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Research paper

Agent based modeling of the effects of potential treatments over the blood–brain barrier in multiple sclerosis☆

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ABSTRACT

Multiple sclerosis is a disease of the central nervous system that involves the destruction of the insulating sheath of axons, causing severe disabilities. Since the etiology of the disease is not yet fully understood, the use of novel techniques that may help to understand the disease, to suggest potential therapies and to test the effects of candidate treatments is highly advisable.

To this end we developed an agent based model that demonstrated its ability to reproduce the typical oscillatory behavior observed in the most common form of multiple sclerosis, relapsing–remitting multiple sclerosis. The model has then been used to test the potential beneficial effects of vitamin D over the disease.

Many scientific studies underlined the importance of the blood–brain barrier and of the mechanisms that influence its permeability on the development of the disease. In the present paper we further extend our previously developed model with a mechanism that mimics the blood–brain barrier behavior. The goal of our work is to suggest the best strategies to follow for developing new potential treatments that intervene in the blood–brain barrier.

Results suggest that the best treatments should potentially prevent the opening of the blood–brain barrier, as treatments that help in recovering the blood–brain barrier functionality could be less effective.

1. Introduction

Demyelinating diseases of the central nervous system are characterized by lesions that are associated with loss of myelin with relative sparing of axons. There are many types of diseases that damage myelin in concert with the destruction of axons: one of these is represented by multiple sclerosis (MS). Autoimmunity plays an important role in the disease outcome and the body's own immune system assaults the myelin sheath causing injury. Numerous genetic factors including HLA-DR15, HLA-A*02 and HLA-DRB1*1501 (Compston et al., 2005; Ramagopalan et al., 2009) are documented to be related to MS.

Relapsing–remitting multiple sclerosis (RRMS) is the most predominant form of MS, around 90% of all patients have RRMS (Sospedra and Martin, 2005), in which disease relapse and remission happens after a certain time period. The degree of the relapse varies from mild to severe based on the course and history of the disease. It is also usual to have a progressive phase of the disease and a large study showed that around 80% of cases were followed by chronic progression within 20 years (Kremenchutzky et al., 2006). Disease progression can be observed by different means including Expanded Disability Status Score (EDSS), Magnetic Resonance Imaging (MRI) lesion and with other physical test including timed 25-Foot Walk, MS Walking Scale-12.

T-cells play a major role in disease progression and it is documented that regulatory T-cells (Treg) drop in the peripheral blood when relapse appears. Conversely, the number of helper T-cells increases in the spinal fluid. It is also conjectured that homeostasis of Treg and effector T cells play a critical role in preventing autoimmunity (Fontenot and Rudensky, 2005; Lund et al., 2008; Carneiro et al., 2007). In particular, lack of functionality or deficiency of Treg may enable negative effects in the peripheral tolerance mechanisms that are supposed to control activation and proliferation of effector T cells (Sakaguchi et al., 1995).

Besides genetic factors, environmental factors are also considered to have a significant role i.e., Epstein–Barr viral infection (Ascherio et al., 2001; Ponsonby et al., 2005; Sundström et al., 2004) and some dietary factors. Vitamin D and turmeric play a protective role in MS and neurodegeneration. Turmeric protects the brain from neurodegeneration and vitamin D is considered one of the most important factors to prevent MS (Goodin, 2009).

For the migration of monocytes, reactive oxygen species (ROS) are crucial but they also initiate lesion development, contributing to lesion persistence in MS by degradation and phagocytosis of myelin and induction of axonal and oligodendrocyte damage (van der Goes et al., 2001; Vladimirova et al., 1998; Hendrikis et al., 2005). To counteract

☆ Availability: The java applet of the model is available through the link: http://www.francescopappalardo.net/MS_VitaminD_BBB_Applet_NLogo/.

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the detrimental effects of ROS, an appropriate antioxidant therapy is considered beneficial for patients who have MS by the limitation of both cellular influx and lesion progression. In the animal model for MS, experimental allergic encephalomyelitis (EAE), protective effects of antioxidants such as flavonoids (Hendriks et al., 2004), catalase (Ruuls et al., 1995), *N*-acetyl-L-cysteine (Lehmann et al., 1994), bilirubin (Liu et al., 2003) and α-lipoic acid (LA) (Marracci et al., 2002; Morini et al., 2004) have been described.

The etiology of the disease is only partially comprehended and the response to treatment cannot easily be envisaged. Symptoms of MS are apparently unpredictable and may differ from person to person, which denotes one of the most disturbing aspects reported by patients. Even in the same patient, symptoms and treatment responses may vary from time to time. Consequently, determining the prognosis of the disease and predicting the response to therapy at the individual level is a real challenge.

In this scenario mathematical/computational models are wished both in knowledge discovery to perform in silico experiments and suggest preclinical experiments and in clinical scenarios to help medical doctors to predict the correct therapy for their patients.

Recently, a computational model that allows the capture of the essential dynamics of RRMS has been presented (Pennisi et al., 2013). Its computational results support the hypothesis that a genetic predisposition alone is not probably sufficient to develop RRMS, and some sort of IS malfunction at a peripheral level is required. A possible cause could be the breakdown of the cross balancing mechanism between T cells (Teff) and regulatory T cells (Treg). In particular a breakdown in the effector T cell (Teff)–regulatory T cell (Treg) cross balancing mechanisms could be the cause. Furthermore, the role of vitamin D (VD) in RRMS has also been investigated (Pappalardo et al., 2014a,b) by taking into account some well known VD-induced immunomodulatory mechanisms.

