. supported this fact, by finding significantly more gray matter affected in SPSM than in RRSM patients [67]. This pattern is related to Inside-Out model which suggests high amount of antigen released from primary cytodegeneration from axon's oligodendrocytes (inside) to myelin (outside). This would promote autoimmunity and inflammatory response, which may lead to further degeneration. This model would explain early neurodegeneration, including gray matter involvement and axonal degeneration in NAWM [68]. High heterogeneity with multifocal lesions is observed when studying plaques in multiple sclerosis. Plaques may be related to different stages of the disease, however in a same patient different type of plaques may be found. In contrast to this disparity, meningeal inflammation was present in all the disease stages, affiliated with a breakdown of the blood–brain barrier (BBB). The BBB may be relatively persistent since vascular changes can be present without concurrent inflammatory cells, and, peripheral cytokines and leukocytes present in older, inactive lesions. Although BBB disruption usually occurs in the development of a new lesion, evidence suggests that it might also arise following neurodegeneration in MS [60,69,70].

However, as mentioned earlier, the ultimate cause initiating the disease remains ambiguous. Postmortem brain tissue of NAWM showed "microglia nodules" with intact BBB not containing leukocyte infiltration, astroglyosis or demyelination but containing clusters of pre active microglia lesions and macrophages that would eventually respond to autoreactive T cells and systemic pro-inflammatory cytokines [71]. With this unusual relation the purpose of activated microglia as an initial pathogenic event in MS must be considered [63,72].

Conclusion # 4: "*Four patterns of demyelination have been discussed, Type I and II are believed to be T cell mediated with a closer association to auto immune demyelination, Type III and IV patterns are seen due to primary oligodendrocyte dystrophy and are reminiscent of virus/toxin induced demyelination rather than autoimmune demyelination, T2 was the most commonly observed demyelination pattern. Gray matter demyelination would correlate with progression of disease and degree of disability. Apart from the demyelination patterns, types of cortical lesions have been described as well, which would include, T1- extending across the white and gray matter, T2- limited with in the cerebral cortex, T3- subpial demyelination extending through adjacent gyri and T4- extending through the full width of the cerebral cortex. T3 lesions were the most commonly observed lesions*".

Immunopathology

MS is thought to be an autoimmune disease which is triggered by an environmental agent in a genetically susceptible person and to be principally mediated by thymus-derived lymphocytes (T cells) [1]. MS is a disease that damages the myelin and causes axonal loss in the CNS. Early in the course of an attack there is perivascular inflammation, dominated by T cells and macrophages containing intracytoplasmic granules of myelin debris. Subsequently, astrocytosis and glial scarring occur. These pathologic processes are generally most conspicuous in the CNS white matter. Nevertheless, cortical and spinal cord gray-matter involvement can also be seen, although usually with less lymphocytic infiltration than in white-matter plaques [9].

Recent work suggests that MS is pathologically heterogeneous and that, perhaps, some cases of MS are due to primary oligodendrocyte pathology rather than to a chronic inflammatory process. For example, one study described four distinct patterns of demyelination, two of which were highly suggestive of a primary oligodendrocyte injury [73].

It is even possible that oligodendrocyte apoptosis is the initial event in the formation of an MS plaque. Because of the complexity and heterogeneity of the mechanism involved in the pathogenesis of the MS we are going to talk about these various factors in this section and then discuss them.

The lesions of multiple sclerosis are characterized as perivenular infiltration of myelin basic protein by the T cells and the macrophages which initiate a chain reaction of autoimmune responses [74].

T cells are only seen in an outer zone of active phagocytosis surrounding the lesion core [75]. Changes in normal-appearing white matter (NAWM) of MS patients are evident in postmortem tissue. The changes observed include a reduction in myelin density, evidence of remyelination, and the presence of reactive microglia in the absence of T cells [76-78]. Also, molecular studies have reported a reduction in myelin-associated glycoprotein in NAWM [79], a change which might interfere with axon–oligodendrocyte associations and thereby reduce the viability of either or both cells. Such abnormalities may be the earliest pathologic changes, preceding both lesion formation and clinical symptoms. The presence of axonal injury and loss in MS plaques has been recognized for well over a century but these changes have been considerably highlighted in recent years [80,81]. Indeed, it is now thought to contribute importantly to the persistent neurologic deficits experienced by patients in the progressive phase of the illness where a threshold of axonal loss is thought to be reached and compensatory CNS resources exhausted. Considerable axonal damage (i.e., transections and bulb formation) has been demonstrated in acute (active) lesions [80,81]. It is also seen to a lesser degree at the active margins of chronic plaques and to an even lesser extent in the core of chronic active lesions [82,83]. Smaller-diameter axons seem to be more sensitive to damage. Although axonal transection is not typically seen in NAWM there is, nevertheless, substantial axonal loss in NAWM tracts [82,83]. Even as early as 9 months into the disease, a 22% loss has been reported in descending tracts distal to a brainstem lesion, with preservation of other tracts [84]. Early in the disease, axonal damage depends primarily on inflammatory processes.

