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Unaltered regulatory B-cell frequency and function in patients with multiple sclerosis

Laure Michel[a,b,c,1], Melanie Chesneau[a,e,1], Philippe Manceau[a], Athenais Genty[a], Alexandra Garcia[a], Marion Salou[a], Annie Elong Ngono[a], Annaïck Pallier[a,e], Marylène Jacq-Foucher[b,c], Fabienne Lefrère[b,c], Sandrine Wiertlewski[b,c], Jean-Paul Soulillou[a,d,e], Nicolas Degauque[a,d,e], David-Axel Laplaud[a,b,c,d,2], Sophie Brouard[a,d,e,*,2]

[a] INSERM, UMR 1064, Nantes, F-44093, France
[b] CHU Nord Laennec, Service de Neurologie, Nantes, F-44093, France
[c] INSERM, CIC 004, Nantes, F-44093, France
[d] Universite de Nantes, Faculte de Medecine, Nantes, F-44035, France
[e] CHU de Nantes, ITUN, Nantes, F-44093, France

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Abstract Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) typically characterized by the recruitment of T cells into the CNS. However, certain subsets of B cells have been shown to negatively regulate autoimmune diseases and some data support a prominent role for B cells in MS physiopathology. For B cells in MS patients we analyzed subset frequency, cytokine secretion ability and suppressive properties. No differences in the frequencies of the B-cell subsets or in their ability to secrete cytokines were observed between MS and healthy volunteers (HV). Prestimulated B cells from MS patients also inhibited $CD4^{+}CD25^{-}$ T cell proliferation with a similar efficiency as B cells from HV. Altogether, our data show that, in our MS patient cohort, regulatory B cells have conserved frequency and function.

Abbreviations: MS, multiple sclerosis; CNS, central nervous system; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; HV, healthy volunteers; RR-MS, relapsing-remitting multiple sclerosis; PP-MS, primary-progressive multiple sclerosis; SP-MS, secondary-progressive multiple sclerosis; BFA, Brefeldin-A.

* Corresponding author at: INSERM 1064, CHU Hôtel Dieu, 30 Bd Jean Monnet, Nantes, 44093, France. Fax: +33 240087411.
E-mail address: Sophie.brouard@univ-nantes.fr (S. Brouard).

[1] Both authors equally contributed to this work.
[2] Both senior authors equally contributed to this study.

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1. Introduction

Multiple sclerosis (MS) is classically thought to be initiated by autoreactive T cells recognizing peptides on myelin sheath proteins [1,2]. However, there is no compelling evidence that the frequency of autoreactive cells is higher in the periphery in MS vs. healthy volunteers and the hypothesis remains controversial [3–20]. Currently, some lines of enquiry suggest a prominent role for B cells in MS physiopathology. While the intrathecal synthesis of immunoglobulins in the CSF of sufferers is a well-known feature of MS, Serafini et al. identified the additional presence of B-cell follicles in the brain meninges of patients [21,22]. Furthermore, peripheral B-cell depletion using anti-CD20 monoclonal antibodies results in a significant improvement in the clinical and radiological inflammatory criteria of MS patients [23,24] that has been associated with the homeostasis of B cells after depletion, favoring a repopulation by B cells with regulatory properties [25].

It has recently emerged that specific subsets of B cells may negatively regulate disease symptoms in mouse models of inflammation, cancer, infection and autoimmunity [26]. In experimental autoimmune encephalomyelitis (EAE), the animal model of MS, B cell μMT-deficient mice (B cells deficient in the μ-chain transmembrane region) have higher EAE severity during the late phase of the disease compared to wild-type mice and do not fully recover [27]. Adoptively transferred wild-type B cells normalize EAE severity in μMT mice, suggesting the existence of a regulatory B-cell sub-population [28]. While the hallmark of these B-cell subsets is the secretion of IL-10, they also have been shown to exert their suppressive properties by other mechanisms: via the secretion of TGFβ [29], granzyme B [30] and/or by contact-dependent mechanisms [31,32].

