Journal of Autoimmunity 54 (2014) 81–92

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Review

Immunomodulation neuroprotection and remyelination – The fundamental therapeutic effects of glatiramer acetate: A critical review

Rina Aharoni*

Department of Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel

ARTICLE INFO

Article history:
Received 20 May 2014
Accepted 23 May 2014
Available online 14 June 2014

Keywords:
Multiple sclerosis (MS)
Experimental autoimmune encephalomyelitis (EAE)
Immunomodulation
Remyelination
Neuroprotection
Autoimmune related pathologies

ABSTRACT

Multiple sclerosis (MS) is a multifaceted heterogeneous disease with various patterns of tissue damage. In addition to inflammation and demyelination, widespread axonal and neuronal pathologies are central components of this disease. MS therapies aim to restrain the pathological processes, enhance protective mechanisms, and prevent disease progression. The amino acid copolymer, glatiramer acetate (GA, Copaxone), an approved treatment for MS, has a unique mode of action. Evidence from the animal model experimental autoimmune encephalomyelitis (EAE) and from MS patients indicates that GA affects various levels of the innate and the adaptive immune response, inducing deviation from the pro-inflammatory to the anti-inflammatory pathways. This includes competition for the binding of antigen presenting cells, driving dendritic cells, monocytes, and B-cells towards anti-inflammatory responses, induction of Th2/3 and T-regulatory cells, and downregulating of both Th1 and Th-17 cells. The immune cells induced by GA reach the inflamed disease organ and secrete *in situ* anti-inflammatory cytokines alleviating the pathological processes. Furthermore, cumulative findings have revealed that in addition to its immunomodulatory activities GA promotes neuroprotective repair processes such as neurotrophic factors secretion and remyelination. This review aims to provide a comprehensive overview on the diverse mechanism of action of GA in EAE/MS, in particular on the *in situ* effect of GA and its ability to generate neuroprotection and repair in the CNS. In view of its immunomodulatory activity, the beneficial effects of GA in various models of additional autoimmune related pathologies, such as immune rejection and inflammatory bowel disease (IBD), are also presented.

1. Multiple sclerosis

1.1. Clinical course and etiology

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS), and one of the most prevalent neurological disorders leading to chronic disability among young adults [1,2]. The disease is heterogeneous in its clinical manifestation and progression, as well as in its pathological mechanisms. The most typical clinical progression pattern is a phase of relapsing and remitting symptoms (relapsing remitting MS, RRMS) that frequently develops to a progressive disease course (secondary progressive MS, SPMS). A fraction of patients shows disease progression from the onset (primary progressive MS, PPMS) which represents a somewhat different pathology [3].

The defined etiology of MS is still unknown, but it is established that complex interactions between environmental factors and multiple genes are involved. Epidemiologic data has identified several infectious and non-infectious factors associated with increased MS risk, of which Epstein–Barr virus (EBV) infection and vitamin D deficiency have gained the greatest corroboration for putative roles in MS pathogenesis [4-7]. Molecular mimicry has been postulated as the initial trigger by which an infectious agent may induce autoimmune tissue damage [8]. Genetic risk factors are mostly confined to the major histocompatibility complex (MHC) class II genes, in particular HLA-DR and HLA-DQ [1,9]. MHC class I alleles as well as genes outside the MHC locus, such as genes encoding for the IL-2 receptor and IFN-γ, were also linked to the disease. Genetic studies in large populations obtained through international collaborations identified a list of more than 50 risk-gene regions associated with MS. The risk associated with each

* Tel.: +972 8 9342997; fax: +972 8 9344141.
E-mail address: rina.aharoni@weizmann.ac.il.

http://dx.doi.org/10.1016/j.jaut.2014.05.005
0896-8411/

individual locus is low, however, collectively they point to the involvement of immune pathways in the etiology of MS [10].

1.2. Animal models of MS

The most intensively studied model of MS is experimental autoimmune encephalomyelitis (EAE). It was discovered as a rare complication of rabies vaccinations when some people developed neurological symptoms upon immunization with desiccated spinal cords of rabies-infected rabbits. The adjuvant-based model, in which rodents or primates are immunized against CNS antigens [11], has been used for about 80 years. Initially brain emulsion or spinal cord homogenate were injected as the encephalitogenic material. Subsequently, various myelin antigens such as myelin basic protein (MBP), myelin proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), and their encephalitogenic regions have been used [12,13]. Applying novel analytical techniques, a variety of additional antigens involved in the pathogenesis of EAE and MS were identified. Some of them are myelin constituents, such as neurofascin NF 155 [14], and some are non-myelin antigens, such as the neuronal cytoskeletal protein neurofilament (NF)-M [15]. As an alternative to the "active EAE model", autoreactive T-cells obtained from immunized animals can be adoptively transferred to naïve animals thus creating a "passive" EAE model.

