Journal of Autoimmunity 36 (2011) 228–238

Contents lists available at ScienceDirect

Journal of Autoimmunity

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

Human T cells induce their own regulation through activation of B cells☆

Sébastien Lemoine [a,b], Ahsen Morva [a], Pierre Youinou [a,b,*], Christophe Jamin [a,b]

[a] EA 2216 "Immunology and Pathology" and IFR 148 ScInBioS at the European University of Brittany and the University of Brest, Brest, France
[b] Brest University Medical School Hospital, Brest, France

ARTICLE INFO

Article history:
Received 23 December 2010
Received in revised form
17 January 2011
Accepted 18 January 2011

Keywords:
Regulatory B cells
T-cell response
IL-10
Regulatory T cells
Autoimmune disease
Systemic lupus erythematosus

ABSTRACT

Regulatory functions for B lymphocytes have been reported in murine models of autoimmune diseases in which B-cell deficient mice were shown to exhibit exacerbated disease. The B cells responsible for the immune regulations were identified as a subpopulation of interleukin 10-secreting cells. However, the mechanism of induction and the characteristics of regulatory B cells in humans have been hardly studied. This study reports that regulation of T cell responses can be induced by B cells following CD40-dependent cognate interaction. T cell proliferation and cytokine production were differentially regulated. Thus, CD40-induced regulatory B cells partially inhibited T cell proliferation following CD40 interaction without requirement of soluble factor. In contrast, modulation of Th1 differentiation resulted from CD80- and CD86-dependent interactions and from IL-10 production. The suppressive effects were mediated by $CD19^{high}IgD^{+}CD38^{high}CD24^{high}CD5^{high}$ B cells and appeared to be indirect, through the induction of regulatory T cells as indicated by the appearance of $Foxp3^{+}CD4^{+}CD25^{+}$T cells. These data suggest that activation signals from T cells initiate regulatory properties in B cells that modulate T cell responses involving regulatory T cells. Finally, studies in autoimmune patients revealed that regulation of T cell proliferation was defective in systemic lupus erythematosus but efficient in other diseases. Restoration of efficient B-cell regulatory activity could provide innovative B-cell based treatment of autoimmune diseases.

1. Introduction

B cells are complex modulators of immunity. They produce antibodies, cytokines, can act as antigen presenting cells and activate T cells [1]. B cells are also involved in the development of autoimmune diseases partly through their ability to produce pathogenic auto-antibodies. New therapeutics approaches based on B cell depletion have been highly successful in the clinic in treating a range of leukemic and autoimmune diseases [2]. However, B cell depletion in murine models of autoimmune diseases such as arthritis and chronic colitis have also led to worsened clinical outcomes suggesting that B cells could also have regulatory roles [3–5]. Mechanisms by which B cells modulate inflammatory responses in murine models, however, appear to depend on the model studied [6–9]. One common feature seems to be the production of IL-10, a well known anti-inflammatory cytokine, by B cells [3,5,10–14]. Nevertheless, other cytokines have been described to trigger B cell-mediated immune regulatory functions. For example, there is evidence that TGF-β induces regulatory B cell functions in NOD mice leading to decreased Th1 responses [15]. Given that Treg cells also produce IL-10 and TGF-β, a close relationship between regulatory B and T lymphocyte populations is, thus, expected.

Studies of murine colitis and experimental autoimmune encephalomyelitis (EAE) have demonstrated that regulatory B (Breg) cells can induce Treg cells to regulate T cell-dependent immune responses [16–18]. However, B cells have also been shown to directly inhibit T cell proliferation through cell–cell contact, even leading to anergy, or apoptosis of T cells [19], and the modulation of the inflammatory response. In this regard, CD40 engagement on B cells appears to be a requisite for the induction of functional Breg cells in mice. Stimulation of CD40 brings about the development of B cells with suppressive properties. Further, signalling in the absence of CD40 makes B cells unable to regulate inflammatory response [3,4].

Abbreviations: Breg, B regulatory cell; EAE, experimental autoimmune encephalomyelitis; MFI, mean fluorescence intensity; PC7, PE-linked to cyanin 7; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T2, transitional type 2 B cell; Treg, T regulatory cell.

☆ This work was supported by a grant from the Ministère de l'Éducation Nationale de la Recherche et de la Technologie.

* Corresponding author at: Laboratory of Immunology, Brest University Medical School Hospital, BP 824, F 29609 Brest, France. Tel.: +33 2 98 22 33 84; fax: +33 2 98 22 38 47.
E-mail addresses: sebastien200109@gmail.com (S. Lemoine), asocum@hotmail.com (A. Morva), youinou@univ-brest.fr (P. Youinou), christophe.jamin@univ-brest.fr (C. Jamin).

0896-8411/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jaut.2011.01.005

The phenotypic nature of Breg cells is still a matter for debate. Two distinct IL-10-producing B cell subpopulations associated with regulatory functions have been identified. One has been recognized as transitional-marginal zone precursor B cells expressing high level of CD21, CD23, CD24, IgM and CD1d, designed as transitional type 2 (T2)-like cells [11,20,21]. The second, described as $CD1d^{hi}$, $CD5^{+}$ and $CD19^{hi}$ B cells has been called "B10" cells since IL-10 is the main cytokine produced by these cells [13].

Recent works have suggested that human B cells can also regulate inflammatory responses [22]. Thus, B cell depletion therapies have been reported to exacerbate ulcerative colitis [23], or lead to the development of psoriasis in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) patients [24]. This indicates the existence of regulatory properties for human B cells. A B cell subpopulation identified in healthy controls but shown to be impaired in patients with SLE, and characterized as $CD24^{high}CD38^{high}$, has been suggested to be responsible for suppressing the differentiation of Th1 cells [21]. However, there is a scarcity of data to permit adequate characterization of human Breg cells.

