

An updated approach to Multiple Sclerosis

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REVIEW

ABSTRACT

Multiple Sclerosis is an inflammatory demyelinating disease of the Central Nervous System, chronic and with unknown pathogenesis. There is no cure for multiple sclerosis and the causes of the disease appear to be related with genetic and environmental components. The most accepted theory assumes a break of the immunoregulatory balance 'active T cells/regulatory T cells' and some evidences show the incidence of oxidative stress in the disease. The therapies that can reduce or stop the clinic symptoms and plaques formation cannot stop the illness progression, that's why the efforts for finding a new and efficient treatment still continue. The complementation of clinical information with molecular analysis could give more accuracy to a specific treatment. Better combinations of drugs and better therapies could be applied if the knowledge about the mechanisms of action of these drugs is improved.

Keywords: multiple sclerosis, autoimmune, beta interferon, T cell, inflammation, central nervous system

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RESUMEN

Un acercamiento actualizado a la Esclerosis Múltiple. La Esclerosis Múltiple (EM) es una enfermedad inflamatoria desmielinizante crónica del Sistema Nervioso Central, y de patogenia desconocida, para la que actualmente no existe cura. Las causas que la desencadenan parecen estar relacionadas a componentes genéticos y ambientales y la teoría más aceptada enuncia que la enfermedad ocurre ante una ruptura del balance inmunorregulatorio 'células T activas/células T reguladoras'; mientras que algunas evidencias muestran también la incidencia del estrés oxidativo en la enfermedad. Las terapias que pueden reducir o detener los síntomas clínicos y la aparición de las lesiones no pueden detener la progresión de la EM, es por esto que numerosos esfuerzos continúan realizándose para encontrar un tratamiento nuevo y eficiente. La unificación de la información procedente de la clínica con la derivada de los análisis moleculares pudiera brindar mayor precisión en la búsqueda de un tratamiento específico. En la medida en que se mejore el conocimiento sobre los mecanismos de acción de estas drogas se podrán aplicar mejores combinaciones de drogas y mejores terapias.

Palabras clave: esclerosis múltiple, autoinmune, interferón beta, linfocito T, inflamación, sistema nervioso central

Introduction

Multiple Sclerosis (MS) is a neurodegenerative, inflammatory demyelinating disease of the Central Nervous System (CNS), with unknown pathogenesis and a chronic evolution. The elimination of the myelin in the axons is one of the events that results from the inflammatory cascade in the MS plaque [1]. The disease is extensively present in the world, but a gradient of geographic distribution is observed from the equator to the poles; in part supporting the idea of the incidence of environmental factors [2].

MS affects near 1 of each 1000 people, 2 women for each man [3] and mainly young persons, between 25-30 years, although there has been early and later cases [4]. The illness is considered chronic, no contagious [5] and has no cure at present.

According to some authors, the MS can be found in different forms: primary progressive (PPMS), relapsing progressive (RPMS), secondary progressive (SPMS) and relapsing-remitting (RRMS), which is the most common (80% of the cases) [6]. Many patients suffer different forms of MS on their life [7]. In general, 1 of 5 patients evolves to a benign stage of the illness without important changes on their life; 1 of 3 patients evolves to an active illness with sequels of consideration that limit the patient's normal development in society, but can still live independent; finally, 1 of 3 patients evolve to a progressive form,

suffering serious sequels as an important mobility reduction that affects substantially the patient's life in some cases [8, 9]. MS is the second more frequently neurologic disease between young adults, after epilepsy, and the most important in the west world [10].

Apparently, environmental and genetic factors are involved in the occurrence of the disease. The identification of Epstein-Barr virus, for example, as a putative environmental trigger of MS is described for Casiraghi in 2011 [11]; the induction of a local breach in the blood brain barrier (BBB) and the attraction of auto-reactive lymphocytes into the brain by the up-regulation of cytokines, chemokines and adhesion molecules is the mechanism proposed in this case. Other ways as molecular mimic appear to be used for some viruses and bacteria [11] as well as the break of immune tolerance of the CNS to auto-antigens, in genetically susceptible persons [12], to trigger the disease.

