



B Cell Functions in the Development of Type I Allergy and Induction of Immune Tolerance

Lisa Naomi Pointner, Fatima Ferreira, and Lorenz Aglas

Contents

- 1 Introduction
- 2 The Contribution of B Cells in the Development of Allergic Sensitization
 - 2.1 Plasma Cells in Type I Allergy
 - 2.2 Memory B Cells in Type I Allergy
- 3 The Contribution of B Cells in the Effector Phase of Type I Allergy
 - 3.1 B Cell-Derived IgE
 - 3.2 IgE Binding to FcεRI: Mediator Release Function
 - 3.2.1 Amplification of the IgE Response
 - 3.2.2 IgE Binding to CD23: FAP and Epitope Spreading
 - 3.2.3 IgE Binding to CD23: Allergen Transport into Mucosal Tissues
 - 3.3 B Cell-Derived IgG in Type I Allergy
- 4 B Cells in Natural and Allergen-Specific Immunotherapy (AIT)-Induced Tolerance
 - 4.1 Altered Antibody Secretion Profile Induced by AIT
 - 4.2 Allergen-Specific Breg Cells
 - 4.2.1 Phenotype of Breg Cells
 - 4.2.2 Function of Breg Cells
 - 4.2.3 The Role of IL-10-Producing Breg in Natural Tolerance
 - 4.2.4 Induction of IL-10-Producing Breg During AIT
 - 4.2.5 The New Breg-Treg Paradigm in AIT

References

Abstract

B cells are key players in the mechanisms underlying allergic sensitization, allergic reactions, and tolerance to allergens. Allergen-specific immune responses are initiated when peptide:MHCII complexes on dendritic cells are recognized by antigen-specific receptors on T cells followed by interactions between

L. N. Pointner · F. Ferreira (✉) · L. Aglas
Biosciences Department, University of Salzburg, Salzburg, Austria
e-mail: fatima.ferreira-briza@sbg.ac.at

costimulatory molecules on the surfaces of B and T cells. In the presence of IL-4, such T-B cell interactions result in clonal expansion and isotype class-switching to IgE in B cells, which will further differentiate into either memory B cells or PCs. Allergic reactions are then triggered upon cross-linking of IgE-FcεRI complexes on basophils and mast cells, leading to cell degranulation and the release of pro-inflammatory mediators.

Mechanisms underlying effective allergen-specific immunotherapy (AIT) involve the induction of Tregs and the secretion of blocking IgG4 antibodies, which together mediate the onset and maintenance of immune tolerance towards non-hazardous environmental antigens. However, the importance of regulatory B cells (Breg) for tolerance induction during AIT has gained more attention lately. Studies in grass pollen- and house dust mite-allergic patients undergoing SCIT reported increased frequencies of IL-10+ Breg cells and a positive correlation between their number and the improvement of clinical symptoms. Thus, Breg are emerging as biomarkers for monitoring tolerance to allergens under natural exposure conditions and during AIT. Further research on the role of other anti-inflammatory cytokines secreted by Breg will help to understand their role in disease development and tolerance induction.

Keywords

Adaptive immunity · AIT · Antibody · B cells · Breg

1 Introduction

B cells play an essential role in type I allergy. In this chapter will be described how B cells contribute to the development of allergic sensitization, to the effector phase via the secretion of high-affinity IgE antibodies but also how they participate in immune tolerance during allergen exposure (Fig. 1).

2 The Contribution of B Cells in the Development of Allergic Sensitization

The development of type I allergies or immediate hypersensitivity reactions are characterized by two phases (van Ree et al. 2014). First, an asymptomatic phase, called *sensitization*, occurs upon first encounter with a specific allergenic source. Subsequent exposures to the same allergen can then trigger the *effector phase*, which corresponds to the manifestation of the disease.

