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# Regulatory B cells and T follicular helper cells are reduced in allergic rhinitis

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### Keywords

Regulatory B cell; T follicular helper cell; Immune tolerance; $T_H2$ lymphocyte; IL-10

## To the Editor

Although the role of human regulatory B cells ($B_{REGS}$) in auto-immunity has been extensively studied, there are limited studies exploring the potential importance of $B_{REGS}$ in allergy.[1–7] A subset of peripheral blood $B_{REGS}$ that produces IL-10 (ie, $CD19^+CD73^-CD25^+CD71^+$ $B_{REGS}$) has recently been demonstrated to inhibit $T_H2$ responses.[1–3] $B_{REGS}$ express the cell surface markers CD19 (a B cell marker), CD25 (the alpha chain of the IL-2 receptor also expressed by regulatory T cells or $T_{REGS}$), and CD71 (a known activation marker of B cells), but express low amounts of CD73 (an ectonucleotidase thought to play a role in T cell suppression).[1,2] Murine *in vivo* studies have demonstrated that antigen specificity is required to develop $B_{REGS}$ capable of secreting IL-10.[8] Human $B_{REGS}$ also exhibit allergen specificity as demonstrated in studies of bee venom allergic subjects in whom lower circulating phospholipase $A_2$-specific $B_{REGS}$ were found in venom allergic patients compared to venom tolerant beekeepers and venom immunotherapy patients.[1] Importantly, T follicular helper ($T_{FH}$) cell production of IL-21 is critical for the maturation of $B_{REGS}$ and their production of IL-10.[8]

In this study we investigated whether subjects with allergic rhinitis had reduced numbers of $B_{REGS}$, as well as reduced numbers of $B_{REGS}$ expressing $CD25^{hi}$, as prior studies of $T_{REGS}$ have identified the $CD25^{hi}$ population as the $T_{REG}$ subset most effective in inhibiting T cell

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responses.[9] In addition to examining levels of $B_{REGS}$ and $T_{FH}$ cells in peripheral blood, we ascertained whether $B_{REGS}$ and $T_{FH}$ cells could be detected in human lymph nodes, a potential site of interaction between $B_{REGS}$, $T_{FH}$ cells, and $T_H2$ cells not previously studied in humans.

To compare circulating levels of $B_{REGS}$ and $T_{FH}$ cells, PBMCs were isolated from 10 allergic rhinitis individuals (mean age: 33.8; gender: 3 males, 7 females) and 7 non-allergic individuals (mean age: 37.9; gender: 2 males, 5 females) in a protocol approved by the University of California San Diego Human Subjects Protection Committee. Allergy status was confirmed by ImmunoCAP specific IgE levels or skin prick testing to cat, dog, cockroach, dust mite, grasses, trees, weeds, and molds (see Table E1 in the Online Repository). Using a previously described gating strategy[1], $B_{REGS}$ ($CD19^+CD73^-CD25^+CD71^+$) were identified in isolated PBMCs by FACS using fluorescently labelled CD19, CD73, CD25, and CD71 antibodies as well as their corresponding isotype controls (eBioscience, San Diego, CA)(Fig 1, A). We also examined the percentage of $B_{REGS}$ expressing $CD25^{hi}$, as prior studies of $T_{REGS}$ have identified the $CD25^{hi}$ population as the $T_{REG}$ subset most effective in inhibiting T cell responses (Fig 1, B).[9] $T_{FH}$-like cells (previously defined in peripheral blood as $CD4^+PD\text{-}1^+CXCR5^+$ T cells)[E1,E2] were also detected in PBMCs by FACS using fluorescently labeled CD4, CXCR5, PD-1 antibodies and their corresponding isotype controls (eBioscience)(Fig 1, A). Human lung lymph nodes were obtained from human lungs post-mortem as previously described at the Arkansas Children's Hospital Research Institute in an IRB exempted protocol.[E3] A single cell suspension was prepared by mechanical disruption of lymph nodes for FACS analysis in order to identify $B_{REGS}$ and $T_{FH}$ cells (as described above for PBMCs). Flow cytometry was performed using the BD Accuri C6 (BD Biosciences, San Jose, CA) and Novocyte Flow Cytometer (ACEA Biosciences, Inc., San Diego, CA) and was analyzed using FlowJo software (version 10.0.7, Tree Star, Inc., Ashland, OR). Statistical analyses were performed using one-tailed t tests and results reported as mean ± SEM.

