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To the Editor

B lymphocytes produce antibodies, act as antigen-presenting cells and produce cytokines.¹ They can be classified into functional subsets according to their distinct expression of surface markers and their profile of secreted cytokines. Regulatory B cells (Bregs), control excessive inflammatory responses primarily through secretion of IL-10. IL-10 inhibits proinflammatory cytokine production and supports regulatory T cell differentiation.²⁻⁴ During the last decade, an essential role has been attributed to IL-10⁺ Bregs in limiting excessive immune reactivity in experimental models of infection, allergic inflammation, autoimmunity, tolerance, tumor growth, and organ transplantation.

² An important *in vivo* functional role for human Bregs has been suggested by the finding that exacerbations of colitis and psoriasis take place after B cell depletion therapy.⁵ An increase in peripheral CD19⁺CD25⁺CD71⁺CD73⁻ Bregs upon allergen-specific immunotherapy of patients has been linked to the suppression of IgE and upregulation of IgG4 production, as well as increased IL-10 production in allergen-specific T and B cells.³ So far, three different IL-10⁺ Breg subsets have been identified in humans. CD19⁺CD5⁺CD1d⁺ and CD19⁺CD24⁺CD38⁺ Bregs were shown to suppress Th1 cells,^{6,7} whereas CD19⁺CD25⁺CD71⁺CD73⁻ Bregs may play a role in allergen-specific immune tolerance. Moreover, Kamekura et al.⁸ have demonstrated significant decrease of CD19⁺CD24^{hi}CD27⁺ Bregs in allergic rhinitis and asthma. In contrast, another study reported an increase of CD19⁺CD24^{hi}CD27⁺ B cells and a decline of CD19⁺CD24^{hi}CD38^{hi} B cells in allergic rhinitis compared to healthy individuals.⁹ However, it is

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noteworthy that none of these studies assessed production of IL-10, which is a crucial feature of Bregs, as a marker of this population. Therefore, in view of this data and the lack of IL-10 measurement in the previous studies, we aimed to compare the percentages of IL-10-producing Breg subsets in peripheral blood from clinically-well characterized asthmatic, allergic rhinitis and healthy 14-15 years old individuals (**Table S1**) using multicolor flow cytometry. Peripheral blood mononuclear cells were stained with antibodies specific for IL-10, CD1d, CD5, CD24, CD38, CD25, CD71 and CD73. Three types of Breg cells were studied according to the gating strategy shown in **Figure 1A**, based on the isotype controls (**Figure S1**). Since IL-10 has been proposed as a key suppressive cytokine of Breg cells *in vivo* and *in vitro*,²⁻⁴ we aimed to compare the percentage of IL-10-producing B cells between patients with asthma, allergic rhinitis and healthy controls at day 0. Donor-dependent variation in the total number of IL-10⁺ B cells (ranging between 1% and 24% of total B cells) was observed and there was no difference between the studied groups (**Figure 1B**). Further comparison of IL-10 expression within the three Breg populations revealed no difference in the percentage of CD19⁺IL-10⁺CD25⁺CD71⁺CD73⁻ (**Figure 1C**), CD19⁺IL-10⁺CD24⁺CD38⁺ (**Figure 1D**), and CD19⁺IL-10⁺CD5⁺CD1d⁺ (**Figure 1E**) Breg cells within or between patients with asthma and allergic rhinitis, and healthy controls at day 0. The comparison of total B cell percentage as well as naïve and memory B cells gated based on the expression of CD27 and IgD (**Figure S2A**) did not show any significant difference between the studied groups (**Figure S2B**). Despite the number of samples being limited, this observation may suggest that there is no deficiency in circulating Bregs in asthmatic and allergic.

As we were interested in the response of B cells to B cell-specific stimulants, we aimed to analyze if there is any difference in the capacity for the induction of IL-10 expression in the three groups after 3 days of stimulation with CpG oligonucleotides (CpG) or anti-B cell receptor (α BCR) and CD40 ligand (CD40L). Cells were gated according to the strategy shown in **Figure 2A**. IL-10 production was significantly induced upon CpG stimulation in patients with asthma and healthy controls. There was a trend towards lower number of IL-10-producing Bregs in patients with allergic rhinitis. Therefore, it

would require a larger patient cohort to determine whether patients with allergic rhinitis have a significant Breg deficiency. One can speculate that even the modest differences in Breg frequencies and function, may have an impact on systemic responses, as Bregs have been associated with the suppression of excessive inflammation, restoration of Th1/Th2 balance, inhibition of Th1 and Th17 differentiation and induction of regulatory T cells, mediated not only through the release of soluble factors, but also via cell-to-cell contact. Moreover, a slight difference in circulating Breg frequencies could reflect a significant difference in tissue-infiltrating Breg cells. Further studies are needed to quantify Breg populations in respiratory mucosal tissues.

