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ASSOCIATION OF MYELIN PEPTIDE WITH VITAMIN D PREVENTS AUTOIMMUNE ENCEPHALOMYELITIS DEVELOPMENT

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Abstract—Multiple sclerosis is a chronic, inflammatory and demyelinating disease of the central nervous system (CNS). As there is no cure for this disease, new therapeutic strategies and prophylactic measures are necessary. We recently described the therapeutic activity of the association between myelin oligodendrocyte glycoprotein peptide (MOG) and active vitamin D3 (VitD) against experimental autoimmune encephalomyelitis (EAE). The objective of this work was to evaluate the prophylactic potential of this association in EAE. C57BL/6 mice were vaccinated with MOG in the presence of VitD and then subjected to EAE induction. Animals were euthanized 7 and 19 days after disease induction and the following parameters were evaluated: body weight, clinical score, inflammatory process in the CNS, amount of dendritic cells (DCs) and regulatory T cells in the spleen and cytokine production by spleen and CNS cell cultures. Vaccination with MOG associated with VitD determined a drastic reduction in clinical score, body weight loss, CNS inflammation, DCs maturation and also in the production of cytokines by CNS and spleen cell cultures. Collectively, our data indicate that this association prevents EAE development. A similar effect from specific self-antigens associated with VitD is expected in other autoimmune conditions and deserves to be experimentally appraised.

Key words: multiple sclerosis, experimental autoimmune encephalomyelitis, myelin oligodendrocyte glycoprotein, vitamin D3, tolerogenic adjuvant.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) affecting mainly young people (Ellwardt and Zipp, 2014). According to a recent update, the estimated number of people with MS is 2.3 million worldwide (Browne et al., 2014). Although etiology and immunopathogenesis of this disease are still not entirely elucidated, it is believed that this pathology is mainly mediated by Th1 and Th17 subsets (Sospedra and Martin, 2005; Luchtman et al., 2014). Cytokines such as interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α) and interleukin (IL)-17, produced by these T cell subsets, mediate inflammation and subsequent axonal degeneration, oligodendrocyte death and neuronal dysfunction (Lucchinetti et al., 2000; Sospedra and Martin, 2005; Furuzawa-Carballeda et al., 2007). Defects in the functional activity of regulatory T cells (Tregs) (CD4 + CD25 +) have been described in MS patients (Viglietta et al., 2004). Genetic predisposition and environmental factors also contribute to MS initiation and development (Lin et al., 2012; Krementsov and Teuscher, 2013).

Experimental autoimmune encephalomyelitis (EAE) has been extensively used to elucidate the pathophysiology and the potential therapeutic measures applied to MS (França et al., 2014; Zorzella-Pezavento et al., 2014; Rahimi et al., 2015). Current therapeutic approaches to control the destructive immune response in autoimmune disease are mainly based on non-specific drugs that systemically suppress the function of many immune effector cells. This extensive immunosuppression often causes serious and sometimes life-threatening side effects (Damal et al., 2013). Therefore, the need for more specific treatments resulting in lower toxicity and long-term effectiveness is highly desirable. Tolerogenic vaccines comprise a new class of vaccines, designed to re-establish immunological tolerance and thereby theoretically able to reverse autoimmune diseases. Substantial advances have been made in the generation of these vaccines that inhibit EAE in a preclinical setting. Some of the most relevant and recent findings in this context include dendritic cells (DCs) vaccines (van Brussel et al., 2014), myelin oligodendrocyte glycoprotein (MOG)-DNA constructions (Fissolo et al., 2012), cytokine-neuroantigen fusion proteins (Mannie et al., 2012) and polymeric biodegradable lactic-glycolic acid particles loaded with MOG plus IL-10 (Cappellano et al., 2014).

An alternative and very straightforward approach could be based on the concept of tolerogenic adjuvants.

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Abbreviations: CFA, complete Freund's adjuvant; CNS, central nervous system; DCs, dendritic cells; EAE, experimental autoimmune encephalomyelitis; Foxp3, forkhead box P3; IFN-γ, interferon gamma; IL, interleukin; MHC-II, major histocompatibility complex class II; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; RPMI, Roswell Park Memorial Institute; TNF-α, tumor necrosis factor alpha; Tregs, regulatory T cells; VitD, 1α,25-dihydroxyvitamin D3.