The sensitization process is orchestrated by the interplay of various immune cells resulting in the induction of effector type 2 T helper cells (Th2) and an enhanced production of Immunoglobulin E (IgE), the hallmarks of the allergic sensitization. During this phase, antigen-presenting cells (APC), mainly dendritic cells (DCs), take up and process allergens. Activated DCs then migrate to the draining lymph nodes to present antigenic peptides in complex with major histocompatibility class II

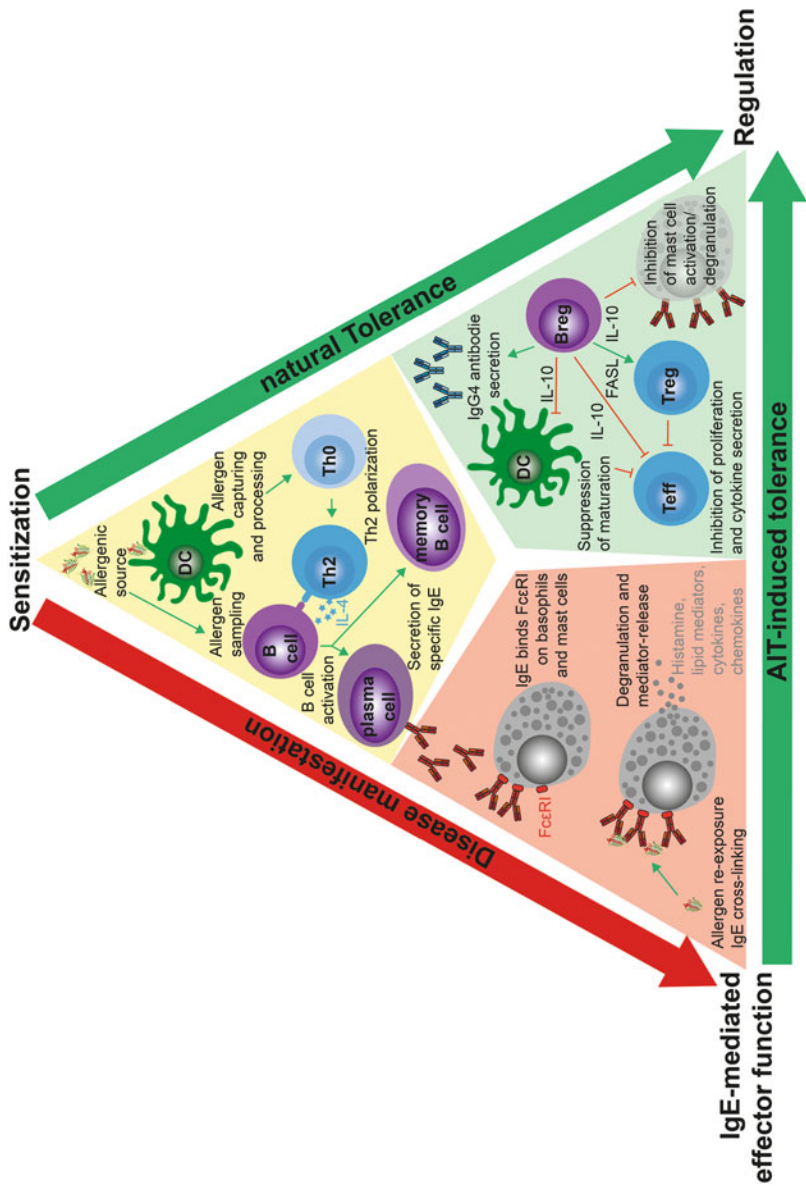


Fig. 1 The B cell function triad in type I allergy. Summary scheme showing the interconnected and versatile role of B cells in three different aspects of type I allergy. During allergic sensitization (in yellow), allergens are taken up and processed by dendritic cells (DC) and B cells. DC present allergenic peptides to

(MHCII) molecules to allergen-specific naïve CD4⁺ T cells. In the presence of an efficient co-stimulation and the appropriate local microenvironment, provided by the cytokine IL-4, they differentiate into Th2 cells that express characteristic cytokines including IL-4, IL-5, and IL-13 (van Ree et al. 2014). Th2 cells then circulate in the blood or reside in specialized T cell zones of peripheral lymphoid organs (spleen and lymph nodes) where they become specialized follicular T helper cells (T_{fh}) waiting to encounter their specific antigen.

