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B cells of multiple sclerosis patients induce autoreactive proinflammatory T cell responses

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ABSTRACT

Antibody-independent B cell functions play an important role in multiple sclerosis (MS) pathogenesis. In this study, B cell antigen presentation and costimulation in MS were studied. Peripheral blood B cells of MS patients showed increased expression of costimulatory CD86 and CD80 molecules compared with healthy controls (HC). In MS cerebrospinal fluid (CSF), 12-fold and 2-fold increases in $CD86^+$ and $CD80^+$ B cells, respectively, were evidenced compared with peripheral blood. Further, B cells from MS patients induced proinflammatory T cells in response to myelin basic protein (MBP). Immunomodulatory treatment restored B cell costimulatory molecule expression and caused significantly reduced B cell induced T cell responses. Together, these results demonstrate the potential of B cells from MS patients to induce autoreactive proinflammatory T cell responses. Immunomodulatory therapy abrogated this effect, emphasizing the importance of B cell antigen presentation and costimulation in MS pathology.

1. Introduction

MS is an inflammatory demyelinating disease of the central nervous system (CNS). Different clinical subtypes exist, including relapsing-remitting MS (RRMS) in which clinical relapses alternate with remission (80–85%), secondary progressive MS (SPMS) with progressive neurological disease in 90% of RRMS patients within 25 years and primary progressive MS (PPMS) with a progressive course from the onset (10–15%) [1]. Research into the role of B cells in MS pathogenesis has mainly focused on the production of autoantibodies. Oligoclonal immunoglobulin bands in the cerebrospinal fluid (CSF) are used for diagnostic confirmation [2]. Several CNS autoantigens that are targeted by the humoral immune response in MS have been described, although disease specificity and pathologic potential of most of the reported autoantibodies is debatable [3]. Antibody-independent B cell functions have gained interest in regard to MS pathology due to the clinical benefit of the B cell depleting anti-CD20 monoclonal antibody rituximab in RRMS and more recently ocrelizumab in RRMS and even PPMS [4,5]. As rituximab does not target antibody-secreting plasma cells, the observed decrease in gadolinium-enhancing lesions on magnetic resonance imaging (MRI) was not due to reduced serum and CSF antibody titers or altered oligoclonal antibodies [6,7]. Instead, the decrease in CSF T cell numbers and peripheral Th1 and Th17 responses following B cell depletion indicated the importance of B cells in T cell regulation in MS pathology [6,8].

Antigen presentation and costimulation are important B cell functions in B-T cell interactions. B cells are professional antigen presenting cells (APC) that are highly efficient in the activation of antigen-specific $CD4^+$ T cells. After B cell receptor (BCR) recognition and processing, antigens are presented on the surface of the B cells by MHC molecules. Moreover, B cells express costimulatory molecules, such as CD86 and CD80, that are necessary to obtain full APC potency [9]. Several *in vitro* studies have shown that human B cells can induce effective T cell responses towards both foreign and self-antigens [10,11]. In MS patients, peripheral B cells showed increased expression of CD80 and MHCII

Abbreviations: APC, antigen presenting cell; BCR, B cell receptor; CM, culture medium; CMV, cytomegalovirus; CNS, central nervous system; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; EDSS, expanded disability status scale; HC, healthy control; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; PE, phycoerythrin; PPMS, primary progressive MS; RRMS, relapsing-remitting MS; SI, stimulation index; SPMS, secondary progressive MS; TT, tetanus toxoid; ΔPF, proliferating fraction.

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during active disease [12]. Memory B cells from the peripheral blood of some RRMS patients induced autoreactive T cell proliferation and IFN-γ production [13]. More information is available from studies using experimental autoimmune encephalomyelitis (EAE), in which B cells promoted the differentiation of myelin oligodendrocyte glycoprotein (MOG)-specific Th1 and Th17 cells [14]. Interestingly, mice selectively deficient in MHCII molecules on B cells were resistant to EAE induction and exhibited diminished Th1 and Th17 responses [15]. Moreover, B cell antigen presentation was proven to be crucial for maximal disease in EAE, further emphasizing the importance of B cells in driving $CD4^+$ T cell autoreactivity [16].

In this study, we measured B cell expression of costimulatory and human leukocyte antigen (HLA) molecules in the peripheral blood, CSF and brain lesions of MS patients and controls and characterized the T cell response (Th1, Th2, Th17) induced by B cells presenting myelin and viral antigens. As the importance of B cell involvement in MS pathogenesis also supports therapeutic B cell targeting, we compared the B cell antigen presentation potential between untreated MS patients and MS patients receiving immunomodulatory treatment. Our findings provide novel insight into the role of B-T cell interactions in MS pathology and elucidate whether current therapies for MS also act on the antigen presentation potential of B cells.

2. Materials and methods

2.1. Human samples

MS patients, diagnosed according to the McDonald criteria [17], and healthy controls (HC) were recruited at the Revalidation & MS Center (Overpelt, Belgium), Zuyderland Medical Center (Sittard, The Netherlands) and Biomedical Research Institute (Diepenbeek, Belgium) after informed consent and the institutes' ethics committees' approval. Samples were stored in the University Biobank Limburg. Clinical data are provided in Table 1.

Flow cytometry was done using 52 HC and 74 MS patients, 41 untreated and 33 receiving immunomodulatory treatment, including IFN-β (n = 13), glatiramer acetate (n = 5), fingolimod (n = 7), natalizumab (n = 4) and others (n = 4). Paired CSF samples were collected from 20 out of 74 MS patients, of which 18 were untreated and 2 received IFN-β treatment. B cell proliferation assay was done for 5 untreated MS patients and 6 age- and gender-matched HC. B:T coculture assay was performed for treated (n = 10) and untreated (n = 9) MS patients and matched HC (n = 10). Treatment included IFN-β (n = 4), dimethyl fumarate (n = 3) and teriflunomide (n = 3).