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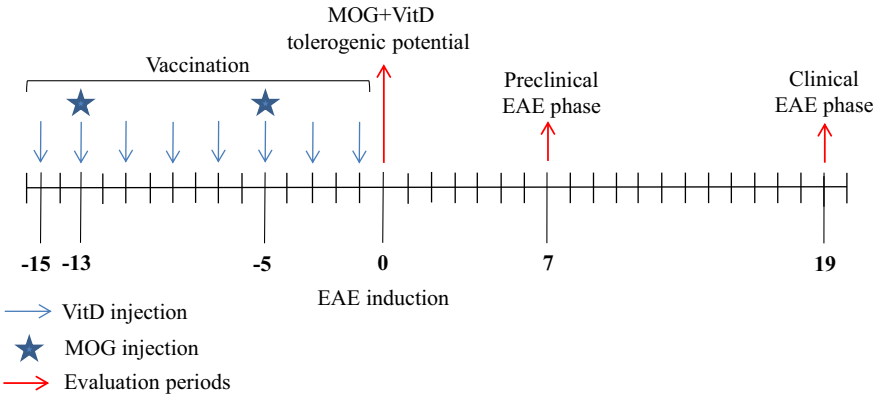
In contrast to the conventional immunogenic adjuvants that intensify the immune response, the so-called tolerogenic adjuvants have the ability to suppress or modify the specific immune response when associated with specific antigens. This concept and its functional application to induce T cell tolerance in autoimmune diseases was conceived by Kang et al. (2008). These authors demonstrated that dexamethasone combined with an insulin peptide and FK506 associated with MOG were prophylactic in diabetes and encephalomyelitis, respectively (Kang et al., 2008, 2009). In this context, we hypothesized that active vitamin D3 (VitD) could also behave as a tolerogenic adjuvant if associated with a specific antigen. This possibility was raised by strong evidences that VitD is able to modulate the immune response at both, innate and adaptive levels. It is capable, for example, to promote antimicrobial response by macrophages through the induction of antibacterial proteins (Wang et al., 2004). On the other hand, it suppresses inflammation and promotes immune tolerance by affecting antigen presentation and T cell proliferation and differentiation (Chun et al., 2014).

In the present study we evaluated if vaccination with MOG in the presence of VitD could be prophylactic and therefore prevent autoimmune encephalomyelitis development. This procedure prevented disease development and triggered reduction in DC maturation, number of Foxp3+ Tregs and proinflammatory cytokine production by spleen and CNS cell cultures. An increased production of TGF-β was observed in the spleen and CNS cell cultures.



EXPERIMENTAL PROCEDURES

Experimental design

Initially, to assess VitD tolerogenic potential, mice were allocated to four groups: 1. CTL group (negative control group) that received only saline by i.p. route; 2. MOG group that was injected only with 2 MOG doses (150 μg by i.p. route); 3. VitD group that was injected only with 8 VitD doses and 4. MOG + VitD group that was injected with both, i.e., 8 VitD doses and 2 MOG doses. VitD tolerogenic potential was tested one day after the last VitD dose and included the evaluation of cytokines, DCs and Tregs determinations. Body weight and serum calcium levels were also measured. To determine the effect of MOG + VitD vaccination strategy in EAE clinical development, mice were first vaccinated with MOG + VitD and then subjected to active induction of EAE (MOG35-55 + Complete Freund's Adjuvant (CFA)). In this case mice were allocated to the following four groups: 1. EAE group (subjected to EAE induction only); 2. MOG/EAE group; 3. VitD/EAE group; 4. M + V/EAE group. Groups 2, 3 and 4 were injected with MOG, VitD or MOG + VitD respectively before EAE induction. Evaluation was performed 19 days after EAE induction, i.e., during the acute encephalomyelitis phase. The following parameters were evaluated: clinical score, body weight and histopathological analysis of the CNS. The effect of vaccination on specific immunity was assessed 7 and 19 days after EAE induction, that is, at the pre-clinical and clinical disease phases, respectively. For this mice were allocated to two groups: 1. EAE group that was subjected to EAE induction and 2. M + V/EAE group that was vaccinated with MOG in the presence of VitD before being subjected to EAE induction. The effect of this vaccination strategy was assessed by cytokine production by spleen cells during pre-clinical and clinical disease phases and also by cytokine production by CNS cells during the clinical disease stage. This experimental design is illustrated below by a timeline scheme.

Animals

Female C57BL/6 mice 5–6 weeks old were purchased from University of São Paulo (USP, Ribeirão Preto, SP, Brazil). The animals were manipulated in accordance with the Ethics Committee for Animal Experimentation – Institute of Bioscience of Botucatu, Universidade Estadual Paulista (protocol number 571).

Vaccination with MOG in the presence of VitD

Mice were i.p. injected with 0.1 μg of VitD from Sigma (St. Louis, MO, USA), every other day during 15 days (on days −15, −13, −11, −9, −7, −5, −3 and −1). On days −15 and −5 the animals were also injected by i.p. route with 150 μg of MOG_{35-55} peptide (MEVGWYRSPFSRVVHLYRNGK) synthesized by Genemed Synthesis Inc. (San Antonio, TX, USA). Mice injected only with VitD or only with MOG were used as