Introduction

Allergic rhinitis (AR) is symptomatized by sneezing, nasal itching, airflow obstruction, and clear nasal discharge caused by IgE-mediated reactions against inhaled allergens. In China alone, data from a recent epidemiology study reported that 25%–38% of people in China suffer from AR. In developed countries, the rate is still on the rise. The molecular mechanism of AR is complex and considered generally caused by an immune tolerance deficiency. As an important kind of immune response, immune tolerance can maintain the immune balance, cut off the individual immune response by the immune system, and avoid the antigen stimulation of lymphocyte proliferation and abnormal immune response. Thus, induction and maintenance of immune tolerance are important mechanisms to maintain immune balance and prevent allergic inflammation. In addition, studies have shown that allergen-specific
regulatory cells (Regulatory T cells, Tregs), their cytokines, and surface molecules are essential for AR to maintain immune balance and self-tolerance.³

B lymphocytes are a major component of the immune system, and their effector functions are best known for producing antibodies, presenting antigens to T cells, and modulating immune responses through cytokine production. In addition to enhancing the immune response, B cells have also been shown to down-regulate the inflammatory response and induce tolerance. Recent studies have shown that the role of B cells in immune regulation cannot be ignored, especially in a subset of B cells, namely regulatory B cells (Bregs), which are characterized by secreting inhibitory cytokines (IL-10, IL-35, and TGF-β) and can regulate the immune response in the body.⁷ Among them, the regulation of allergen tolerance mediated by Bregs is regarded as an important immune tolerance mechanism of AR. Therefore, the present study aims to preliminarily explore the possible mechanism between Bregs and AR, and the prospects for the clinical application of Bregs in the treatment of AR.

Origin and phenotype of Bregs

B cells can be divided into two subgroups: B1 cells producing IgM and participating in the innate immune response⁴ and B2 cells participating in humoral immune response.⁶ The immature B cells, initial B cells, and plasma cells can be induced to differentiate into Bregs with immunosuppressive function in a specific immune microenvironment. There have been no unique Breg cell markers so far. However, Breg cells have many different phenotypes and can induce immune tolerance by inhibiting pathogenic T cell responses. A number of different Bregs have been identified based on phenotypic and functional characteristics in both mice and humans. In mice, several types have been found, such as CD5+B1a cells, CD1d+CD2+CD23+ transition phase 2 peripheral zone progenitor cells, CD5+CD1dhiB cells, peripheral zone B cells, CD19+CD138+ plasma blasts, CD19-CD138-plasma cells, and phenotypic CD5+CD19+CD1dhiB cells are more recognized as Bregs.⁷ In humans, there are mainly two types of Bregs, including CD19+CD24hiCD38hi and CD19+CD24hiCD27+.⁸ In addition, Toll-like receptors (TLR)-2, TLR-4, TLR-9 and B cell receptor, BCR signaling, CD40, CD80/CD86, or B-cell activating factor (BAFF) have been shown to induce B cells with inhibitory activity (Table 1).⁹,¹⁰ Parasites such as Schistosoma Mansoni, Wuchereria Bancrofti, Leishmaniais, and Plasmodium, bacterium such as Vibrio cholerae, and Escherichia coli, and viruses such as hepatitis B virus and human immunodeficiency virus (d HIV), all can induce the production of Bregs and IL-10.¹¹

Immunomodulatory Function of Bregs

Bregs are involved in multiple mechanisms to regulate immune responses and target various different immune cell types, such as dendritic cells (DC),¹² macrophages,¹³ and various lymphocytes. The most significant effector function is the production of the potent immunosuppressive cytokine IL-10. However, different subpopulations can also produce TGF-β and IL-35 or inhibit target cells through a cell contact dependent mechanism (Figure 1).

Release of cytokines

Bregs mainly secrete immunomodulatory cytokines: IL-10, TGF-β, and IL-35. IL-10 has a broad inhibitory effect on different cell types and widely recognized as an immu-ntolerance inducer in patients with other chronic inflammatory diseases. and ,and ,The regulatory effect of IL-10 is mainly to activate Jak1 and Tyk2 by binding with receptors, further phosphorylate STAT3, STAT1, and STAT5, and induce the expression of target genes.¹⁴ As a landmark cytokine of Bregs, IL-10 mainly inhibits Th1 and Th17 cell responses and promote the proliferation of Tregs and enhance their expression of the transcription factor Foxp3, while suppressing antigen presentation and proinflammatory cytokine production by DCs, monocytes, and macrophages, maintaining the number and function of iNKT cells and inducing IgG4 production.¹⁵ Transforming growth factor-β (TGF-β) is also considered a Bregs-related inhibitory molecule and it is involved in many processes, including tissue remodeling and immune regulation. TGF-β can activate several signaling pathways, including drosophila mothers against the decapentaplegic protein (SMAD), extracellular signal regulated kinase (ERK), C-Jun amino-terminal kinase (JNK), P38, phosphatidylinositol 3-kinase (PI3K), and protein kinase B (AKT).¹⁶ The mechanisms of TGF-β-Bregs regulating the immune response include: (1) Inhibit the differentiation and function of Th1, Th2, Th17 and cytotoxic