Table 1
Characteristics of MS patients and HC.

	Number	Age[a]	Gender F:M (%F)[b]	MS type[c] RR	SP	PP	EDSS[d]
Flow cytometric analysis							
HC	52	35.7 ± 13.0	34:18 (65%)		N.A.		N.A.
MS total	74	45.6 ± 12.0	56:17 (77%)	53	14	7	3.4 ± 1.9
MS untreated	41	47.6 ± 8.5	31:10 (76%)	27	7	7	3.4 ± 1.6
MS treated[e]	33	42.2 ± 14.6	25:7 (78%)	26	7	0	3.3 ± 2.2
B cell proliferation assay							
HC	6	32.2 ± 9.9	6:0 (100%)		N.A.		N.A.
MS	5	42.8 ± 21.5	3:2 (60%)	4	1	0	3 ± 3.3
B:T coculture assay							
HC	10	43.2 ± 14.4	5:5 (50%)		N.A.		N.A.
MS untreated	9	47.7 ± 16.3	5:4 (55%)	5	4	0	3.8 ± 2.2
MS treated[f]	10	42.9 ± 11.6	8:2 (80%)	10	0	0	2.1 ± 1.7

[a] In years, mean ± SD.
[b] F: female, M: male.
[c] RR: relapsing-remitting, SP: secondary progressive, PP: primary progressive.
[d] Expanded disability status scale, mean ± SD.
[e] IFN-β (n = 13), glatiramer acetate (n = 5), fingolimod (n = 7), natalizumab (n = 4), other (n = 4).
[f] IFN-β (n = 4), dimethyl fumarate (n = 3), teriflunomide (n = 3).

2.2. Cell isolation

PBMC were isolated from whole blood by density gradient centrifugation (Lympholyte, Cedarlane Laboratories, Sanbio B.V., Uden, The Netherlands). Cells were collected by centrifugation of the CSF for 12 min at 250*g*. CSF samples contaminated with red blood cells were excluded from analyses.

B cells were purified from PBMC using magnetic negative (STEMCELL Technologies SARL, Grenoble, France) or positive (Miltenyi Biotec B.V., Leiden, The Netherlands) selection. T cells were enriched by magnetic negative selection (STEMCELL Technologies SARL). Purity was confirmed on a FACSAria II flow cytometer (BD Biosciences, Erembodegem, Belgium).

2.3. Flow cytometric analysis

PBMC or CSF cells were stained using the following anti-human fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)- or peridinin chlorophyll protein (PerCP)-labelled monoclonal antibodies: CD19, HLA-DR/DP/DQ, HLA-A/B/C, CD86, CD80 or CD40 (all from BD Biosciences). Isotype controls were used for gating strategies (BD Biosciences). Samples were analysed on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences).

2.4. Immunohistochemistry

Formalin-fixed paraffin-embedded sections from MS brain tissue (n = 4, Netherlands Brain Bank) were deparaffinized and rehydrated. Following antigen retrieval in citrate buffer pH 6.0, sections were stained overnight at 4 °C with anti-CD20 (1:100, Dako, Heverlee, Belgium) and 1 h at room temperature with anti-HLA-DR/DP/DQ (1:100, Dako) or anti-CD80 (1:100, Abcam, Cambridge, UK). After washing, sections were incubated with appropriate secondary antibodies (Life Technologies, Gent, Belgium) for 1 h at room temperature. Antibodies were diluted in PBS/1% BSA. Cell nuclei were labelled with DAPI and autofluorescence was blocked by 0.1% Sudan Black in 70% ethanol. Stained sections were evaluated on a Nikon Eclipse 80i microscope using standard objectives and NIS Elements BR 3.10 software (Nikon).

2.5. B cell proliferation assay

CFSE-labelled (1 μM, Life Technologies) B cells were seeded at 1×10^5 cells with 1×10^5 autologous irradiated (8275 Rad) PBMC in triplicate. Culture medium (CM) was RPMI-1640 (Lonza, Verviers, Belgium) supplemented with 10% foetal bovine serum (FBS, Life Technologies), 1% sodium pyruvate, 1% nonessential amino acids, 50 U/ml penicillin and 50 μg/ml streptomycin (all from Sigma-Aldrich, Diegem, Belgium). For stimulation, 2,5 limit of flocculation (Lf)/ml tetanus toxoid (TT, RIVM, Bilthoven, The Netherlands), 1 μg/ml cytomegalovirus (CMV, BD Biosciences), 40 μg/ml human myelin basic protein (MBP, purified as described [18]) or 10 μg/ml human MOG peptides 1–22, 34–56, 64–86, 74–96 (Severn Biotech Ltd., Worchester, UK) were added. Unstimulated B cells or 1 μg/ml CpG2006 (Invivogen, Toulouse, France) with 50 U/ml IL-2 (Sigma-Aldrich) were used as a negative or positive control, respectively. After 13 days, B cell proliferation was assessed by flow cytometry using anti-human HLA-A/B/C peridinin chlorophyll protein (PerCP) (Biolegend, London, UK), 7-aminoactinomycin D (7-AAD), HLA-DR PE and CD80 PE (all from BD Biosciences). The Δ proliferating fraction (ΔPF, %) was calculated by subtracting the mean background proliferation from the mean proliferation in response to antigen and was positive when ≥ 2%. Analysis was done on a FACSAria II flow cytometer and FACSDiva software (BD Biosciences).