In humans, some regulatory B cells have recently been identified. However, the study of these cells is still in its infancy and their phenotype and mechanisms of their action need more comprehensive description. In MS, several studies have reported a decreased secretion of IL-10 by B cells [25,33,34], but these results remain contested [35] and the function of these cells has never fully been explored. The aim of our study is to characterize the frequency and functional properties of regulatory B cells (Bregs) in the blood of MS patients by comparison with healthy volunteers (HV). We show that cytokine secretion following a 48 h stimulation of B cells was the same for our MS patients and HV. Moreover, these prestimulated B cells from MS patients were able to inhibit $CD4^+CD25^-$ responding T cell proliferation in vitro in the same manner as B cells from HV. We show that this suppressive B-cell activity is independent of IL-10 and TGFβ but dependent on contact between the T and B cells. Altogether, our data do not suggest that a lack of peripheral B-cell regulation contributes to the physiopathology of MS, at least in our cohort of patients.

2. Patients, materials and methods

2.1. Patients and healthy volunteers

The 63 patients included in the study suffered from MS defined by the revised MacDonald criteria 2005, as indicated in Table 1 [36]. Different forms of MS were included: relapsing-remitting (RR-MS, $n = 47$), primary progressive (PP-MS, $n = 10$) and secondary progressive MS (SP-MS, $n = 11$). Fourteen patients were suffering relapses at the time of sampling. The mean age was 43.1 ± 1.5 years (range: 18–73 years). All patients had been without treatment for at least 3 (immunomodulatory treatment) or 6 months (immunosuppressant drugs). Some of the patients, being recently diagnosed, had not yet been treated, some were refusing treatment, and others had low disease activity and did not wish to be treated.

Sixty-one healthy volunteers were studied in parallel as controls. The mean age, 39 ± 1.7 years (range: 18–68 years), was not significantly different from that of the MS patients. Patients and HV were recruited after obtaining informed consent. The University Hospital Ethical Committee and the Committee for the Protection of Patients from Biological Risks approved the study.

2.2. Human cell isolation and phenotype analysis

Patient and HV blood samples (100 ml) were always collected at the same time and used for parallel experiments. Fresh PBMCs were separated on a Ficoll (Eurobio®) gradient layer. 10^7 PBMCs were stained with anti-CD19-PC7 (clone SJ25C1), anti-CD24-PE (clone ML5), anti-CD38-FITC (clone HIT2), anti-CD5 APC (clone UCHT2), anti-CD27-alexa700 (clone M-T271) and anti-CD10-APC (clone HI10a) (BD Biosciences, San Jose, USA). Viability staining was performed using the live/dead cell aqua staining kit (Life Technologies, Invitrogen, Carlsbad, USA). Cells were analyzed using a LSRII flow cytometer (BD Immunocytometry Systems) and data were analyzed using FlowJo Version 9.0.1 (TreeStar).

2.3. Cell culture

B cells were purified using the B-cell isolation Kit II, Human (Miltenyi, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Purity was higher than 94%. B cells were then seeded in complete RPMI 1640 in 96-well U-bottom plates at a final concentration of 10^6 cells/ml. Stimulation using CD40 ligand (1 μg/ml, RD Systems, Minneapolis, USA) and CpG oligonucleotide (ODN 2006, 10 μg/ml) was performed for 48 h at 37 °C, 5% CO_2 PMA (250 ng/ml). Ionomycin (1 μg/ml) and Brefeldin-A (10 μg/ml) (Sigma-Aldrich, St Louis, USA) were added for the last 5 h of culture. Culture supernatants were removed and stored after 48 h to wells in which BFA had not been added. Control B cells were cultured for 48 h without stimulation.

2.4. Analyses of B-cell cytokine production and phenotype of IL-10^+ B cells

After the 48 h of culture, viability staining was first performed using the live/dead cell staining kit aqua (Invitrogen, Life Technologies). B lymphocytes were then stained with anti-CD19-PC7, anti-CD24 FITC (clone ML5), anti-CD27-alexa700, anti-CD38 PC5 (clone HIT2), and anti-CD5-APC (BD Biosciences). Cells were then washed, fixed, and permeabilized using the permeabilization/fixation kit (BD Biosciences). Fc gamma receptor inhibitor (Ebiosciences, San Diego, USA) was used to avoid non-specific staining. The different IL-10, IL-2,