### Table 1  Types of Bregs in mice and humans.

<table>
<thead>
<tr>
<th>Types of Breg cells</th>
<th>Mouse model</th>
<th>Human</th>
<th>Suppressor molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2-MZP</td>
<td>CD19+CD21hi</td>
<td>—</td>
<td>IL-10</td>
</tr>
<tr>
<td></td>
<td>CD23hiCD24hi</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Tim-1+ B cells</td>
<td>Tim-1+CD19+</td>
<td>—</td>
<td>IL-35</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>CD138+MHC-1loB220+</td>
<td>—</td>
<td>IL-10</td>
</tr>
<tr>
<td>MZ B cells</td>
<td>CD19+CD21hiCD23-</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>B10 cells</td>
<td>CD5+CD1dhi</td>
<td>CD24hiCD27+</td>
<td>IL-10</td>
</tr>
<tr>
<td>Plasmablasts</td>
<td>CD138+CD44hi</td>
<td>CD19+CD24hiCD27int</td>
<td>IL-10</td>
</tr>
<tr>
<td>Immature cells</td>
<td>—</td>
<td>CD19+CD24hiCD38hi</td>
<td>IL-10</td>
</tr>
<tr>
<td>BR1 cells</td>
<td>—</td>
<td>CD19+CD25hiCD71hi</td>
<td>IL-10 IgG4</td>
</tr>
</tbody>
</table>
Roles of regulatory B cells in the pathogenesis of allergic rhinitis

To IgG subtypes and promote the differentiation of B cells to IgA+B cells. 17 IL-35 is mainly secreted by Tregs and Bregs and mediates signal transduction through pSTAT1/pSTAT4 in T cells and pSTAT1/pSTAT3 in B cells. 18

Figure 1  (A) Bregs can modulate T cell responses by suppressing effector T cells while promoting the proliferation of Tregs. These effects are mediated by secreted factors such as IL-10, TGF-β, IL-35, and membrane-bound molecules at the B cell and T cell interface including CD1d, CD40, CD80, CD86, PD-L1, FasL, and MHC on the B cell, and TCR, CD40L, CD28, PD-1, Fas on the B cell. (B) IL-10/Jak/STAT signaling pathway, IL-35/Jak/STAT signaling pathway, and TGF-β/SMAD signaling pathways in Bregs. Bregs, regulatory B cells; Tregs, regulatory T cells; PD-L1, Programmed death-ligand 1; FasL, Fas ligand; MHC, major histocompatibility complex; TCR, T-cell receptor; SMAD, drosophila mothers against decapentaplegic protein; JAK, Janus Kinase; STAT, signal transducer and activator of transcription; P, Phosphorylation.

CD8+ T cells, enhance the expression of Foxp3 and CTLA-4 in CD4+Tregs, induce the production of CD8+ Tregs. (2) Inhibit the activity of dendritic cells and induce the tolerance of dendritic cells (TolDCs). (3) Inhibit the conversion of B cells to IgG subtypes and promote the differentiation of B cells to IgA+B cells.
these B cells has an autocrine effect, which can further expand Bregs and then enhance the activity of Foxp3+Treg cells induced by T cells. It can also enhance the proliferation of Th1 and Th17 cells by blocking the G1 phase of cell division and inhibiting the development of Th2 cells by inhibiting GATA3 and IL-4. In addition to the above cytokines, Bregs can also produce indoleamine 2, 3-dioxygenase (IDO), which alter T cell activation by breaking down the essential amino acid tryptophan. Bregs may also induce T cell death by secreting granase B, which are mainly secreted by CD38+CD1d+IgM+CD147+B cells and up-regulated by IL21.