This study was aimed at exploring the possibility that T cells can induce their own B cell-dependent regulation through cell-to-cell contact. Further, it was hoped that the study could determine whether Breg functions are restricted to a specific subpopulation of B cells. The results indicate that T cells activate B cells through CD40 engagement to bring about inhibition of T cell proliferation and differentiation to Th1-type cells. Co-stimulation of B cells through CD40 and TLR9 was found to enhance the regulatory effects of the resulting Breg cells. Interestingly, the results also reveal that regulation of T cell proliferation was distinct from the differentiation of T cells to Th1 cells. Thus, while regulation of proliferation only required cell–cell contact involving CD40 engagement, Th1 cell differentiation was dependent on CD80 and CD86 interactions and on the production of IL-10. Although we have identified the $CD19^{+}IgD^{+}CD38^{high}CD24^{high}CD5^{+}$ subpopulation as the B cell subset exhibiting efficient regulatory functions, all B cells induced to express CD5 appeared to be able to acquire regulatory capacity. Finally, the appearance of $Foxp3^{+}CD4^{+}CD25^{+}$ T cells in the presence of appropriately-activated B cells suggests that B cell-dependent suppressive effects are associated with the generation of Treg cells.

2. Materials and methods

2.1. Isolation of cells

Peripheral blood from controls was donated by healthy laboratory staff donors. Blood samples from 4 patients with RA, 4 with SLE and 7 patients with primary Sjögren's syndrome (pSS) were collected after obtaining informed consent. All patients fulfilled the criteria of the respective disease. Tonsils were obtained from children undergoing routine tonsillectomy. The tissues were minced up, and filtered to remove tissue fragments and clumps. Blood samples and tonsillar cell suspensions were layered onto Ficoll-Hypaque and centrifuged. Mononuclear cells were incubated with neuraminidase-treated sheep red blood cells and T cells depleted by a second 30-min round of centrifugation. T cells were further purified by negative selection with T cell enrichment kit (Stem Cell Technologies) according to the manufacturer procedure. All preparations were >95% pure $CD19^{+}$ B cells and >98% pure $CD19^{-}$ $CD5^{+}$ T cells, respectively.

2.2. Cell culture

B cells were cultured for 3 days in 24-well plates in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine (Invitrogen Life Technologies), 200 U/ml penicillin, and 100 μg/ml streptomycin. For stimulation, B cells were seeded at 5×10^5 cell/ml on 5×10^5 NIH-3T3 fibroblasts transfected or not with human *CD40L* gene and treated with mitomycin C, with or without anti-IgM Ab-coated beads (Irvine Scientific), or CpG-ODN 2006 (Cayla-InvivoGen).

T cells were seeded at 2×10^5 cell/ml on anti-mouse IgG-Fc coated 96-well plates in complete RPMI 1640 medium. The cells were stimulated with 1 μg/ml anti-CD3 and anti-CD28 mAbs. For the co-culture experiments, resting B cells, or B cells pre-stimulated for 3 days were added to T cells for 5 days at 4B:1 T ratio, or as otherwise indicated. Blocking experiments were carried out using 1 μg/ml anti-CD40L (R&D Systems), 1–10 μg/ml anti-CD80, anti-CD86, or anti-IL-10 mAbs (ImmunoTools).

2.3. Proliferation assays

Freshly isolated T cells were labelled with 5 μM CFSE before stimulation. T cell proliferation was evaluated by flow cytometry on Epics XL or FC500 (Beckman Coulter) measuring the decrease in the mean fluorescence intensity (MFI) of CFSE. In co-culture experiments, cells were stained with PE-linked to cyanin 7 (PC7)-conjugated anti-CD19 mAb, and CFSE MFI analyzed in CD19-negative cells.

2.4. ELISA

IFNγ and TNFα in the supernatants of cultured cells were measured at different dilutions by commercial ELISA kits using paired Abs (Beckman Coulter). RPMI 1640 complete medium was used as negative control.

2.5. Flow cytometry

All mAbs were purchased from Beckman Coulter, unless otherwise specified. We used FITC-conjugated anti-CD27, anti-CD25 (BD Biosciences), PE-conjugated anti-CD24 (BD Biosciences), anti-CD5, anti-CD25, anti-IgD (BD Biosciences), PE-linked to cyanin 5 (PC5)-conjugated anti-CD38, anti-CD4 and PC7-conjugated anti-CD19. Intracellular staining for IL-10 or TGFβ was performed after permeabilisation of the cells using cytofix/cytoperm permeabilization kit (BD Biosciences), with FITC-conjugated anti-IL-10 or PE-conjugated anti-TGFβ (R&D Systems). Foxp3-positive T cells were determined using PE-conjugated anti-Foxp3 (BD Biosciences) according to manufacturer's instructions.

2.6. Statistical analysis

All data were expressed as the mean ± SD. Statistical analyses were performed with Prism4 Graphpad Software using chi-squared test for comparisons of percentages and the Mann–Whitney *U*-test, or Wilcoxon test for comparisons of quantitative values. Significance was assessed at $p < 0.05$.

3. Results

3.1. Human T cells induce their own-regulation through stimulation of B cells

To assess whether T cells were capable of inducing feedback regulation through activation of B cells, we used an *in vitro* system to study their proliferation in the presence of B cells. T cells were labeled with CFSE and induced to proliferation in the presence of anti-CD3 and anti-CD28 mAbs. Proliferation was assessed by flow cytometry (Fig. 1A). The extent of T cell proliferation was also