During sensitization, allergens are also recognized by B cells, which are lymphocytes expressing cell surface antigen-specific immunoglobulins (Ig), also called B-cell antigen receptors (BCR) (LeBien and Tedder 2008). During their development in the bone marrow, B cells undergo a structured rearrangement of their immunoglobulin gene segments in order to generate a huge repertoire of BCR recognizing over a billion different antigenic epitopes (Gould and Wu 2018). Fully developed B cells express the isotype IgM and IgD on the cell surface and migrate into lymphoid follicles of peripheral lymphoid tissues for antigen surveillance (Pieper et al. 2013; LeBien and Tedder 2008).

The binding of the specific antigen to the BCR of a naïve immature B cell triggers the activation of specific intracellular signaling pathways, internalization of the antigen-BCR complex, and the subsequent presentation of antigenic peptides in the MHCII groove at the cell surface. The antigen-activated B cell is concomitantly guided towards T cell zones in order to facilitate the meeting with the cognate T_{fh} cell (Cyster and Allen 2019). The communication between both cells involves a cell-to-cell contact via the interaction of the peptide:MHCII complex and the antigen-specific T cell receptor as well as through the interaction of the costimulatory molecules CD40 and CD40 ligand (CD40L) and OX40L and OX40 on the B cell and T cell surfaces, respectively (Akiba et al. 1999). In addition, a *soluble factor*, IL-4, provided by the Th2-like T_{fh} cells drives the switching to IgE. IL-4 acts via the type I IL-4 receptor and triggers the activation of the STAT6 signaling pathway (Dullaers et al. 2012; van Ree et al. 2014). In consequence, these signals result in clonal expansion and isotype class-switching into IgE-expressing B cells, which will

Fig. 1 (continued) allergen-specific naïve T cells (Th0) and induce their polarization into type 2 effector T cells (Th2). Allergen-loaded B cells interact with cognate Th2 cells, which triggers their activation and differentiation into IgE-secreting plasma cells (PC) or memory B cells. Allergen-specific IgE binds to the high-affinity receptors FcεRI on mast cells and basophils in tissues and blood circulation, respectively. During allergen challenge (in red), the cross-linking of IgE-FcεRI complexes triggers cell degranulation and release of pro-inflammatory mediators such as histamine, lipid mediators, cytokines, and chemokines and leads to manifestation of allergic symptoms. The concomitant upregulation of FcεRI, interleukin 4 (IL-4) and CD40 ligand (CD40L) leads to an enhanced and sustained allergic immune response. Regulatory B cells (Breg) are key players in natural- and allergen-specific immunotherapy (AIT)-induced immune tolerance (in green). Breg cells secrete allergen-specific IgG4 antibodies that compete with IgEs for allergen binding. Through the secretion of IL-10, Breg cells display a pleiotropic immunoregulatory function. IL-10 can block the function of effector T cells (Teff) either directly or indirectly; on the one hand via the induction of regulatory T cells (Treg), and on the other hand by the inhibition of antigen-presenting cells. Finally, IL-10 can inhibit degranulation and activation of mast cells

differentiate into either plasma or memory B cells (Janeway et al. 2001). The mechanisms underlying this choice are still under investigation; however, studies have reported that the isotype of the BCR can influence the differentiation path, in which the IgE BCR promotes a spontaneous preferential differentiation into plasma cells (PCs) (Cyster and Allen 2019).

The defined state of a sensitized individual is then when the secreted allergen-specific IgEs have primed mast cells in tissues and basophils in the blood circulation by binding to their high-affinity receptors, FcεRI.