**Cell contact inhibition**

In addition to cytokine secretion, some B cell surface molecules are involved in the suppressive function of Bregs. CD1d plays an active role in Bregs-mediated inhibition. Studies have shown that the up-regulation of CD1d on B cells is associated with the protection of intestinal mucosal inflammation mediated by B cells. Costimulatory molecules are involved in the inhibitory mechanism of B cells. B cells express CD40 on their surface and inhibit T cell-mediated inflammatory response by interacting with T cell surface CD40L. B7 costimulatory receptors CD80 and CD86 have also involved in B cell contact dependent inhibition. The interaction between the B7 surface receptor and its inhibitory ligand cytotoxic T lymphocyte protein 4 (CTLA-4) or CD28 on target cells is crucial for regulating T cell activation. Programmed death ligand 1 (PD-L1) is a transmembrane protein that binds to the inhibitory molecule PD-1 to activate immune cells. It was found that B cells expressing PD-L1 and PD-L2 inhibited inflammatory T cells by up-regulating PD-1 expression, and activation of macrophages and microglia cells played a protective role. Other membrane-associated molecules include Fas ligand (FasL), CD19, CD62L and MHC-II, T-cell immunoglobulin and mucin domain 1 (TIM-1), intracellular signal transduction and transcription activator, and myeloid differentiation response gene 88 (MyD88) are also associated with Bregs mediated inhibition.

**Progress of Bregs in airway allergic diseases**

Previous studies on Bregs have focused on autoimmune models, and recent studies have also shown that Bregs can reduce Th2-based immune diseases, such as airway allergies. An allergy refers to a dysregulation of the immune response to allergens, resulting in the expansion of polarized Th2 cells, increasing IgE production, and eosinophilia. In an allergic disease, B cells preferentially convert to IgE subtypes in the presence of localized IL-4, which forms the core of an acute inflammatory response to an allergen. Allergen-specific IgE binds to FC Receptors (FCR) on mast cells and basophils, and subsequent exposure to the same allergen results in degranulation and inflammation.

**Allergic asthma**

Allergic asthma is a chronic inflammatory disease dominated by the Th2 type response, characterized by a high reactivity to inhaled allergens in the lower respiratory tract. The number of CD24hiCD27+B cells in patients with asthma was down-regulated, and the ability to secrete IL-10 was significantly lower than that of normal subjects after LPS stimulation, suggesting that the number and function of Bregs in asthmatic patients were defective. An in-depth analysis of the Bregs phenotype showed that CD9 was a specific marker for mouse and human Bregs. In HDM mouse models, adoptive CD9+B cells secreted IL-10 through mitogen-activated protein kinase signaling pathway to inhibit Th2 and Th17 inflammation, restore Treg/Th2 ratio, and induce the CD3+CD4+CD25+ effector T cell apoptosis to restore immune balance in lung tissues. In OVA-sensitized mouse airway inflammation, CD5+CD1dhiB cells inhibited the development of allergic airway inflammation by producing TGF-β to promote the accumulation of Foxp3+Treg cells in the lung and finally induced in vivo tolerance to allergens. In addition, Bregs can also control cockroach allergen induced inflammation by inducing FASL-mediated apoptosis of CD4+T cells. The CD1dhi Bregs prevents the development of allergic airway inflammation by interacting with Tregs.

**Allergic rhinitis**

Allergic rhinitis (AR) is an IgE mediated hyper-reactive inflammation of the upper respiratory tract in response to inhaled allergens, characterized by itchy nose, sneezing, runny nose, and nasal congestion. The level of Bregs in AR patients is lower than that in healthy subjects and increased after immunotherapy. Kim et al. reported that both CD19+CD25+CD71+CD73+Bregs and CD19+CD25hiCD71+CD73+Bregs decreased in AR patients, and there were fewer T follicular helper (Tfh) cell-like cells (CD4+PD-1+CXCR5+) in AR patients. It is suggested that the recovery of nasal mucosal immunity and Bregs can be promoted by regulating the proportion and function of Thf cells. A recent study reported that the proportion of CD19+CD24hiCD38hi and CD19+CD5hiCD1d+Breg cells in seasonal AR patients was lower than that in healthy controls, but significantly increased after specific immunotherapy (SIT). In addition, TGF-81+Bregs was down-regulated in OVA-induced wild-type BALB/C AR mouse model in animal experiments, but increased with the increase of OVA stimulation times, indicating that the immune tolerance of AR mice is related to the increase of TGF-81+Bregs. Activation of PAR2 inhibited the expression of IL-10 in B cells, and treatment of B cells with Bcl2L12shRNA liposomes reversed this inhibition, suggesting that the regulation of Bcl2L12 may be a new way to treat AR. Sema3A is highly expressed in Bregs, and treatment of AR mice with recombinant Sema3A reduced the density of nerve fibers in the lamina propria of the turbinate, showing that Breg can alleviate allergic symptoms by inhibiting excessive neural activity with Sema3A. Studies have shown that the expression of IL-12A and EBI3mRNA and serum IL-35 level are decreased in AR patients. Existing
literature suggests that IL-35 is involved in immune tolerance, but the mechanism of Bregs-derived IL-35 in allergic reactions and SIT needs further study.