In the dynamics of MS an important aspect is covered by the role of the blood–brain barrier (BBB), a diffusion barrier necessary for the normal function of the central nervous system and it is made of endothelial cells, astrocyte end-feet and pericytes. Moreover, tight junctions situated between cerebral endothelial cells, selectively exclude most blood-borne substances from entering the brain (Ballabh et al., 2004). During brain diseases such as multiple sclerosis, the disorganization and consequently the impermeability of this brain solute barrier is compromised. The mechanisms of breakdown of BBB are not yet clear, but the involvement of inflammatory cytokines released from $CD4^+$ T cells seems possible, during the immune attack on the myelin–oligodendrocyte complex (Minagar and Alexander, 2003).

In the present paper we investigate the role of the BBB by extending our previously developed agent-based model (ABM) in order to evaluate the effects of possible interventions of potential treatments that regulate the mechanisms involved in the breakdown and functional recovery of the BBB.

2. Materials and methods

2.1. The role of BBB in MS

Before gaining access through BBB, immune competent cells have to perform various actions. The classic flowchart of capture, rolling-tethering, activation, arrest-crawling and transmigration have been extensively described in the literature (Mrass and Weninger, 2006; Engelhardt, 2006; Owens et al., 2008; Man et al., 2007). Each step involves interaction of BBB-endothelial cells and leukocytes via expression of cellular adhesion molecules (CAMs) by BBB-endothelial cells (BBB-ECs), such as intercellular adhesion molecules 1 (ICAM-1) and vascular cell adhesion protein 1 (VCAM-1). Resting T cells have a very limited ability to enter the CNS parenchyma. Conversely, activated T cells can migrate into the CNS regardless of their antigen specificity (Ludowyk et al., 1992; Hickey, 2001). This is particularly due to the pro-inflammatory cytokines by activated T cells that also increase permeability, which favors subsequent leukocyte infiltration (Biernacki et al., 2004; Seguin et al., 2003).

The access of pro-inflammatory leukocytes into the CNS is thus related to an early phenomenon that can trigger the events leading to neuroinflammation, BBB disruption and MS plaque formation (Engelhardt, 2006; Raine et al., 1990). It has also been observed that immune cells of MS subjects also express reactive oxygen species (ROS) and enzymes that can accelerate their migration to the CNS by influencing BBB function, either directly or indirectly.

Tumor necrosis factor alpha (TNF-α) and interferon-gamma (IFN-γ) levels are often elevated in the serum of MS subjects (Ensoli et al., 2002; Hohnoki et al., 1998), especially during relapses (Kahl et al., 2002; Frisullo et al., 2008), although this remains controversial (Ozenci et al., 2000; van Boxel-Dezaire et al., 2001; Durelli et al., 2009). These cytokines act together to regulate the expression of several chemokines, cytokines and CAMs. It has been shown that intravenous administration of TNF-α to mice results in an increased BBB permeability (Tsuge et al., 2010), In Larochelle et al. (2011), in vitro TNF-α and IFN-γ stimulation alters the architecture of junction proteins on primary cultures of BBB-ECs and, more recently, TNF-α treatment of human BBB-ECs was reported to cause a strong upregulation of Toll-like receptors (TLRs)-2 and −3, with subsequent downregulation of tight junction protein expression (Nagyoszi et al., 2010) even if this remains to be confirmed.

Combination of TNF-α and IFN-γ influence the expression and secretion of numerous chemokines by BBB-ECs (Wosik et al., 2007; Lombardi et al., 2008, 2009; Subileau et al., 2009; Ifergan et al., 2006; Chui and Dorovini-Zis, 2010). These chemokines promote both adhesion of leukocytes to endothelial cells (indirectly through avidity-maturation of integrins) and migration of leukocytes across BBB-ECs (Holman et al., 2011), causing firm adhesion, crawling, polarization and extravasation of T cells across the BBB (Engelhardt, 2006).

According to the neurovascular mechanisms and disruption phenomena in which the BBB is involved and which it may precede, accelerate or play a part in the chronic disease processes, how can the BBB be protected?

Specific targets for preserving and restoring BBB functionality could include transport of leukocytes across the activated BBB, BBB breakdown, decreased release of proinflammatory cytokines and antioxidant therapy.

New therapies based on blocking transport of central memory T cells, effector memory T cells, and activated monocytes with Natalizumab (therapeutic neutralizing monoclonal antibody to a4 integrin) across the BBB have been reported (Ransohoff, 2007). T cells express a4b1 integrins on the cell surface, and their transport across the BBB into the cerebrospinal fluid (CSF) and into the MS lesions is blocked by Natalizumab. Natalizumab binds and inactivates the integrin molecule in leukocytes. Therapeutic effects include dramatic reductions of the BBB breakdown in recipients with active MS.

At the BBB level, a number of carrier-mediated transporters, such as those for neuropeptides, choline, thyroid hormones, vitamins, and nucleobases, contribute to susceptibility or progression of neurodegeneration, then manipulation of such transport systems and barrier function may offer neuroprotective and treatment strategies. Future challenges will include the understanding of the crosstalk between non-neuronal cell types (e.g., glia and microglia), cells of the vessel wall (e.g., endothelium and pericytes), and neurons. Moreover the crosstalk between neurons and peripheral hematopoetic cells, vascular niches, and neurogenic loci in the brain should also be investigated. Identifying how these cells respond to, process, synthesize different receptors, and identify the ligands that mediate their interactions, are critical to understanding how these cells regulate the neuronal milieu (Zlokovic, 2008).

Glatiramer acetate (La Mantia et al., 2010), a synthetic polymer of amino acids, denoted Copolymer 1 (Cop 1), composed of L-alanine, L-lysine, L-glutamic acid and L-tyrosine, own several immunological mechanisms to prevent the interaction of myelin basic protein (MBP)