The incidence of genetic factors in the beginning of the illness has been widely studied for many authors; maybe the most important founded MS association is with many HLA haplotypes (from the extended major histocompatibility complex) [13]. For example, the reduction in the expression of HLA-DRB1*15 in the thymus in early life, appear to be related to the loss of central tolerance and the risk of autoimmunity in later life [14]. A significant association between

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HLA-DRB1*1501 and susceptibility to MS have been reported for Shahbazi in 2011 [15]. HLA-DRB1*1501 is also related with a specific tumor necrosis factor alpha polymorphism (308) that is involved in MS susceptibility [15].

The Innate Immune Response, the first line of response against microbial pathogens, has been also identified recently as a factor involved in the regulation of the antigen-specific adaptive immune response, in the MS pathogenesis [16].

Some aspects of MS pathogenesis

An autoimmune attack against the white matter of the self-CNS induces the lesions [17] which can be followed by multi-systemic effects as paralysis [18]. T cells (CD8+) with receptors for myelin epitopes, from healthy immune repertoire, can pass across the BBB under a pathological activation. These T cells enter to the CNS parenchyma and activate a sequence of events that leads the formation of a typical multiple sclerosis plaque [19].

The autoimmune activation of T cells is produced when they recognize auto-antigens presented by the local antigen-presenting cells and start secreting pro-inflammatory mediators at the same time that they recruit and activate macrophages. The role of macrophages is important in the acute neuronal dysfunction; first, they attack the myelin sheaths and the oligodendrocytes, becoming responsible of the demyelinating process. Second, the macrophages can attack the naked axons obtaining direct damage and indirect neuronal degeneration [20].

The early lesions, besides the local damage effects, succeed the proliferation, activation and circulation of new self-reactive T cells. These cells can re-enter the CNS and establish a pro-inflammatory loop that carries out successive damages to the axons and oligodendrocytes. Irreversible damage to the axons and cells of the glia avoids the re-myelinating process and leads to persistent neurological damage [21].

T helper (Th) cells with predominant generation of interleukin (IL)-17 (Th17 cells) attach to brain endothelial cells better than Th1 (with predominant generation of IFN γ) cells which is at least in part due to the presence of CD146 on the Th17 cells. Moreover, Th17 cells express high levels of molecules such as CCR6 and CD6, which enhance entry of infiltrating T cells into the CNS and have an important role in the development of experimental autoimmune encephalomyelitis (EAE) and probably MS [21].

The IL-17-producing T cells (CD4+ or CD8+) have been detected in both active and chronic MS, and the central role of Th17 produced cytokines (IL-17A, IL-17F, IL-6, IL-9, IL-21, IL-22, IL-23, IL-26 and TNF α) is the induction of inflammatory reactions. High frequency of CNS auto-reactive Th17 cells has been detected in the immune periphery before onset of clinical disease, but not in the CNS. In acute EAE, the large number of CNS auto-reactive Th17 cells is present in the inflamed CNS. In recovery from an acute EAE, high levels of CNS auto-reactive Th17 cells are still present in the immune periphery, but not in the CNS. Moreover, the frequency of Th17 cells is significantly higher in the cerebrospinal fluid (CSF) of RRMS patients during relapse, in comparison with

RRMS patients in remission or patients with other non-inflammatory neurological diseases.

Apparently, the main function of IL-17 in immunopathogenesis of MS is the breakdown of BBB. Generation of IL-17 enhances the activation of matrix metalloproteinase-3 (MMP-3) and attracts neutrophils to the site of inflammation. Neutrophil-mediated activation of enzymes such as MMPs, proteases and gelatinases participates in BBB disruption. IL-17 increases the generation of reactive oxygen species (ROS) in brain endothelial cells. The oxidative stress mediates activation of the endothelial contractile machinery. Activation of the contractile apparatus is responsible for the loss and disorganization of tight junction proteins, which consecutively leads to BBB disruption [21].

Pro-inflammatory cytokines as gamma interferon and tumor necrosis factor beta, released by activated T cells, can induce the expression of surface molecules in antigen-presenting cells and adjacent lymphocytes. The expression of MS antigens, mainly components of myelin, by these cells, can activate the immune response against the antigens or provoke anergy [6]. The auto-antibodies against myelin basic protein, and other myelin related proteins, have been found in MS patients [22].

Recent studies provide a link between micro-RNA (miRNA) functions and neurodegeneration. Complete loss of miRNA expression in the brain leads to neurodegeneration in several animal models; other evidences from patients showed that miRNA dysregulation could, indeed, contribute to neurodegenerative disorders [23]. Thus, miRNAs are rapidly appearing to be key regulators of neuronal development and function, as well as important contributors to neurodegeneration.