**Bregs-regulated immune tolerance in allergic rhinitis**

Studies have shown that SIT of AR can enhance Bregs-mediated immune tolerance. The process includes increased Bregs level of specific IL-10 production, induction of Tregs l proliferation, significant increase of IgG-4 antibody level, inhibition of Th2 response, thus improving symptoms of AR and producing immune tolerance (Figure 2).

**Inhibitions of antigen presenting cell function**

**DC cells**

Several studies have reported that Bregs can prevent DCs from producing IL-12 through IL-10 and suppress the maturation of monocyte-derived DCs. DC is the most potent antigen-presenting cell (APC). The functions of DC consist of ingesting, processing, presenting antigens, and activating the differentiation of initial T cells in the immune response. The association between AR and Bregs can be verified by the fact that IL-10 can inhibit the secretion of IFN-α from DC cells, suppress the maturation of DCs, and finally hinder the occurrence of AR.

**Macrophages**

Macrophages are a kind of antigen-presenting cells, usually induced by Th1 cells. They initiate and modulate cellular immune responses but also synthesize and release IL, TNF, NO, and other active substances. TNF-α, as an important cytokine produced by macrophages, displays a broad spectrum of biological responses. Research shows that TNF-α can destroy the epithelial cell integrity and play an important role in the development of AR. Bregs can prevent the macrophages from releasing TNF-α by secreting IL-10. On the one hand, it can weaken the role of macrophage antigen presenting cells and on the other hand, it can alleviate the occurrence and development of AR by inhibiting macrophage differentiation of inflammatory factors such as TNF-α and IFN-γ.

**Inhibitions of helper T cells response**

**Th2 cells**

Excessive activation of Th2 cells is the underlying cause of AR. In general, Th1 cells secrete inflammatory cytokines such as IFN-γ and IFN-α, which mediate cellular immune responses, whereas Th2 cells secrete IL-4, IL-5, IL-13 etc., which mediate humoral immunity in the body. In the peripheral blood of normal people, Th1 and Th2 cells maintain a normal immune response by keeping a relative balance in numbers. When sensitive individuals exposed to allergens, the allergens would be processed by antigen-presenting cell (APC) and then the signal of antigen peptide could be presented to T cells, which makes the differentiation of Th initial cells shift and leads to the Th1 reaction switch to Th2 reaction. The immune response of Th2-Th1 is the cause of AR. Bregs can inhibit the immune response mediated by Th2 cells by secreting IL-10 and TGF-B cytokines, reducing the imbalance of Th1/Th2 ratio in patients with AR and preventing the occurrence of AR.

![Figure 2](image_url)

Figure 2 Role of Bregs in the regulation of AR. Bregs inhibit DCs and effector Th cells, promote the generation of Tregs, upregulate IgG-4 antibody levels. Bregs, regulatory B cells; DC, dendritic cells; Th, helper T cell; Tfh, follicular helper T cell; NK cell, natural killer cell.
Th17 cells
Th17 cells are a unique subset of Th cells that can secrete IL-17. In recent years, accumulating evidence has shown that compared with normal people, patients with AR have a higher proportion of Th17, which indicating that Th17 is negatively correlated with the occurrence of AR. Bregs can inhibit the differentiation and proliferation of primary T cells to Th17 cells by mediating IL-10 and IL-35, resulting in the reduction of Th17 cells, the secretion of IL-17, and the alleviation of symptoms in AR patients. Therefore, the inhibitory effect of Bregs on Th17 cells may provide a new idea to explain the pathogenesis and treatment of AR.

Follicular T helper cell
Bregs have inhibitory effect on follicular T helper cell (Tfh), which can hinder the differentiation of initial T cells into Tfh cells. Tfh is a subset of CD4+ T cells, which could assist B cells in producing antibodies and expressing chemokine receptor type 5 (CXCR5) and interleukin-21 (IL-21). Some studies have found that Tfh cells played an important role in AR-related immune processes by facilitating the transformation and maturation of IgE, IgG-1 antibodies, and promoting Th2 reaction. Bregs can inhibit the function of Tfh cells by secreting IL-10, thereby reducing the occurrence of AR.

