IL-10–overexpressing B cells regulate innate and adaptive immune responses

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Background: Distinct human IL-10–producing B-cell subsets with immunoregulatory properties have been described. However, the broader spectrum of their direct cellular targets and suppressive mechanisms has not been extensively studied, particularly in relation to direct and indirect IL-10–mediated functions.
Objective: The aim of the study was to investigate the effects of IL-10 overexpression on the phenotype and immunoregulatory capacity of B cells.
Methods: Primary human B cells were transfected with hIL-10, and IL-10–overexpressing B cells were characterized for cytokine and immunoglobulin production by means of specific ELISA and bead-based assays. Antigen presentation, costimulation capacity, and transcription factor signatures were analyzed by means of flow cytometry and quantitative RT-PCR. Effects of IL-10–overexpresing B cells on Toll-like receptor–triggered cytokine release from PBMCs, LPS-triggered maturation of monocyte-derived dendritic cells, and tetanus toxoid–induced PBMC proliferation were assessed in autologous cocultures.
Results: IL-10–overexpressing B cells acquired a prominent immunoregulatory profile comprising upregulation of suppressor of cytokine signaling 3 (SOCS3), glycoprotein A repetitions predominant (GARP), the IL-2 receptor α chain (CD25), and programmed cell death 1 ligand 1 (PD-L1). Concurrently, their secretion profile was characterized by a significant reduction in levels of proinflammatory cytokines (TNF-α, IL-8, and macrophage inflammatory protein 1α) and augmented production of anti-inflammatory IL-1 receptor antagonist and vascular endothelial growth factor. Furthermore, IL-10 overexpression was associated with a decrease in costimulatory potential. IL-10–overexpressing B cells secreted less IgE and potently suppressed proinflammatory cytokines in PBMCs, maturation of monocyte-derived dendritic cells (rendering their profile to regulatory phenotype), and antigen-specific proliferation *in vitro*.
Conclusion: Our data demonstrate an essential role for IL-10 in inducing an immunoregulatory phenotype in B cells that exerts substantial anti-inflammatory and immunosuppressive functions. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *IL-10 overexpression, regulatory B cells, immune regulation, anti-inflammatory effects, immunoregulatory capacity*

B lymphocytes display a unique role in immune response through the production of antibodies, representing the humoral arm of the adaptive immune response. In addition, B cells substantially contribute to the full magnitude and fate of the normal immune response through antigen presentation, cytokine secretion, and lymphoid tissue organization. Consequently, the importance of complex B-cell biology was recognized in altered/inadequate pathologic immune responses, such as (1) asthma and allergies, a chronic immune reactivity to innocuous antigens (allergens) in sensitized subjects; (2) autoimmunity, a lack of control of pathologic immune response to self-antigens; (3) antitumor immunity with insufficient immune response to tumor antigens; and (4) acceptance or rejection of transplanted organs.[1,2]

A growing body of evidence has attributed an essential role for B cells in limiting excessive immune reactivity. IL-10–mediated immune regulation by B cells has been described in experimental models of infection, allergic inflammation, autoimmunity, tolerance, tumorigenesis, and organ transplantation.[3-11] A functional role for human regulatory B cells was further supported by the finding that B cell–depleting therapy was associated with exacerbations of colitis and psoriasis induction and neutropenia in patients undergoing organ transplantation.[12-14] Furthermore, altered numbers, function, or both of regulatory B-cell subsets in patients with chronic inflammatory and autoimmune diseases, as well as insufficient antitumor immunity, was associated with locally increased numbers of IL-10–producing B cells. Allergen-specific immunotherapy typically leads to suppression of IgE and upregulation of IgG_4 production, as well as increased IL-10 production, in allergen-specific T and B cells. These observations have stimulated scientific interest to study the role and capacity of regulatory B cells and their underlying immunosuppressive mechanisms.[15-18]

Distinct human IL-10–producing B-cell subsets with an *in vivo* demonstrated role have been described. Recently, we reported that IL-10^+ B regulatory 1 (Br1) cells that were enriched among $CD25^+CD71^+CD73^{lo}$ B cells could potently suppress antigen-specific $CD4^+$ T-cell proliferation.[19] These Br1 cells were comprised of 2 temporally distinct but spatially linked immunosuppressive functionalities: increased IL-10 production and subsequent preferential IgG_4 secretion. These findings were demonstrated in phospholipase A2-specific B cells of beekeepers,

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Abbreviations used
APC: Antigen-presenting cell
BLIMP-1: B lymphocyte-induced maturation protein 1
Br1: B regulatory 1
DC: Dendritic cell
FITC: Fluorescein isothiocyanate
GARP: Glycoprotein A repetitions predominant
G-CSF: Granulocyte colony-stimulating factor
IL-1Ra: IL-1 receptor antagonist
IRF-4: Interferon regulatory factor 4
MDDC: Monocyte-derived dendritic cell
MIP: Macrophage inflammatory protein
PD-L1: Programmed cell death 1 ligand 1
PE: Phycoerythrin
SOCS: Suppressor of cytokine signaling
TLR: Toll-like receptor
TLR-L: Toll-like receptor ligand
TT: Tetanus toxoid
VEGF: Vascular endothelial growth factor
XBP-1: X-box binding protein 1

representing a human antigen-specific *in vivo* model of tolerance upon high-dose antigen exposure. These findings particularly highlight a role for regulatory B cells in the development and maintenance of antigen-specific peripheral tolerance. The suppressive mechanisms of most regulatory B-cell subsets thus far described are, at least in part, IL-10 dependent.[19-22]

