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Decreased Regulatory B Cells in Pediatric Patients with Asthma

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Emerging research has focused on the role of regulatory B cells (B regs) in allergic asthma.^{1–3} B regs are involved in immunomodulation and have been demonstrated to suppress inflammatory responses in autoimmunity, allergy, infection, transplantation, and cancer.² B regs secrete IL-10, an anti-inflammatory cytokine, that is theorized to have a beneficial effect on asthma pathophysiology by suppressing the IgE-mediated allergic cascade and decreasing airway inflammation.⁴ In murine models, a deficiency of B regs has been associated with elevated serum IgE, eosinophilia, and increased type 2 cytokines in bronchoalveolar lavage fluid.^{4,5} Adult studies have demonstrated decreased B reg numbers in the peripheral blood of patients with allergic asthma as compared to healthy controls,^{6,7} however, no similar study has been performed in pediatric asthma where the allergic phenotype is dominant. This pilot study investigated the hypothesis that levels of B regs in children and adolescents with asthma are decreased compared to healthy control children.

We recruited 11 children and adolescents (ages 3–18 years) with asthma and obtained blood samples from 10 age-matched control children without asthma or allergies. Peripheral blood mononuclear cells were isolated from whole blood using centrifugation and Ficoll gradient separation, rested in cell culture media overnight, and treated with the golgi complex inhibitor brefeldin A prior to staining. A live/dead stain was used to identify dead cells for exclusion. To enumerate B reg subsets, cells were stained with fluorescent antibodies against CD19, CD24, CD25, CD27, CD38, CD71, CD73, and IL-10. Data was acquired

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Conflicts of Interest: CMB is a co-founder and scientific advisory board member of Mana Therapeutics and Catamaran Bio, board member of Cabaletta Bio, and has stock in NexImmune and Repertoire Immune Medicines. She also serves as a DSMB Member for SOBI and has served on advisory boards for Pfizer, BMS and Roche. The other authors do not have and conflicts of interest.

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on a Beckman Coulter Cytoflex S flow cytometer and analyzed using FlowJo software. B cells were identified by CD19+ staining. We identified and enumerated three distinct subsets of B cells shown to produce IL-10: (a) CD24+ CD38+ B cells, (b) CD73- CD25+ CD71+ B cells, and (c) CD38+ CD27+ B cells. Within each of these three subsets, we identified the percentage of B regs, defined as those cells within each subset of B cells that were IL-10+. Our primary outcome was the percentage of IL-10+ B regs within each of the three identified subsets. These percentages were compared between asthma participants and control participants using the Wilcoxon Rank Sum Test (Mann Whitney U Test). The study was approved by our local Institutional Review Board.

Among the 11 participants with asthma, the median age was 11.0 years (range 3–18 years), 55% were male, 82% were Black, and 18% were Hispanic. Among the 10 control participants, the median age was 13.5 years (range 7–17 years), 50% were male, 50% were Black, and 30% were Hispanic. All the asthma participants had moderate or severe persistent asthma treated with at least medium-dose inhaled corticosteroids. None of the asthma participants had been treated with systemic corticosteroids in the past 3 weeks or an asthma biologic therapy in the past 12 months. Within the asthma group, 91% had allergic asthma defined by evidence of recent positive aeroallergen sensitization testing and/or current history of seasonal rhinitis.

As shown in Figure 1, participants with asthma had significantly lower levels of CD24+ CD38+ B regs as compared to non-asthmatic control participants. Specifically, the frequency of IL-10-producing CD24+ CD38+ B reg cells expressed as a percentage of all CD24+ CD38+ B cells was approximately 3-fold lower in participants with asthma as compared to the control group (median = 0.57% vs. 1.50%, $p=0.036$). There was not a statistically significant difference when comparing B reg percentages in asthma participants versus control participants for the other two evaluated B cell subsets (CD73- CD25+ CD71+ $p=0.062$ and CD27+ CD38+ $p=0.42$). Frequencies of B cell subsets did not differ between the two groups.

In summary, we discovered lower levels of circulating CD24+ CD38+ B reg cells in pediatric asthma participants as compared to healthy controls. This finding suggests that lower levels of B regs in children with asthma may be associated with suboptimal control of allergic inflammation contributing to the development of asthma and/or excess morbidity from asthma. Appropriate B reg numbers and function may be essential in controlling asthma and allergic disease by releasing suppressive signals to decrease TH2 inflammation. Additionally, the persistent inflammation observed in severe, difficult-to-control asthma, may be due to impaired immunoregulation caused by a deficiency of B regs. Finally, this work may aid in the development of novel immune-based therapies to increase B reg functionality in these patients.