However axonal loss can continue to occur in the later, less inflammatory stages of MS, even in patients with an apparent cessation of their clinical and radiologic relapses [85]. Therefore, it seems likely that disease mechanisms independent of inflammation may contribute to the ongoing axonal loss in MS.

Inflammatory demyelination is considered to be the main finding in MS, but recent studies have shown an actual neuro-axonal degeneration and synaptic pathology [86]. Considering the outside-in model, the lesions begin to develop from the myelin (outside) to the axon (inside). In recent studies axonal injury and gray matter lesions in normal-appearing white matter have been demonstrated and in spinal cord sections, immunostaining of the neurofilaments show that demyelination in animal models for MS precedes axonal injury with apoptosis of the oligodendrocytes, indicating that axonal injury could be a response to demyelination, here it is seen that the lesions develop

from the axon (inside) to the myelin (outside) (Inside-Out model) [87].

According to Dendrou *et al.* [88], it can still be argued whether the inception of the autoimmune response begins in the periphery or the central nervous system. In the peripheral model, autoreactive T cells activated at the peripheral sites, travel to the CNS along with monocytes and activated B cells. This model is consistent with experimental autoimmune encephalomyelitis (EAE) in animals where emulsified CNS antigen and immune stimulants are introduced, leading to the production of pathogenic TH1 and TH17 cells in lymph nodes. After crossing the blood brain barrier, these cells enter the circulation and launch an autoimmune response within the CNS.

For the most part multiple sclerosis was thought to be a T cell autoimmune condition, but recent studies have shown the presence of B cells and plasma cells directed towards the oligodendrocytes, myelin and neurons in the peripheral blood and the CSF of individuals with multiple sclerosis, suggesting that B cells might play an important role as the precursors of antibody secreting plasma cells and the activation of T cells by acting as antigen presenting cells. As the role of B cell in the pathogenesis of multiple sclerosis is elucidated this could lead to the emphasis being laid on monoclonal antibodies against CD 20 molecule [89,90].

Clinically, the majority of MS patients present with relapsing-remitting course and within a few years, a large number of these patients with or without treatment with immunomodulatory agents enter in another phase of disease known as secondary progressive MS. Another arm of the immune system, the humoral immune system-autoantibodies as well as activated complement also play a significant role in the pathogenesis of MS [91,92]. The combined activation of the cellular and immune wings coupled with disruption in the blood brain barrier, activation of cerebral endothelial cells and loss of the adherent endothelial junctions [93-95] this could be responsible for the development of perivenular demyelinating lesions discussed earlier.

Increased frequencies of antiphospholipids antibodies (APLA) are observed in autoimmune conditions apart from systemic lupus erythematosus (SLE), which are not necessarily associated with thrombosis, as seen in, immune thrombocytopenic purpura (ITP) [96] and multiple sclerosis.

Antiphospholipid antibodies (aPL) and endothelial microparticles in MS

It has long been recognized that aPL occur at high frequency in many disorders other than APS and SLE, especially those known to be immune-mediated, such as immune thrombocytopenic purport (ITP), multiple sclerosis (MS), and rheumatoid arthritis (RA). However, the significance of aPL in these disorders has been generally dismissed as non-specific or epiphenomenal, partly because the aPL did not appear to be related to symptoms, and perhaps also because aPL in these disorders is inconsistent with the paradigm that the pathological significance of aPL is limited to thrombosis. This review was motivated in part by recent findings which indicate that aPL are in fact associated with symptoms in non-APS (Bidot et al 2009), non-SLE disorders in humans.

The reported frequencies of positive APLA in MS have ranged from 10% to 44% and 88% [97-101]. One of our article published in 2007 in reference to the presence of APLA in MS, we found a high correlation of several APLA-IgM associated with exacerbations in MS comparing with remission.

Several reviews of the neurological symptoms of APS/SLE are available [102-104], and many case reports, e.g., cerebral ischemia [105]. However, MS is not thought to involve ischemia, although elements of the coagulation cascade are present in MS lesions, including fibrinogen and recently, tissue factor and protein C inhibitor [106].