Micro-RNAs are involved in adult neurogenesis which may imply the possible role of some miRNAs in endogenous repair mechanisms in MS. The modulation of these miRNAs may stimulate the differentiation of neural stem/progenitor cells into mature neurons that can replace neurons lost through the disease process in MS. New evidences have identified a number of new transcriptional regulators and miRNAs as having key roles in oligodendrocyte differentiation and CNS myelination, providing new targets for myelin repair [23]. miRNA mediated regulation is essential for immune homeostasis and the prevention of autoimmune diseases. So, as biomarkers for the disease or maybe included in a specific therapy, miRNAs could be important in the characterization and therapy in MS in the near future.

Regulatory T cells can be induced in periphery under an autoimmune response [24]. Their capacity to suppress immune response has been observed through direct interaction with antigen-presenting cells and converting them tolerogenic [25, 26]. The most accepted theory about autoimmune inflammation of CNS assumes a break of the immune-regulatory balance between activated T cells and regulatory T cells. Dysfunctions of certain regulatory T cells have been reported for MS [27]; this field became a goal for researchers looking for the efficient therapy in MS.

Venken in 2008, describe the existence of damaged regulatory T cells, joined to a reduced expression of

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FOXP3, in RRMS patients [28]. Damage to regulatory T cells CD4+CD25+FOXP3+ could be the explanation for changes in the tolerance to autoantigens that brings susceptibility to MS and the autoimmune course of the disease [29, 30].

The treatment with Interferon β -1a (IFN- β) improves the expression of the tolerogenic molecule B7-H1 in dendritic cells, changing their inhibitory properties and contributing to relevant immunoregulatory mechanisms in MS [31]. Then, IFN- β functions as an inducer of regulatory T cells through the interactions of T cells with dendritic cells [32].

Apart from the generalized theory about the autoimmune cause of MS, Zamboni *et al.* have recently proposed a new theory that relates MS cause with a chronic cerebrospinal venous insufficiency [33]. Although it has been created a wave of expectation in many patients, some studies are now in progress in order to verify the theory; that is the case of those led by Doepp *et al.* [34] and Sundstrom *et al.* [35] in 2010, which not confirm the results obtained by Dr Zamboni.

Oxidative stress in MS

Healthy cells have several mechanisms of self-defense against the damage induced by free radicals; when these mechanisms fail the cells are under the oxidative stress phenomenon. The oxidative stress may cause cellular damage and cell death due to oxidation of essential bio-molecules [36].

Neurons are particularly vulnerable to damage induced by free radicals, they have low levels of antioxidants as glutathione [37, 38] and reduced enzymatic activities of detoxification as performed by catalase or superoxide dismutase [39, 40]. In addition, neurons cannot replicate themselves, so, alterations induced on this kind of cells may lead to irreversible damage on CNS [41].

The reactive oxygen species are particularly active in brain; the neurotransmitters and excitatory amino acids, unique in the brain, are highly ROS producers. Metals as Iron, catalytic for free radical reactions, are present at elevated concentrations in several regions of the brain; with reduced levels of transferrin. Other oxidative stress sources are generated by the constant use of oxygen in the mitochondria and other auto-oxidation enzymatic pathways. ROS attack glia and neurons leading to neuronal damage [42].

Some studies explore the effect of antioxidants in the EAE model, in mice [43-45]; alpha lipoic acid for example, shows the ability to pass through the BBB and reduce inflammation, demyelination and axonal damage in the spinal cord [46]. However, these results have not been reproduced in humans and the few clinical trials for this kind of molecules have not proved to delay MS progression [42].

A good antioxidant drug for MS has to be administered at an early state of the disease; before the irreversible neuronal damage takes place. Also, the drug must be able of penetrate the BBB to gain a therapeutic level in the CNS [42].

The immunomodulatory effects of Luteolin, a flavonoid with antioxidant activity [47], seems to be caused by the modulation of regulator components of cytoskeleton like Rho GTPase family, a group of

proteins that inhibit NF-kappaB, a protein that has been associated to a high number of inflammatory diseases [48]. This kind of inhibition may lead to reduce the expression of MMP9 [49, 50] a protein involved in oxidative stress mechanisms.

MS treatments

Immunomodulatory or immunosuppressive therapies, capable of to halt or reduce clinical symptoms and plaques development in MS, generally cannot stop the illness progression. Drugs like IFN- β and Mitoxantone are modifier therapies licensed to be used in SPMS patients. The finding of restorative and neuroprotective therapies is now one of the main goals for researchers at the MS field, due to the neurodegenerative component of the disease [17].

