Clinical Immunology (2015) **160**, 133–141

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Breast regression protein-39 is not required for experimental autoimmune encephalomyelitis induction

Ester Cantó [1], Carmen Espejo*,[1], Carme Costa, Xavier Montalban, Manuel Comabella**

Servei de Neurologia-Neuroimmunologia, Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Institut de Recerca Vall d'Hebron (VHIR), Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain
Universitat Autònoma de Barcelona, 08193 Bellaterra, Cerdanyola del Vallès, Spain

Received 25 March 2015; accepted with revision 7 June 2015
Available online 12 June 2015

KEYWORDS
EAE;
BRP-39;
Multiple sclerosis

Abstract Increasing evidence points to a role for chitinase 3-like 1 (*CHI3L1*) in multiple sclerosis (MS). Here, we aimed to explore the potential involvement of *CHI3L1* in the animal model of MS, experimental autoimmune encephalomyelitis (EAE). EAE was induced by immunization with MOG_{35-55} peptide in wild-type (WT) and knock-out (KO) mice for breast regression protein 39 (*BRP-39*), the mouse homologue of human *CHI3L1*. Immunological responses in splenocytes were assessed by means of polyclonal and antigen-specific proliferation assays. Central nervous system pathology and chitinase gene expression were also investigated. *BRP-39* expression was increased in WT MOG_{35-55}-immunized mice compared to saline-immunized controls. No differences were found between WT and *BRP-39* KO mice regarding EAE clinical course, day of disease onset, mortality rate, splenocyte proliferative responses or histopathological findings. These results do not support a role of BRP-39 in the pathogenesis of EAE.

Abbreviations: AMCase, acidic mammalian chitinase; BRP-39, breast regression protein 39; CHI3L1, chitinase 3-like 1; CHI3L3, chitinase 3-like 3; CHIT-1, chitotriosidase; CIS, clinically isolated syndrome; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; GFAP, glial fibrillary acidic protein; HE, hematoxylin and eosin; KB, Klüver–Barrera; KO, knock-out; LEA, lycopersicum esculentim agglutinin; LPS, lipopolysaccharide; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; PBS, Phosphate buffered saline; PHA, phytohemagglutinin; p.i, post-immunization; WT, wild-type.

* Correspondence to: C. Espejo, Servei de Neurologia-Neuroimmunologia, Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Vall d'Hebron Institut de Recerca (Ed. Mediterrània, Lab. 115), Pg Vall d'Hebron 119-129, 08035 Barcelona, Spain. Fax: +34 932746084.
** Correspondence to: M. Comabella, Servei de Neurologia-Neuroimmunologia, Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Vall d'Hebron Institut de Recerca (Ed. Mediterrània, Lab. 114), Pg Vall d'Hebron 119-129, 08035 Barcelona, Spain. Fax: +34 932746084.
E-mail addresses: carmen.espejo@vhir.org (C. Espejo), manuel.comabella@vhir.org (M. Comabella).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.clim.2015.06.004
1521-6616/

1. Introduction

Mouse breast regression protein 39 (*BRP-39*) and its human homologue Chitinase 3-like 1 (*CHI3L1*) are members of the mammalian chitinase family, which is a family of proteins containing a glyco-18 domain. For these proteins, chitin is the only documented substrate. Only chitotriosidase (*CHIT-1*) and acidic mammalian chitinase (*AMCase*) have demonstrated a chitinolytic activity, while chitinase-like proteins can bind chitin, but do not have chitinolytic activity [1].

CHI3L1 is produced by a variety of cells including chondrocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, hepatic stellate cells, airway epithelial cells, neutrophils, inflammatory macrophages and different types of cancer cells [2–9]. Regarding the central nervous system (CNS), its expression has also been described in astrocytes and microglia of encephalitic monkeys and human immunodeficiency virus encephalitis patients as well as in samples of human brain infarction [10,11]. In a recent study conducted by our group, CHI3L1 expression was found in reactive astrocytes and microglia of chronic active lesions in multiple sclerosis (MS) patients, but not in non-neurological controls [12].

Increased levels of CHI3L1 have been reported in a wide variety of disorders such as rheumatoid inflammatory bowel disease [13], arthritis [14], systemic lupus erythematosus [15], asthma [16], sarcoidosis [17], atherosclerosis [18] and type 2 diabetes [19], which are characterized by chronic inflammation, and circulating levels of the protein correlated in some cases with disease activity and severity [13–17, 19,20]. High levels of CHI3L1 have been previously reported in plasma, serum and CSF of MS patients [11,21,22]. Additionally, CHI3L1 levels were found increased in cerebrospinal fluid (CSF) samples of patients with clinically isolated syndrome (CIS) who later developed MS [12,23], supporting the idea of a prognostic role of this protein in the disease. In experimental animal models, both *BRP-39* knock-out (KO) mice and mice overexpressing *BRP-39* have shown potential roles for this protein in the induction of inflammatory responses in a murine allergic asthma model and in a model of cigarette-smoking emphysema [24–26]. In the present study, we aimed to investigate the role of *BRP-39* in the outcome of experimental autoimmune encephalomyelitis (EAE), the animal model of MS.