2.1 Plasma Cells in Type I Allergy

The differentiation of B cells into PCs can occur in two ways, depending on the duration of allergen exposure. After a short contact with the allergen, activated B cells can differentiate into short-lived PCs that produce low-affinity IgE antibodies (Roth et al. 2014). Mouse studies have shown that under certain conditions, the production of IgEs of low affinity can be sufficient to induce allergic reactions and occurs in extra follicular areas of the draining lymph nodes (Jimenez-Saiz et al. 2017; Wu and Zarrin 2014). However, in case of a persisting antigen exposure, the sustained B cell activation gives rise to follicles followed by the formation of germinal centers (GCs), which are sites of intense B cell proliferation, selection, maturation, and death in secondary lymphoid tissues. In GCs, B cells initially continue to proliferate as plasmablasts and then undergo important maturation steps leading to high-affinity IgE-secreting PCs and memory B cells (Tedder; Medina et al. 2002; Oracki et al. 2010; Klein and Dalla-Favera 2008). In fact, the huge variety of antigen specificity is determined by the two variable domains of the heavy and light chains of the immunoglobulin whereas the function is determined by the isotype of the constant (C) region of the heavy chains; C epsilon (Cε) being the heavy chain in IgE (Pieper et al. 2013). Three major modifications occur at the genetic level of differentiating B cells, which aim to further diversification of their BCR:

- Firstly, point mutations, termed *somatic hyper-mutations*, mediated by cytidine deaminase (AID), occur in the variable regions of the expressed immunoglobulin gene in order to increase the affinity of the antibody to its respective antigen (Geha et al. 2003).
- Secondly, in a process called *affinity maturation*, B cells expressing a BCR with high affinity are selected for proliferation and survival by competing with follicular dendritic cells for antigen presentation to Tfh cells (Gould and Wu 2018). Mutations that improve Ig's affinity facilitate the efficient activation of the B cell, whereas cells bearing lower affinity Igs undergo apoptosis. The GCs are therefore a site of both, high B cell death and proliferation (Janeway et al. 2001; Vinuesa et al. 2010).
- Thirdly, selected B cells undergo *class-switch recombination*, also driven by AID, in order to change the isotype of the expressed Ig while retaining antigen's

specificity. In regard to IgE responses, the synergistic effect of CD40L-mediated co-stimulation and IL4 triggers the genetic rearrangement in the genomic locus of the heavy chain to replace the constant C region of IgM (C μ) by the constant region of IgG (C γ) and subsequently of IgE (C ϵ); the IgE gene being located downstream of the IgG gene (Geha et al. 2003).

The final differentiation process is controlled by different TFs (e.g., the B cell specific activating protein and the positive regulator domain I-binding factor 1) and associated with phenotypic changes (Roth et al. 2014; Dullaers et al. 2012). Even though PCs still respond to differentiation and survival signals provided by T cells, such as IL6 and CD40L, they become unable to express MHCII, making them incapable of presenting antigens to T cells (Saunders et al. 2019). Once their differentiation is completed, PCs can home into different immune compartments. PCs that reside in secondary lymphoid organs behave as short-lived PCs. In contrast, PCs that migrate through the circulation back to the bone marrow and the lamina propria of inflamed tissues survive in niches as long-lived resident cells, thus, representing a form of IgE memory (Moutsoglou and Dreskin 2016; Roth et al. 2014).

IgE-secreting PCs are key players of type I allergic reactions due to their capacity to secrete large amounts of high-affinity and isotype switched IgE antibodies (IgE proteins represent 10–20% of all synthesized proteins in a PC) (Medina et al. 2002).

2.2 Memory B Cells in Type I Allergy

The humoral immune memory is mostly characterized by the generation of memory B cells, which are quiescent, antigen-experienced, long-lived B cells originating from GCs and persisting long after antigen challenge in order to act in the surveillance for antigen in the circulation and mucosal tissues. Under basal conditions, memory B cells express surface Ig antibodies but secrete them at low rate. Upon antigen re-exposure, they can mount a rapid and high-affinity secondary humoral immune response that is more efficient than the primary response (Saunders et al. 2019; He et al. 2017).