We found that percentages of $CD25^+$ $B_{REGS}$ (2.45 ± 0.41 vs 5.05 ± 1.42)($P < 0.05$) and CD25hi $B_{REGS}$ (0.25 ± 0.05 vs 0.49 ± 0.06)($P < 0.01$) were lower in allergic rhinitis individuals compared to non-allergic controls (Fig 2, A). The lower levels of $B_{REGS}$ in allergic rhinitis subjects tended to cluster in a similar range, while non-allergic individuals exhibited a wider distribution. Our studies are consistent with reports showing that $B_{REGS}$ are reduced in allergic individuals compared to controls.[1,2,4–5,7]

Levels of $T_{FH}$-like cells ($CD4^+PD\text{-}1^+CXCR5^+$) were significantly lower in allergic rhinitis individuals (1.40 ± 0.26) compared to non-allergic individuals (2.81 ± 0.51)($P < 0.01$)(Fig 2, B). As $B_{REGS}$ have been recently demonstrated to undergo expansion under the direction of IL-21 produced by $CD4^+CXCR5^+PD\text{-}1^+$ $T_{FH}$ cells in autoimmunity[E4], the reduced numbers of $T_{FH}$-like cells in allergic rhinitis may contribute to the reduced numbers of $B_{REGS}$ observed. We further determined that $B_{REGS}$ and $T_{FH}$ cells are detectable in human lung lymph nodes as has been demonstrated in secondary lymphoid organs in autoimmunity.[E1,E2] Thus, $B_{REGS}$ and $T_{FH}$ cells are present in human lung lymph nodes and may regulate adaptive $T_H2$ responses in allergic inflammation in asthma (see Fig E1 and E2 in the Online Repository).

To determine whether the $B_{REG}$ and $T_{FH}$ cells we quantitated by FACS expressed prototypic cytokines characteristic of $B_{REGS}$ (i.e. IL-10) and $T_{FH}$ cells (i.e. IL-21), we used cell sorting to obtain purified populations of $B_{REGS}$ and $T_{FH}$ cells (>99% pure populations) from one non-allergic blood donor who we had previously noted had increased numbers of these cells (Figures 1 and 2). Using these cell sorted populations, we demonstrated *in vitro* increased IL-10 protein production by $B_{REGs}$ stimulated with CpG (see Figure E3, A in the Online Repository), as well as increased IL-21 mRNA expression by $T_{FH}$ cells stimulated with anti-CD3 and anti-CD28 antibodies (see Figure E3, B in the Online Repository). A previous study[4] in allergic rhinitis and asthma has also noted a reduced % of $B_{REGS}$ and $T_{FH}$ cells which were not phenotyped as in this study to determine whether they expressed prototypic cytokines (IL-10, and IL-21). Another difference between the two studies is the use of different cell surface markers to phenotype $B_{REGS}$. In this study we used cell surface markers ($CD19^{+}CD73^{-}CD25^{hi}CD71^{+}$) previously used to characterize $B_{REGS}$ expressing IL-10 in allergic disease[1], whereas the $B_{REGS}$ investigated in another study of allergic disease[4] used $B_{REG}$ cell surface markers ($CD3^{-}CD19^{+}CD24^{hi}CD27^{+}$) previously characterized in auto-immune diseases.[E5] $B_{REGS}$ identified in auto-immune disease may or may not function the same as $B_{REGS}$ identified in allergic disease and further studies are required to investigate the functional similarities and differences in these $B_{REG}$ subsets. Interestingly, CD38 has recently been reported to identify IL-10 producing $B_{REGS}$ in allergic disease.[2] In this study, we did not examine antigen-specific IL-10 production by $B_{REGS}$. However, previous studies have demonstrated that $B_{REGS}$ (using the same surface markers we used) had antigen-specificity to Phospholipase A2 in bee tolerant beekeepers.[1]