In 2000, our collaborative investigation demonstrated elevated endothelial microparticles (EMP) during exacerbations of MS [107,108]. Those findings motivated further investigations, this time of aPL in MS, with the hypothesis that aPL might be involved in endothelial activation in MS. Several prior reports had established that aPL commonly occur in MS, but in most of them the patient population was heterogeneous or inadequately defined, and there was no indication of a relation between aPL and the pathophysiology of MS.

To examine the relationship more closely, we tested samples of well-defined, treatment-naive MS patients in either exacerbation or remission, documented by neurological as well as brain MRI with and without contrast. The central finding was that all aPL measured were significantly elevated in acute phases *vs.* remission, and correlated strongly with MRI imaging, $p = 0.002$ [74]. The antigens tested included β2GPI, FVII, and four pure PL (CL, PC, PS, PE). Of interest, aFVII was never detected in remission but was present in 60% of acute MS; and anti-β2GPI was positive in 80% of acute MS. It is possible that unidentified and possibly MS-specific auto antibodies were also present, judging by the strong reaction to the pure PL in acute, but not remission, cases. Unexpectedly, aPL in MS were exclusively of IgM class, with no IgG detected.

Because that work showed a direct relation between aPL and clinical state in MS, it is plausible to suspect that aPL may be involved in the pathogenesis of MS. Of course, the possibility exists that aPL in acute MS are epiphenomenal; but the same argument could be levelled against the hypothesis that aPL cause thrombosis. In further support, Shoenfeld and colleagues clearly demonstrated neuropathological effects of aPL in animal models [109-111]. Since some aPL have been identified with anti-endothelial (anti-EC) antibodies (earlier cited), and since our group [107] and others have documented endothelial activation in MS, it is relevant to note that anti-EC have been detected in MS and were proposed to contribute to its pathogenesis. In 1989, Tsukada et al. found anti-EC in 75% of active MS but in only 4% of remission [112]. However, a 1992 report found only 13% positive [113] and a later report found only 10% reactive to human umbilical vein EC (HUVEC) [114]. On the other hand, another report around the same time, but using brain microvascular EC rather than HUVEC, found that 12/16 active vs 0/15 inactive MS reacted to EC [91]. This suggests that anti-EC in MS are specific for brain microvessels, and would be consistent with the fact that CNS lesions in MS tend to develop around brain microvessels (Dawson fingers) [115].

Conclusion # 6: "*To summarize, perivascular inflammation of the myelin leading to inflammatory demyelination is considered the hallmark of MS, these lesions are dominated by the presence of T cells and macrophages and most of the disease is limited to the white matter. However, recent studies unearthing new evidence point towards gray matter involvement and axonal injury. Another interesting finding is the possibility of B cell involvement in the pathogenesis of MS, which has led to the development of newer Anti CD20 medications. It can still be argued whether the pathogenesis of MS begins in the periphery or the central nervous system. Contrary to the popular belief that APLA were only associated with thrombotic conditions like ITP, in light of recent*

studies APLA can also be associated with the pathogenesis of MS. More studies about the effect of the endothelial microparticles must be done, to know the role of them in the MS pathogenesis"

Other hypothesis and controversies

Theory of Cerebral spinal fluid volume (CSF): A few studies suggest that patients with MS had slightly increased levels of CSF regurgitant fraction when compared to the control group, however, that being said no statistical significance was found, this slight difference in the CSF regurgitant factor could be attributed to an atrophy-dependent ventricle volume increase in the MS patients leading to an increased mild hyperdynamic situation independent of the venous theory. Chiang et al demonstrated a relationship between ventricular morphology and aqueductal CSF flow in healthy subjects versus individuals with communicating hydrocephalus [58].

However, another study failed to detect differences in few of the parameters while comparing the CSF of individuals with MS to that of the control group, the parameters were no difference was found were mean velocity, peak systolic and diastolic velocities and regurgitant fraction but were able to demonstrate differences in the craniocaudal and caudocranial CSF flow volumes and stroke volume, which were found to be higher in patients with relapsing remitting multiple sclerosis compared to the control sub group [116].

Another theory regarding MS is that basically it is a degenerative disorder, similar to Parkinson's and Alzheimer's diseases and other progressive degenerative CNS disorders of unknown etiology. As with many of these conditions, the degenerative process probably began many years or possibly decades before the initial symptoms of disease presented. Kerbrat et al supports this claim by saying that the adolescents with MS have a significantly reduced brain volume as well as a reduced head size [117], implying that the inception of the disease was many years ago while the skull was still in the development phase [86].