Despite the importance of IgE in the pathogenesis of type I allergies, the mechanisms underlying the generation of IgE memory are still unclear and intensively studied. Most studies support the existence of a memory for IgE-mediated allergic responses in mice and humans and claim that this memory is attributed to IgG1+ memory B cells that can, upon activation, differentiate into IgE-secreting plasma cells by de novo class-switching to IgE (Talay et al. 2012; He et al. 2013). In fact, isotype class-switching towards IgE seems to be a sequential procedure in which a B cell undergoes a successive isotype switch from IgM to IgE via one or more intermediate isotypes (IgG1 and IgG4). Since, after a primary response, IgG precursor cells are selected after somatic hyper-mutations and persist for weeks in the GCs, the sequential de novo switching generates IgE antibodies of high affinity (Gould and Wu 2018; He et al. 2013).

Alternatively, IgE class-switching can occur directly from an IgM precursor cell resulting in a low IgE affinity and B cell apoptosis during selection in the GC, which was observed in IgG1-deficient mice that upon repeated immunizations developed IgE-secreting PCs derived directly from IgM precursor cells. The cells failed to produce high-affinity IgEs although the levels were comparable to those of wild type mice, suggesting that IgG1 cells are essential for the generation of high-affinity IgE influenced by Th2 conditions (Erazo et al. 2007).

The existence of IgE memory B cells is still a matter of debate. Although IgE+ memory B cells have been reported in human, their functionality and cell fate remain unclear (Talay et al. 2012; Gould and Wu 2018). In mice, IgE memory B cells are very rare and the reason might be the transiency of GCs containing IgE-expressing B cells that quickly disappear during the primary response. Indeed, IgE BCRs are expressed at low levels compared to IgG BCRs, which reduces the ability of these cells to capture and present antigens and, thus, might explain the poor competition ability of IgE B cells within GCs (Cyster and Allen 2019).

3 The Contribution of B Cells in the Effector Phase of Type I Allergy

3.1 B Cell-Derived IgE

In the absence of disease, IgE is present in very low concentrations in the serum (the lowest of all five isotypes), has a short half-life and, because of its highly active and rather invasive biological nature, the expression of IgE is tightly regulated (Wu and Zarrin 2014). IgE antibodies normally serve to protect the host from parasitic infections such as helminths as well as to neutralize venoms and toxins, preventing tissue damages of the host. Individuals mounting a protective IgE response have elevated serum IgE levels but they remain below IgG levels indicating a well-regulated IgE synthesis. In contrast, when the IgE response is inappropriately initiated in response to allergen exposure, these antibodies become deleterious in the pathogenesis of allergic disorders.

3.2 IgE Binding to FcεRI: Mediator Release Function

During the sensitization phase, IgE antibodies secreted by PCs enter the circulation and, due to their small size (190 kDa), diffuse easily into periphery such as blood circulation and mucosal tissues. There, IgE molecules bind via their constant region to the high-affinity receptor, FcεRI, on the cell surface of circulating basophils and tissue-resident mast cells. Such sensitized immune cells react sensitively to allergens even when the concentration of circulating IgE is low (Dullaers et al. 2012).

Upon re-exposure, the specific allergen binds to the variable regions of adjacent cell-bound IgE molecules resulting in physical cross-linking of IgE-FcεRI complexes. This interaction triggers activation of downstream signaling pathways

leading to cell degranulation and release of pro-inflammatory mediators. The early release of pre-synthesized mediators (e.g., histamine, serine proteases, carboxypeptidase A, and proteoglycans) is responsible for the immediate phase reaction of the allergic immune response that develops within minutes. Four to eight hours thereafter, newly sensitized lipid mediators such as arachidonic acid products (e.g., leukotrienes and prostaglandin), as well as cytokines and chemokines (e.g., IL-4, IL-5) are released in the delayed phase reaction that is accompanied by the recruitment of additional inflammatory cells (such as eosinophils). The resulting effects are inflammation, vasodilation, increased permeability, smooth muscle contraction, and further tissue damages (van Ree et al. 2014).

The manifestations of IgE responses are very variable regarding the magnitude, the localization, and the type of clinical symptoms (e.g., sneezing, itching, coughing, eczema). They usually develop at the entry sites as local reaction (the skin, in the mucosal tissues of the airways, and in the gut) whereas a strong IgE response associated with a massive mast cell degranulation can cause a potentially lethal systemic reaction, called anaphylaxis (Gould and Sutton 2008).