In summary, our findings show that $B_{REGS}$ (total and $CD25^{hi}$) are reduced in subjects with allergic rhinitis. We have demonstrated that these $B_{REGS}$ produce IL-10 and as such, could suppress $T_H2$ responses and play an important role in tolerance induction. We also demonstrated a significant reduction in levels of $T_{FH}$-like cells and their corresponding IL-21 production in allergic subjects, and made the novel observation that these cells are present in human lung lymph nodes. At present, the relative contribution of $B_{REGS}$ compared to the $T_{REGS}$ in inducing tolerance to allergens is unclear. Interestingly, $B_{REGS}$ are able to induce $T_{REGS}$ suggesting that $B_{REGS}$ can have both a direct IL-10 effect on inducing tolerance, as well as an indirect effect through the induction of $T_{REGS}$.[3] Future studies of $B_{REGS}$ and $T_{FH}$ cells in allergic disease may identify their relative importance as compared to $T_{REGS}$ in natural and acquired tolerance induction by allergen immunotherapy.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Abbreviations

| | |
|---|---|
| **$B_{REG}$** | Regulatory B cell |
| **$T_{REG}$** | Regulatory T cell |
| **$T_{FH}$** | T follicular helper cell |

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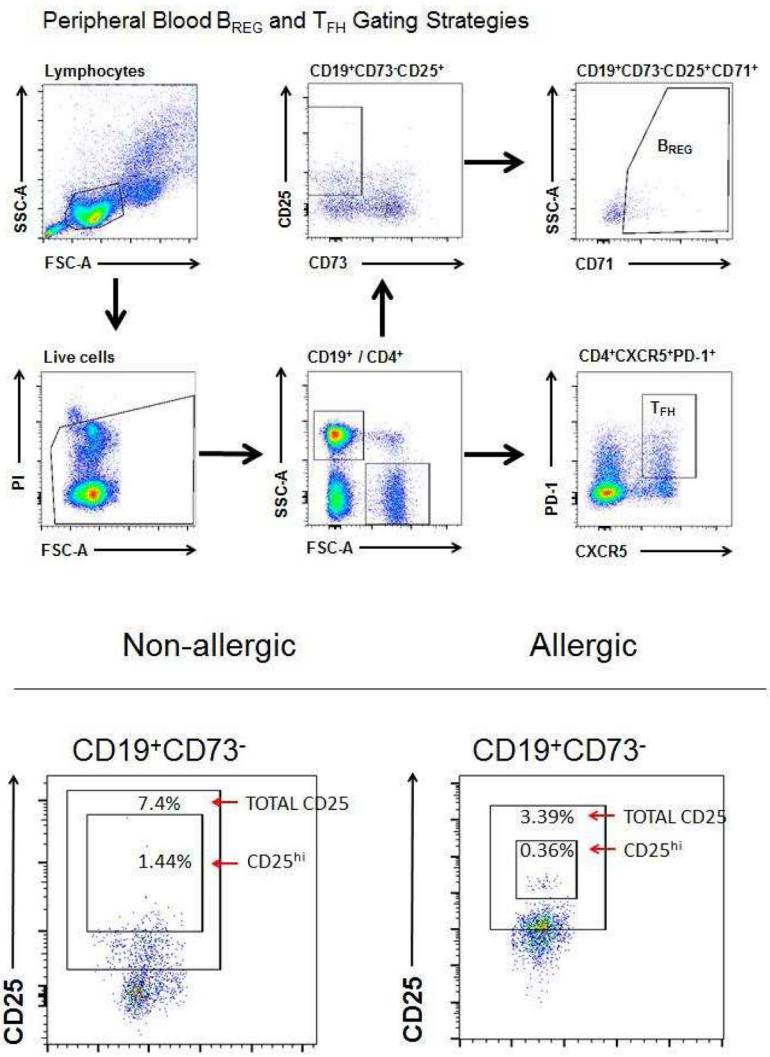