2. Materials and methods

2.1. Animals

Eight- to twelve-week-old female and male *BRP-39* KO mice on a C57BL/6 background (kindly provided by JA Elias, Yale University School of Medicine, New Haven, [24]) and their wild-type (WT) littermates were maintained on a 12 h light–dark cycle with food and water provided *ad libitum*. All experiments were performed in strict accordance with European Union and governmental regulations (Generalitat de Catalunya Decret 214/97 30th July). The Ethics Committee on Animal Experimentation of the Vall d'Hebron Research Institute approved all procedures described in this study (protocol number: 38/09 CEEA).

2.2. EAE induction and clinical follow-up

Anesthetized female and male mice were immunized by subcutaneous injections of phosphate buffered saline (PBS) containing 50 μg of $MOG_{35–55}$ (Proteomics Section, Universitat Pompeu Fabra, Barcelona, Spain) emulsified in CFA (Sigma Chemical, Saint Louis, MO, USA) containing 3 mg/ml *Mycobacterium tuberculosis* H37RA (Difco Laboratories, Detroit, MI). On days 0 and 2 post-immunization (p.i.) mice received 150 ng of *Bordetella pertusis* toxin (Sigma Chemical) intravenously. Mice immunized in the same way using PBS without the peptide were included as controls. All animals were weighed and examined daily for neurological signs according to the following criteria: 0—no clinical signs; 0.5—partial loss of tail tonus for two consecutive days; 1—paralysis of whole tail; 2—mild paraparesis of one or both hind limbs; 2.5—severe paraparesis of hind limbs; 3—mild tetraparesis; 4—tetraparesis; 4.5—severe tetraparesis; 5—tetraplegia; 6—death (modified from [27]). Score 5 and weight loss > 30% were defined as endpoint criteria to minimize the suffering and to guarantee the animal welfare. Mice meeting endpoint criteria were euthanized and were assigned a score of 6 until the end of the experiment. Experiments were performed in a blinded manner. All data presented are in accordance with the guidelines suggested for EAE publication [28].

2.3. Splenocyte proliferation assay

On days 12–16 after EAE induction, a total of 14 WT and 15 *BRP-39* KO mice were euthanized with CO_2 (>70%) and spleens were removed. Spleen cell suspensions were prepared by grinding the spleens through a nylon mesh and cultured in 96-well plates at 2×10^5 cells/well in a total volume of 200 μl of Iscoves modified Dulbecco's medium (IMDM) (PAA Laboratories GmbH, Pasching, Austria) supplemented with 10% HyClone FetalClone I (Thermo Fisher Scientific, Waltham, MA, USA), 50 μmol/l of 2-mercaptoethanol (Sigma Chemical), 2 mmol/l of glutamine, 50 U/ml of penicillin and 50 mg/ml of streptomycin, all obtained from Gibco BRL (Paisley, UK). Cultures (five replicas) were stimulated with 5 μg/ml of $MOG_{35–55}$, 5 μg/ml of PHA (Sigma Chemical), or 15 μg/ml of LPS (Sigma Chemical), alone or with 50 ng/ml recombinant BRP-39 (mouse chitinase 3-like 1, R&D, Minneapolis, USA). Cells cultured without any stimulus were used as baseline controls. Cultures were incubated in a humidified atmosphere at 5% CO_2 and 37 °C for 48 h. After stimulation, cells were cultured for additional 18 h in the presence of 1 μCi/well of [3H]thymidine (Perkin Elmer, Waltham, MA, USA). Cells were then harvested by aspiration onto glassfiber filters and levels of incorporated radioactivity were determined using a scintillation counter *2450 Microplate counter MicroBeta$^2_{TM}$ (Wallac, Turku, Finland). Proliferation index was expressed as mean of counts per minute (cpm) of the replicas from the stimulated cultures divided by the cpm mean of baseline control cultures.*

2.4. *BRP-39*, *CHI3L3*, *CHIT-1* and *AMCase* gene expression

Mouse spinal cords were obtained at days 12 and 31 after immunization by insufflation with PBS, snap-frozen in liquid