IL-10 is a pivotal anti-inflammatory cytokine that protects the host from excessive tissue damage during host defense to pathogens and acts as one of the key molecules critically involved in the development and maintenance of immune tolerance and homeostasis.[23,24] IL-10 deficiency leads to the development of spontaneous colitis in mice.[25] IL-10 suppresses the production of proinflammatory cytokines and chemokines, as well as antigen presentation.[24] In B cells IL-10 enhances survival, proliferation, and differentiation and modulates class-switch recombination through suppression of IL-4–induced IgE and induction of IgG_4.[24,26,27]

Plasmid-driven IL-10 transfection was performed to reveal the role of IL-10 on the phenotype and functions of B cells. IL-10 overexpression was sufficient for acquisition of a notable immunoregulatory phenotype in B cells. In conjunction with secreted IL-10, these B cells further extend their immunosuppressive functions on both innate and adaptive immune responses.

METHODS

Human B cells were purified from PBMCs by means of negative selection with immunomagnetic separation and transfected either with control (backbone) plasmid (ctrl_tr) or pORF–hIL-10 to overexpress *IL-10* (IL-10_tr) or left nontransfected (non-tr). Plasmid-mediated gene transfer in B cells using nucleofection was efficient and resulted in IL-10 overexpression (see Fig E1, *A* and *B*, in this article's Online Repository at www.jacionline.org). *IL10*-transfected B cells have been cultured in medium alone before cytokine and immunoglobulin secretion was quantified with ELISA and a bead-based multiplex assay, gene expression with quantitative RT-PCR, and expression of surface molecules with flow cytometry. Preliminary experiments were performed to determine the optimal time for measurements, which were used as indicated in the figure legends. IL-10–overexpressing B cells were stimulated with Toll-like receptor (TLR) 9 ligand for 72 hours before transfection and then cocultured with (1) autologous PBMCs stimulated with either TLR ligands (TLR-Ls) to induce proinflammatory cytokine secretion or antigen for induction of specific proliferation or (2) monocyte-derived dendritic cells (MDDCs) stimulated with LPS for their maturation capacity to address their suppressive potential.

A detailed description of the materials and methods used in this study is available in the Methods section in this article's Online Repository at www.jacionline.org.

Statistical analysis

Presented data are expressed either as individual values of each donor or as means ± SEMs. Statistical analysis was performed with GraphPad Prism 5.0 software (GraphPad Software, La Jolla, Calif), and paired *t* tests (if not stated differently) were used for assessment of statistical significance. Statistically significant scores are indicated on the graphs as asterisks, representing significantly changed values between samples having *P* values of at least less than .05. The grade of statistical significance is displayed in the figure legends.

RESULTS

IL-10–overexpressing B cells show decreased production of proinflammatory cytokines with increased IL-1 receptor antagonist and VEGF levels

Under optimized transfection conditions (see Fig E1, *A* and *B*) and after 24 hours of culture in medium, IL-10_tr B cells upregulated *IL10* mRNA expression (approximately 3500-fold) and secreted IL-10 (approximately 100-fold) compared with levels seen in ctrl_tr B cells (Fig 1 and Fig 2, *A*, top left panels). The transfection procedure did not alter the stimulatory effect of TLR9-L on IL-10 induction compared with that seen in non-tr B cells (see Fig E1, *C*). However, lack of further increase in IL-10 production in IL-10_tr B cells stimulated with TLR9-L at the protein level was accompanied by significant downregulation of *IL10* gene transcription 24 hours after transfection (see Fig E1, *D*; similar data were observed after 48 hours [data not shown]). This implies that after reaching maximal production capacity, IL-10 might limit/downregulate its own expression through a negative feedback mechanism at the transcriptional level. High IL-10 production in IL-10–overexpressing B cells was accompanied by a significant decrease in production of the proinflammatory cytokines TNF-α, IL-8, and macrophage inflammatory protein (MIP) 1α (Fig 1). In contrast, levels of the anti-inflammatory cytokines IL-1 receptor antagonist (IL-1Ra) and vascular endothelial growth factor (VEGF) were significantly increased compared with those in control cells. Furthermore, there was a tendency for inhibition of MIP-1β and interferon-inducible protein 10, whereas detectable levels of IL-6, IFN-γ, GM-CSF, and RANTES were not significantly changed (see Fig E2 in this article's Online Repository at www.jacionline.org). Additionally, although there was no measurable Semaphorin3A in *IL10*-transfected B cells after 48 hours, secreted levels of TGF-β were detectable but did not significantly change in relationship to IL-10 overexpression.

These data demonstrate that IL-10 overexpression suppresses proinflammatory cytokines and induces anti-inflammatory cytokines in B cells.

IL-10–overexpressing B cells acquire an immunoregulatory phenotype characterized by expression of CD25, GARP, PD-L1, and CD38

In IL-10_tr B cells there was a significant increase in mRNA expression of IL-2 receptor α (*IL2RA*, CD25) and glycoprotein A