**FIG 1. FACS Gating strategy to detect $B_{REGS}$, $CD25^{hi}$ $B_{REGS}$ and $T_{FH}$ cells in peripheral blood**

**A,** Circulating $B_{REGS}$ and $T_{FH}$-like cells were identified from the circulating lymphocyte population as $CD19^{+}CD73^{-}CD25^{+}CD71^{+}$ and $CD4^{+}PD\text{-}1^{+}CXCR5^{+}$ respectively using the gating strategy shown. **B,** Example of FACS of circulating $B_{REGS}$ from a non-allergic individual (*left*) and an allergic individual (*right*) including isotype controls were used to quantitate their total $CD25^{+}$ and $CD25^{hi}$ subsets.

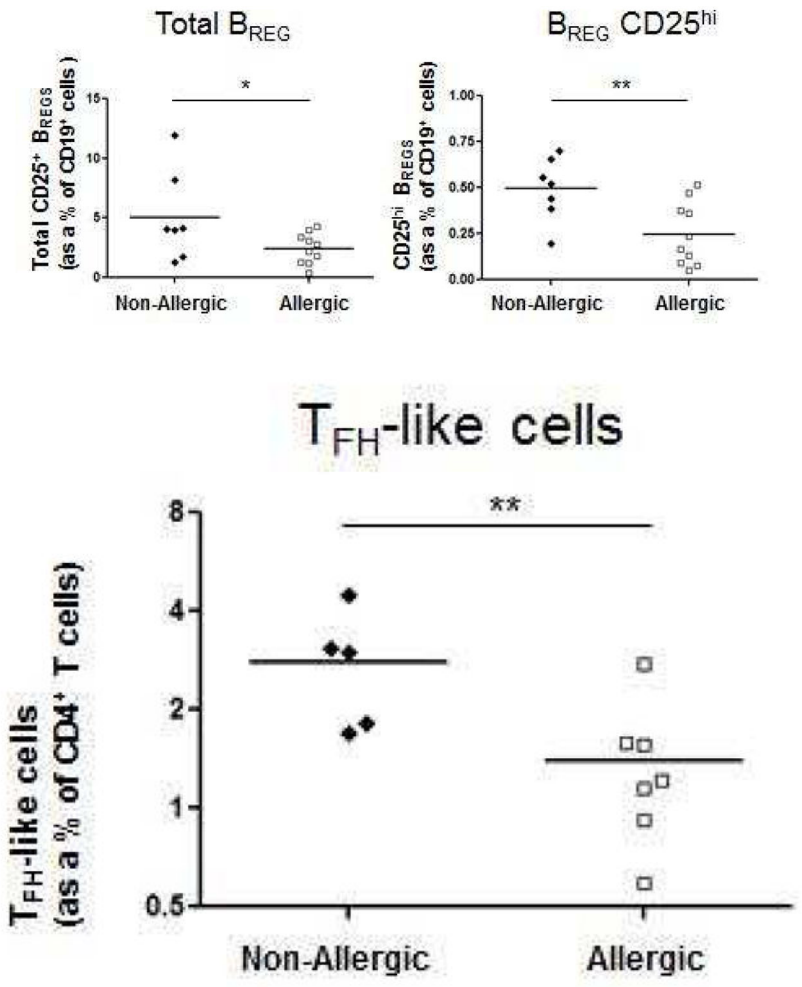


**FIG 2. FACS Quantitation of $B_{REGS}$, $CD25^{hi}$ $B_{REGS}$, and $T_{FH}$-like cells in allergic rhinitis subjects and controls**

**A**, Peripheral blood total $CD25^+$ (*left*) and $CD25^{hi}$ (*right*) $B_{REGS}$ from allergic rhinitis (n = 10) and non-allergic individuals (n = 7) were quantitated as percentages of $CD19^+$ cells. **B,** Circulating $CD4^+PD\text{-}1^+CXCR5^+$ $T_{FH}$-like cells from allergic rhinitis (n = 7) and non-allergic individuals (n = 5) were quantitated as percentages of $CD4^+$ T cells. $*P < .05$, $**P < .01$.