Our significant finding in children and adolescents agrees with Oliveria et al., who also found lower levels of circulating CD24+ CD38+ B reg cells in adult patients with allergic asthma.⁷ This indicates that B regs may be important in both younger and older patients with allergic asthma. In children and adolescents, allergic asthma is the most common phenotype. It is possible that B reg deficiencies may be more common in allergic asthma as compared

to non-allergic asthma. We were unable to investigate this further given that our cohort of asthma participants was predominantly allergic. Furthermore, we measured B reg levels in peripheral blood and not specifically within the airways; however, future research could investigate associations to determine if blood levels are a good surrogate marker for airway levels.

A unique feature of our study is that our cohort was predominantly non-white. While this limits the generalizability of our findings, this accurately reflects our local demographics where Black children account for the majority of referrals to our asthma clinical programs. A study focused on other races will be important to evaluate if deficiencies of B regs are consistent across different racial groups. Alternatively, levels of B regs may not be consistent across racial groups. Since asthma is a racially disparate health condition with severe and poorly controlled asthma disproportionately affecting Black children,⁸ it is possible that Black children with asthma may have a greater deficiency of B regs contributing to the disparity.

While we found significant differences in levels of B regs within the CD24+ CD38+ subset, we did not find statistically significant differences in the other two B cell subsets that we investigated. These two subsets were not examined in the adult asthma study by Oliveria et al.;⁷ however, CD73- CD25+ CD71+ B regs have been demonstrated to be decreased in patients with allergic rhinitis⁹ and increased after venom immunotherapy.¹⁰ Our non-significant finding for these two subsets may be simply related to statistical power as this was a small pilot study. Alternatively, it may be possible that specific subsets of B regs may be more specific to inflammation in pediatric asthma. Further investigation of the role of B regs in both pediatric and adult asthma is needed. If it is determined that deficiencies in B regs are a key contributing factor to the pathophysiology of severe asthma, then future therapeutics may be designed to increase B reg counts and/or function to reduce asthma morbidity.

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Abbreviations/Acronyms:

B regs	Regulatory B cells
IgE	Immunoglobulin E
IL-10	Interleukin 10

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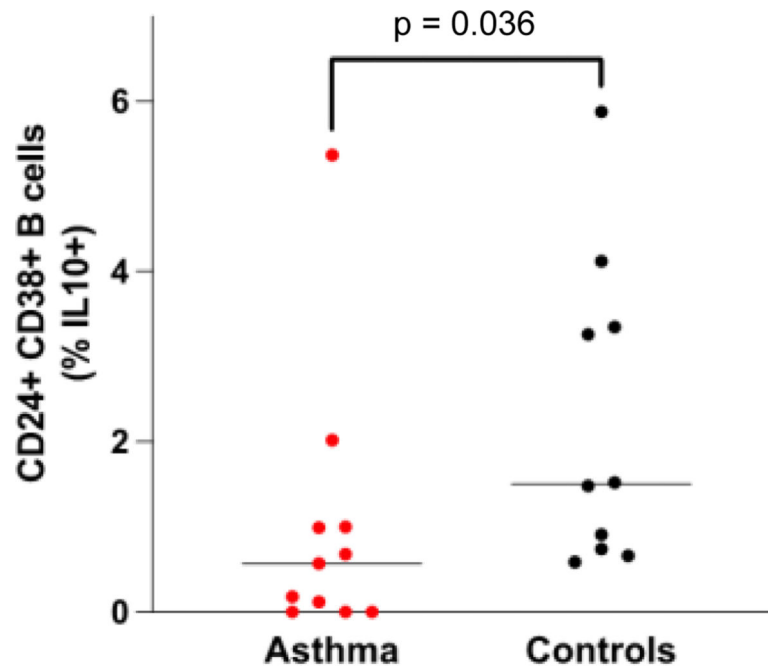


Figure 1: Blood levels of regulatory B cells (B regs) in children with asthma versus control children without asthma or allergies. Values displayed are the percentage of IL10+ cells within CD24+CD38+ B cells. Horizontal bars represent the median value for each group. P-value was calculated using the Wilcoxon Rank Sum Test.