The availability of multiple inbred mice strains facilitated the development of a wide spectrum of EAE models. Different clinical manifestations can be obtained depending on the genetic background and the antigen used for immunization. Thus, the characteristic clinical symptoms manifested as ascending flaccid paralysis can occur in an acute monophasic manner (e.g. in SJL/J mice immunized by peptide 89–101 of MBP) [16], chronic manner (e.g. in C57BL/6 mice immunized by peptide 35–55 of MOG) [17] or in a relapsing remitting manner (e.g. in SJL/J mice by peptide 139–151 of PLP) [18]. Importantly, different pathological patterns are involved in different EAE models. The relapsing-remitting model is characterized by widespread myelin damage, whereas the chronic model is characterized by axonal and neuronal damage [18]. A major difference between MS and EAE is that whereas MS occurs spontaneously, the classic EAE models require external immunization, mostly with adjuvant enriched by bacterial components which heavily boost the immune system. A breakthrough in this respect was the development of transgenic mice strains that manifest spontaneous EAE. In particular, the MOG 35-55 specific TCR-transgenic strain on a C57BL/6 background (2D2) is widely used in multiple studies and combined models [19].

Another category of animal models used to study MS is those induced by toxic agents, like the copper chelator cuprizone, that cause demyelination with a relative absence of inflammation or axonal damage [20]. This model is employed to investigate principal features of de- and re- myelination in the CNS, but due to the lack of an autoimmune inflammatory component, it poorly resembles MS. Viral models, such as Theiler's murine encephalomyelitis virus (TMEV), are used in view of the suspected role of viruses in MS pathogenesis. Intra cerebral inoculation of TMEV results in an early subtle disease phase. Thereafter, susceptible mouse strains develop brain and spinal cord inflammation, demyelination and axonal damage [21]. The versatile animal models, in particular the various EAE forms, have been highly valuable tools for studying pathological mechanisms involved in MS and for drug development.

1.3. Pathological hallmarks

Traditionally, MS has been considered an autoimmune disease in which an aberrant immune response leads to an inflammatory attack on the myelin component in the CNS. Immune cells of both the adaptive and innate systems are involved in the inflammatory network that mediates the disease [1,22–24]. T-helper (Th)-1 and Th-17 cells, cytotoxicT-cells, B-cells and macrophages enter the CNS through the blood brain barrier (BBB) and the plexus choroideus, secreting pro-inflammatory cytokines, chemokines and other inflammatory substances [25–28]. The penetration of inflammatory cells into the CNS, especially in the relapsing-remitting stage, is associated with profound disturbances of the BBB as detected by contrast-enhanced MRI [29]. Microglia, the resident CNS antigen presenting cells, are stimulated upon tissue damage and further facilitate T-cell activation [30]. All these cell populations maintain the inflammatory milieu and mediate tissue injury. Immune cells such as Th2/3 and T-regulatory (Treg) cells that mediate anti-inflammatory protective pathways could potentially suppress the disease. However, reduced amounts of Th2/3 and Foxp3 expressing Treg cells were found in active lesions of MS brains [31]. Furthermore, Treg cells were shown to have functional defects in MS patients and even secrete pro-inflammatory cytokines such as INF-γ and IL-17 [32,33].

The immune attack leads to the destruction of the myelin sheaths and to the formation of the primary MS hallmark, sclerotic demyelinated lesions (plaques). The lesions typically display inflammation, demyelination, oligodendrocyte loss, and variable degrees of axonal loss [34,35]. Astrocytic activation is also a characteristic feature of the lesions, resulting in the formation of a glial scar [36]. In addition, diffused molecular and cellular changes in the normal-appearing white matter (NAWM) have recently been recognized as a component of MS pathology. Indeed, profiling of normal-appearing white matter from MS brains revealed upregulation of genes associated with high oxidative stress and inflammation [37,38]. It was therefore suggested that endogenous inflammatory reactions throughout the entire white matter affect and facilitate lesion formation in early as well as in chronic MS. Cortical demyelination is common in progressive MS, and correlates with irreversible disability and cognitive impairment [3,24,39–42]. Furthermore, grey matter pathology is not restricted to the chronic stage and may even precede the appearance of white matter plaques [41–43].

MS was traditionally considered an inflammatory demyelinating disease, which leaves the axons largely intact. However, accumulated findings indicate that neurodegeneration, defined as a relentless process of death and decay of neural structures, plays an important role in the pathogenesis of MS. During the disease development, demyelinated axons are prone to degeneration due to lack of trophic support and increased vulnerability to the immune attacks. Neuronal damage ranges from subtle changes, such as the loss of dendritic ramification, to direct cell death [44,45]. In MOG-induced EAE, axonal degeneration, motor neuron loss, and alterations in diffusion tensor imaging (DTI) and magnetization transfer ratio (MTR) indicate structural tissue damage that may account for the chronic clinical course [18,46]. In MS patients, accumulation of damage markers, such as amyloid precursor protein (APP) as well as increased nonphosphorylated neurofilament-positive ovoids have been demonstrated [47]. Significant fiber transection was detected even in patients with a short disease duration, indicating that axonal loss can occur at MS onset [48]. Novel imaging methodologies have revealed constant reduction in whole-brain volume (atrophy) indicating tissue loss, evolution of persistent T1-hypointense lesions (black holes), and changes in MTR and DTI parameters indicating a reduction in structural tissue integrity [49]. MRI spectroscopy has shown a decline in the resonance intensity of the neuronal metabolite N-acetyl-aspartate, a marker of axonal and neuronal integrity [49]. These findings support the recognition of the neurodegenerative aspects of MS.