Although, α BCR+CD40L stimulation resulted in increase of IL-10⁺ B cells in some individuals, there was no significant change (**Figure 2B**). The patients who responded strongly to α BCR+CD40L did not have less severe symptoms compared to the rest of the subjects in allergic rhinitis group. There was no significant difference in the percentage of viable CD19⁺ B cells in total cells between the studied groups. The CD19⁺CD25⁺CD71⁺CD73⁻ Bregs showed a very profound response to CpG and α BCR+CD40L stimulations in patients with asthma, whereas in patients with allergic rhinitis this increase was observed only after CpG treatment (**Figure 2C**). However, a significant induction of CD19⁺CD25⁺CD71⁺CD73⁻ Bregs was influenced by donor-dependent variation in response to CpG stimulation. The other two subsets, CD19⁺CD24⁺CD38⁺ Bregs (**Figure 2D**) and CD19⁺CD5⁺CD1⁺ (**Figure 2E**) were decreased upon stimulation with both CpG and α BCR+CD40L. In line with a previous study, we observed an increase in IL-10⁺ B cells upon stimulation with CpG³ and α BCR+CD40L⁷. Although CpG is a potent stimulus for inducing IL-10 production by B cells, there was no significant difference between the groups. The observed decrease in the percentage of CD19⁺CD24⁺CD38⁺ Bregs upon stimulation with both CpG and α BCR+CD40L may be explained by the fact that CD19⁺CD24⁺CD38⁺ Bregs develop from transitional B cells and they might have already differentiated after three days of stimulation into a less CpG responsive B cell population. Interestingly, we found that CD24⁺CD38⁺ Breg cells express surface

markers associated with other described Breg subsets. Most prominently, CD24⁺ CD38⁺ Breg cells also express CD71 and CD5 (data not shown).

In conclusion, our results showed no differences in numbers of peripheral Bregs both at baseline and after stimulation in patients with asthma and allergic rhinitis compared to healthy controls. Although we observed a trend towards decreased induction of IL-10-producing B cells in response to stimulation in patients with allergic rhinitis, due to limited number of study subjects, we cannot conclude whether there is a deficiency. It is likely that we did not observe an impaired induction of Bregs in response to stimulation as both patients with asthma and allergic rhinitis were under treatment. Nonetheless we cannot exclude that exacerbation of asthma or allergic rhinitis may influence the Breg responses. It is likely that frequency of Bregs in peripheral blood may not reflect the potential impairment in local regulation of Bregs. Direct responses should be studied in the nasal tissue and induced sputum. It has been demonstrated that IgE⁺ B cells were existing in sputum but not in blood and bone marrow. Further research on a larger population focusing on IL-10-producing Bregs in the periphery and affected organs may be necessary to conclude whether there is a deficiency in Breg subsets in patients with allergic rhinitis and asthma.

References

1. Lund FE. Cytokine-producing B lymphocytes-key regulators of immunity. *Curr Opin Immunol*. 2008;20(3):332-338.
2. van de Veen W, Stanic B, Wirz OF, Jansen K, Globinska A, Akdis M. Role of regulatory B cells in immune tolerance to allergens and beyond. *J Allergy Clin Immunol*. 2016;138(3):654-665.
3. van de Veen W, Stanic B, Yaman G, et al. IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. *J Allergy Clin Immunol*. 2013;131(4):1204-1212.
4. Rosser EC, Mauri C. Regulatory B cells: origin, phenotype, and function. *Immunity*. 2015;42(4):607-612.

5. Dass S, Bowman SJ, Vital EM, et al. Reduction of fatigue in Sjogren's syndrome with rituximab: results of a randomised, double-blind, placebo controlled pilot study. *Ann Rheum Dis*. 2008.
6. Yanaba K, Bouaziz JD, Haas KM, Poe JC, Fujimoto M, Tedder TF. A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. *Immunity*. 2008;28(5):639-650.
7. Blair PA, Norena LY, Flores-Borja F, et al. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. *Immunity*. 2010;32(1):129-140.
8. Kamekura R, Shigehara K, Miyajima S, et al. Alteration of circulating type 2 follicular helper T cells and regulatory B cells underlies the comorbid association of allergic rhinitis with bronchial asthma. *Clin Immunol*. 2015;158(2):204-211.
9. Luo J, Guo H, Liu Z, et al. Analysis of Peripheral B Cell Subsets in Patients With Allergic Rhinitis. *Allergy Asthma Immunol Res*. 2018;10(3):236-243.
10. Carter NA, Rosser EC, Mauri C. Interleukin-10 produced by B cells is crucial for the suppression of Th17/Th1 responses, induction of T regulatory type 1 cells and reduction of collagen-induced arthritis. *Arthritis Res Ther*. 2012;14(1):R32.

Figure Legend

Figure 1. Percentages of Breg cell subsets and their expression of IL-10 are similar in patients with asthma (A) or allergic rhinitis (R) compared to healthy individuals (H) at day 0.

A. Gating strategy of three different Breg cell subsets in an asthmatic donor. **B.** Percentage of IL-10⁺ B cells within total B cells. Kruskal-Wallis test was used to compare differences between the groups. **C-E.** Three different Breg cell subsets: **C.** CD19⁺CD25⁺CD71⁺CD73⁻ **D.** CD19⁺CD24⁺CD38⁺ **E.** CD19⁺CD5⁺CD1d⁺ measured as a percentage of total IL-10⁺ B cells. Wilcoxon matched-pairs signed rank test was used to analyze changes in response to different stimulation. H (n=6), A (n=6), R (n=6). Each bar represents the mean ± SEM.

Figure 2. Three Breg cell subsets are responding differently to CpG and αBCR+CD40L stimulations compared to unstimulated control (U.S.) in patients with asthma (A) or allergic rhinitis (R) and healthy individuals (H) at day 3.

A. Gating strategy of three different Breg cell subsets shown in an asthmatic donor.

B. Increase in IL-10 expression in total B cells. (n=8), A (n=6), R (n=8). C-D. Three different Bregs

subsets: C. CD19⁺CD25⁺CD71⁺CD73⁻ D. CD19⁺CD24⁺CD38⁺

E. CD19⁺CD5⁺CD1d⁺ measured as a percentage of total IL-10⁺ B cells. Wilcoxon matched-pairs signed rank test was used to analyze changes in response to different stimulation. *p<0.05; **p<0.01. H (n=5-6), A (n=6), R (n=6). Each bar represents the mean ± SEM.

Letter to the Editor

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