All the established drugs for treating MS are only partially effective, besides their serious potential side effect; that is why many studies are nowadays in progress to find new agents or to optimize actual therapies. Doses modifications, changes in the route of administration and time or duration of the treatments are some of the factors studied [51].

Among approved MS treatments are: IFN- β [52], Copaxone or Glatiramer Acetate (GA) [53], Immunoglobulins intravenous injection [54], plasmapheresis [55], Natalizumab [56, 57]. Other treatments proposed or still being studied are: chemokines receptor antagonists [58], immune therapies based on auto-antigens [59], stem cells transplant [60], strategies involving B cells [61] and T cells [62], and some others studying the effect of immunomodulatory and immunosuppressive compounds; that is the case of FTY720, Fingolimod [63] and Treosulfan [64], between others. According to Fox RJ in 2010, seven therapies were approved for FDA to treat RRMS and a dozen are now under study [65].

IFN- β , a molecule with anti-inflammatory properties, is the most widely used drug for treatment of MS. The downregulation of T helper 1 cytokines and the inhibition of the migration of inflammatory T cells into the CNS are important factors in the therapeutic effects of IFN- β .

According to recent reports, osteopontin and IL-17 play significant roles in the pathogenesis of MS [66, 67]. Osteopontin is a T-bet-dependent proinflammatory cytokine produced by Th1 cells and dendritic cells, which regulates expression of downstream inflammatory cytokines such as IL-10, IL-12, IL-17, IL-23 and IL-27. IL-17 is expressed by a distinctive cell lineage named Th17 cells, also recognized as a key mediator of MS. Recent findings indicate that IFN- β downregulates expression of osteopontin and the differentiation of IL-17-secreting Th17 cells in MS [68].

GA is a random polypeptide made up of four amino acids (L-glutamic acid, L-lysine, L-alanine, and L-tyrosine) in a specific molar ratio, that causes an immune deviation from a Th1 to a Th2 phenotype and induces antigen-specific T suppressor cells that cross-react with putative autoantigens in the CNS, and inhibits antigen presentation [69].

Natalizumab is a humanized monoclonal antibody that binds to integrins and prevents their interaction with their ligands. Integrins mediate the migration of T cells in the CNS and constitute a pre-requisite for

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transmigration of immune cells across the blood-brain barrier [70, 71].

Evidence exists that implicates B cells in the development and perpetuation of MS [72, 73]. Some strategies that have B lymphocytes as a target are now under study, for example, that one that considers B cells depletion using specific monoclonal antibodies, as Rituximab [74]. In this experiment, the effect of B cells elimination was observed in peripheral blood but not in cerebrospinal fluid, depending on the levels of expression of Rituximab target antigen CD20. The efficiency or not of this or other experiments affecting B cells needs to be validated and studied deeply. The serious potential side effect of any new drug has to be considered and the cost-benefit relation for the patients must be well established.

Neuroprotection and neural reparation are new strategies recently inserted in clinical trials, although the impact of neuroprotective agents on the immune system may not always be beneficial, as recently demonstrated for sodium channel blockers in EAE [75]. Protecting axons and glia from inflammatory damage and facilitating repair are also future directions for MS therapy. Theoretically, two strategies are in study: boosting of endogenous repair mechanisms and cell replacement therapies. Also, the identification of auto-antigens that can induce axonal injury and an immune attack versus both glial and neuronal cells [76] is valuable information for neural reparation strategies.

Prostaglandins (autacoids derivatives of arachidonic acid) are implicated in the modulation of many physiological systems, such as CNS and the immune system. The relation between these molecules and MS and other pathologies has been observed, for example, implicating some of them (PG-D2, PG-E2/EP4) in the inhibition or activation of T lymphocyte proliferation and consequently, the inflammatory response, apparently depending on the prostaglandin concentration. Several studies tried to measure the prostaglandins levels in CSF and serum of MS patients, and more of them suggest that prostaglandins are increased in CSF of MS patients. PG-E is one of the major effective factors in pathogenesis and treatment of MS and evidences show that PG-E2 may influence the remyelination process. Some studies have discovered that 15d-PG-J2 decrease the function of macrophages, monocytes, microglial cells; inhibits Th1 differentiation and leads to the amelioration of EAE [77]. Thus, the possibility of use these molecules, in particular those which have proved potent anti-inflammatory properties, might be a method to considerate for combined therapies in MS.