Louapre et al claim that neurodegeneration in multiple sclerosis is an event independent of inflammation and that it occurs early in the disease making it a significant predictor of clinical disability. Advanced neuropathological and imaging studies have thrown light on the sustained neurodegenerative mechanisms early in the disease, including neuronal and neuritic injury. It can still be argued that between inflammation and neurodegeneration, which process is primary and which process is secondary and how they correlate over the course of the disease. Evidence points out that myelin loss in inflammatory white matter lesion can induce axonal degeneration locally and that neuronal and axonal loss happen outside areas of inflammation, making neurodegeneration an independent event as a possible explanation. They theorize that neurodegeneration could evolve independent of inflammation, being influenced by genetic and metabolic backgrounds and that there is a need to develop animal models of MS neurodegenerative pathology and an in vivo biomarker of neuronal function to test for neuroprotective agents in progressive MS [118].

In another study done by Scott *et al.* [119], it is theorized that monitoring relapse phenotype can be imperative in determining the course and the outcome of the disease. Earlier studies suggest that the predictive value of relapses becomes mostly or completely obsolete after the first 2 to 5 years [120,121].

Based on the most commonly used disability scale, worsening cases of MS have a higher preponderance towards motor symptoms when compared to sensory symptoms after initial attacks [122,123] indicating that relapses affecting the motor system are more likely to contribute to long term outcomes even after initial relapses, however long term dataset was not analyzed in these studies. Gettings et al; and Skoog et al noticed that relapses of the motor system and relapses with residua in later stages of MS were more likely to have a progressive outcome with rapid progression of disability [124,125].

Studies done recently may not be in concordance with earlier studies regarding the importance of the temporal placement of various types of relapses within the course of MS, that being said, there is a general consensus that the mode and severity of initial symptoms has an impact on the course of the disease. The difference in the finding of the studies could be explained by the exclusion of the mode and severity of attacks in the earlier studies when compared to more recent studies and the importance of motor attack over sensory attack, incomplete recovery over complete recovery and later MS over early MS have only been started to be considered in studies recently. Monitoring long term natural history cohorts can be a challenge as well, as it can be hard to find available detailed information on each relapse in every patient.

Skoog et.al suggested that motor symptoms without complete recovery occurring at any time during the course of the disease have the potential to modify the risk of transition to secondary progressive multiple sclerosis [129]. It was also stated that there was higher long term risk of poor prognosis when slow worsening disease course with confirmed sequelae due to relapse occurred [124].

In another study [126] discussing the pathology of multiple sclerosis, emphasis was laid on antibodies that interfere with the process of re-myelination, primarily attacking the proteins that are vital to re-myelination, namely the myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte protein (MOG). Anti MOG antibodies were seen in high quantities from the brain tissue acquired during autopsy of diseased individuals. Another protein mention in this study was AN2, which is a cell surface glycoprotein expressed on oligodendrocyte progenitor cells in the developing and developed CNS [127]. Anti AN2 antibodies have been detected in high quantities in the CSF of certain patients reporting active relapses [128]. The manner of working of the anti AN2 antibodies was studies in vitro and it was found that these antibodies caused issue with re-myelinating process by blocking the migration of oligodendrocyte precursor cells, myelin synthesis, which could eventually lead to the destruction of oligodendrocytes, the presence of these auto antibodies could be a fair explanation to the inability to re-myelinate. It is also interesting to note the discussion of another set of auto antibodies, the antibodies against the axon, as these antibodies have been detected in high quantities in the CFS of multiple sclerosis patients and were also present in the autopsy tissue of diseased individuals, these antibodies could be a possible explanation for the axonal pathology seen in MS, as it was seen that these antibodies were not only seen in the active lesions but had infiltrated the white matter without any apparent demyelination and axonal damage was independent of oligodendrocyte pathology [129].

Contribution of PTX3 to antiinflammatory effects in MS and its animal model EAE.

The PTXC3 is an Acute Phase reaction protein in neuroinflammatory diseases and recent studies revealed the induction of the phagocytosis of the myelin, locally; but do not point to a role for PTX3 in controlling the development of autoimmune neuroinflammation [130].

The use of autologous hematopoietic stem cell transplant (HSCT)

as second-line therapy for relapsing multiple sclerosis (MS) is debated by two experts on MS.

One of the experts advocates for using HSCT as a second-line therapy for patients with a high level of disease activity and resistant to standard treatment, being polemic the real meaning of "standard treatment".

The other professor advocates for not using HSCT as a second-line therapy for MS. He refers that the efficacy of the monoclonal therapies (even the recently approved or anticipated monoclonal therapies (daclizumab and ocrelizumab) and HSCT is fairly similar, being the treatment-associated mortality much higher for HSCT, suggesting HSCT for third-line therapy [130].

Conclusion #7: *"The enunciation of several other hypothesis strongest suggest the multifactorial mechanism in the pathogenesis of the MS".*

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