Promotion of regulatory T cell function
Bregs can promote the expression of Tregs mainly by secreting IL-10. Some studies have found that Bregs can provide an appropriate microenvironment for the induction and differentiation of Tregs by secreting TGF-β. Tregs, as an important subgroup of T cells, are involved in immune suppression by secreting IL-10, etc. Tregs cooperate with Th17 and its cytokine IL-17 to regulate the development of AR. It has proven that Treg/Th17 imbalance is a mechanism in AR. Anderson AE et al. found that desensitization therapy brings with higher Tregs and better clinical symptoms in AR patients, which may be explained by the fact that Tregs can reduce the proliferation of Th2 response by inhibiting the activation of T cells. In addition, it has been reported that phospholipase A2-specific Bregs can upregulate CCR5 expression, a marker expressed by Tregs, in both healthy people and patients undergoing allergen-specific immunotherapy. Bregs can regulate the production and function of Tregs in various ways. Therefore, it can be deduced that Bregs can inhibit the occurrence of AR by increasing the production of Tregs.

Promotion of natural killer cell function
Natural killer (NK) cells, as the main type of cells in natural immunity, can secrete a large number of cytokines, regulate the immune response, and exhibit certain cytotoxicity, which is a non-specific immunity of the human body. When activated, NK cells secrete IFN-γ, which can promote Th0-Th1 differentiation and inhibit Th0-Th2 differentiation. As an immunomodulatory disorder, the core of AR is also the balance of Th1/Th2 cells, and NK cells can regulate the response of Th1 and Th2, so they play an important role in the occurrence and development of AR. Mahr B et al. found that Tregs could enhance the function of NK cells, while Bregs could enhance the function of Tregs. Therefore, Bregs can indirectly promote NK cells to secrete IFN-γ and increase Th1, finally hinder the occurrence of AR.

Promotion of IgG4 expression
It was found that Bregs could promote IgG-4 expression by secreting IL-10. The increasing IgG-4 can competitively block the binding of IgE to the surface of allergen and mast cells, which hinders IgE-mediated AR. It is well known that AR is a non-infectious inflammatory disease, which involves IgE-mediated mediators (mainly histamines), and various immune-active cells and cytokines. Bregs may hinder IgE-mediated AR development through increasing the expression of IgG-4.

Advances in Bregs-based immunotherapy
Allergen specific immunotherapy (AIT) is a long-term treatment option for AR, allergic asthma and allergic conjunctivitis. In AIT, the dose of allergen vaccine gradually increases, until the symptoms disappear. It is also the only etiology-oriented treatment strategy recommended by the WHO. At present, the mechanism of immunotherapy is to restore the body's immune tolerance by reducing allergen-induced IgE. Bregs were initially observed in healthy beekeepers and patients allergic to bee venom. After 4 months of venom immunotherapy, the level of IL-10-producing Bregs increased by two- to five-folds, as high as that observed in healthy beekeepers. A similar phenomenon was also found in AR patients. After AIT treatment, the clinical symptoms of AR patients were significantly relieved, whereas the levels of Tregs and Bregs in the peripheral blood were significantly increased, and that of IgE was significantly reduced, indicating that AIT treatment could achieve the therapeutic efficacy by promoting the generation of Bregs in AR patients. Currently, there is no therapy for AR based on Bregs in clinical practice. Previous animal studies have confirmed the possibility of Bregs expansion in vitro, and stimulating the proliferation of IL-10-producing Bregs using CD40 in vivo. Therefore, it can be expected that Bregs-based immunotherapy may be developed to treat AR in the near future.

Future recommendations
AR is a common non-infectious inflammatory disease of the upper respiratory tract, and it is difficult to obtain satisfactory results in clinical treatment. The mechanism of the occurrence and development of the disease is complex. Therefore, extending the study to Bregs is more conducive to in-depth and comprehensive understanding of the disease. However, there are few studies on the regulatory mechanism of Bregs in AR at present, and most of them focus on the immune tolerance mechanism of Bregs, which leads to the limitation of clarifying the mechanism of Bregs in AR. In addition, questions on which cytokines induce Bregs in AR and whether there are Bregs-specific transcription factors remain to be further studied. It is believed that further research on Bregs will contribute to the discussion of the pathogenesis of AR and provide new ideas and
approaches for the prevention, diagnosis, and treatment of AR.

Conflict of interest

The authors have no conflict of interest to declare.

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