At the same way, advances in imaging techniques, proteomics, pharmacogenomics, metabolomics and transcriptomic must be integrated and used in order to make better designs of drugs and improve the applicability of future therapies in MS.

The combination of two drugs or compounds as a new strategy for potentiate particular effects and lead to more efficient treatments, is a way investigated today looking for access to superior levels of MS patients life. For example, combination of IFN- β and Luteolin increases the immunomodulatory effects of IFN- β , obtaining a major efficacy in clinic and re-

ducing neutralizing antibodies and other factors that affect the IFN- β treatment [78]. Nevertheless, lower intestinal absorption of flavonoids limits the combination expected beneficial effects [79].

Among the aspects to improve in new therapies are the reduction of brain lesions, the relapses and the prevention of CNS permanent damage. At this moment, patients continue having relapses and MS progression, still under IFN- β or GA treatment [80]. The combination of IFN- β , the more accepted and used drug for the treatment of MS, with other compounds that potentiate the neuroprotective effect, is a way to consider and study deeply.

Searching for new drugs and therapies, there is a need to learn from past and present research including clinical and pre-clinical experiments. Although EAE model has proven to be immensely valuable for studying many aspects of MS, there remains a great need to identify the next generation of therapeutics that will particularly target the unmet need of treatment for the progressive phase of disease. According to Vesterinen in 2010, at this time the testing in animals of candidate interventions for MS has potentially been confounded by limited internal validity (with little reported use of randomization, blinding and power calculations) and by limited external validity (with few treatments given at clinically appropriate time points) [81].

Pharmacogenomics in MS

Current strategies for treating MS are limited by the complex and heterogeneous character of the disease in the field of genetics, not completely elucidated. That is why hypothesis in study require advances in the knowledge of those factors involved in the MS pathogeny at genomic and transcriptomic levels. A serious study on these fields could give relevant information about the mechanisms involved in response to drugs and other important aspects, and could complete the information obtained with the clinic. Identification of genetic variants and biomarkers that may predict the treatment response is very useful in order to guarantee a good response and patient satisfaction [82].

Contemporary definition of pharmacogenomic includes genetic variants related with drug response, pharmacogenetic, and the effects induced at the RNA level by drugs [83]. The necessity for genome wide techniques has led to the creation and evolution of DNA microarrays and other high throughput techniques [84-86].

Since some years ago, several studies have explored the fields of pharmacogenomic and MS. Microsatellite tests in the genome of affected families [87, 88] detected at least 60 genomic regions potentially related with disease susceptibility. Although, there is no clear reproduction of this results. DNA microarray studies describing the MS lesions have seen anomalous behavior of inflammation related genes in active lesions [89-91].

The majority of pharmacogenomic studies published in 2010 on MS therapies has been related with IFN- β and has identified expression correlations between IFN- β and possible new biomarker genes; valuable information that could constitutes inclusive criteria for managing MS treatment decisions. Using mainly DNA microarrays and single nucleotide

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polymorphisms analysis, mostly genes with immune-related functions were found to be up- or down-regulated under the application of IFN- β . All these studies underscore the complex and pleiotropic actions of IFN- β , a drug whose precise mechanism of action in the rejection of the disease is not yet fully understood [92]. The study of substances as GA and Natalizumab shows equally the incidence of sets of multiple genes in patient response to the treatments. Thus, it is mandatory to estimate aggregate effects of those genes on drug treatment to correctly generate new therapies.

Comparatively, the literature published about MS pharmacogenomic is lower than the literature published on pharmacogenomic of other diseases; but efforts of the scientists all over the world, looking for new strategies in MS treatment, would revert this situation in the next few years.

Conclusions

Multiple Sclerosis is a complex disease; some evidences relate environment influence over genetically susceptible persons as the factors involved in the beginning of MS. The inflammatory response -mediated by lymphocytes, cytokines, macrophages -and the incidence of oxidative stress, are very important in the pathogenesis of the disease and must be considered in the strategies for new treatments. Drugs that are now in the market are not completely effective and show potentially negative side effects; the combination of drugs seems to be an alternative to explore and the application of high throughput screenings can be used to continuously improve knowledge about the disease and treatments in study. The necessity of more efforts is present, patients deserve it.

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