3.2.1 Amplification of the IgE Response

Once initiated, the IgE response can be further amplified at the site of inflammation by the recruitment and activation of other immune cells expressing FcεRI such as basophils, mast cells, and eosinophils. The binding of allergens to the related IgE-FcεRI complex on these cells enhances the expression of FcεRI on the cell surface resulting in a positive feedback-loop that increases the sensitivity to the allergen. Moreover, it induces the expression of CD40L and the secretion of IL-4. Therefore, like Th2 cells, these cells can drive class-switching of B cells to IgE, thus, enhancing the allergic response (Gould and Sutton 2008).

3.2.2 IgE Binding to CD23: FAP and Epitope Spreading

IgE also binds to the low-affinity receptor, FcεRII (or CD23), which is expressed on antigen-activated B cells as well as other immune cells (dendritic cells, epithelial cells, and eosinophils) (Dullaers et al. 2012). Binding of allergens to the IgE-CD23 complex improves the antigen-presenting capacity of B cells by ameliorating their aptitude of allergen internalization and presentation to T cells, hence maintaining the activation of the immune system (Gould and Sutton 2008; Acharya et al. 2010). Indeed, this process called CD23-mediated “*facilitated antigen presentation*” (FAP) is known to be as efficient as the antigen presentation by dendritic cells via FcγR, and much more effective than BCR-mediated internalization by B cells (Wypych et al. 2018).

Activated B cells expressing CD23 can bind other pre-existing circulating IgE via their constant region and thus, present unrelated allergens to cognate T cells regardless of the specificity of the B cell's own BCR. This phenomenon, called *epitope spreading*, leads to an enhanced allergic sensitization as well as to the simultaneously development of allergic reactions towards several allergens (Gould and Sutton 2008).

3.2.3 IgE Binding to CD23: Allergen Transport into Mucosal Tissues

An additional role for CD23 is the transport of free and allergen-bound IgE across the epithelial barrier into the underlying mucosa (Acharya et al. 2010). In a process, called transcytosis, IgE secreted into the gut lumen was found to bind food-derived allergens, which were further delivered to immune cells from the mucosal gastrointestinal tract, to trigger food allergic reactions (Gould and Sutton 2008).

3.3 B Cell-Derived IgG in Type I Allergy

As previously mentioned, an IgE response is usually associated with IgG production; IgG1 and IgG4 production in humans, and with IgG1 production in mice. These IgG antibodies are playing a significant role in allergy. However, in contrast to IgE, they are involved in protection and regulation of type I allergic reactions by contributing to immune tolerance, which will be discussed in detail below.

4 B Cells in Natural and Allergen-Specific Immunotherapy (AIT)-Induced Tolerance

Many advances have been achieved in the field of allergy research over the last centuries such as the successful implementation of biomarkers (either cellular, humoral or functional) for the interpretation of clinical outcomes and treatment efficacy, the development of hypoallergenic vaccine candidates, and the characterization of hundreds of allergenic molecules and sources. Still, up to now AIT represents the only treatment option for allergic diseases providing a long-term if not permanent relief of symptoms. The underlying cellular mechanism enabling an effective AIT depends on the onset and maintenance of immune tolerance towards non-hazardous environmental antigens mainly via inducing Treg cells and the secretion of allergen-specific IgG4 blocking antibodies. At present, limited information is available concerning the role of regulatory B cells (Breg) in induction of immune tolerance in course of AIT but gains more attention by the day. Here, we describe the involvement of B cells in allergen tolerance and in AIT with special focus on Breg, an immunosuppressive subset of B cells.

4.1 Altered Antibody Secretion Profile Induced by AIT

The production of antibodies is a crucial function of plasma cells, hence inevitably associated with the induction of allergen tolerance by AIT mostly characterized by a 10- to 100-fold increased secretion of serological, allergen-specific IgG blocking antibodies (Jutel et al. 2005; Reisinger et al. 2005). A successful AIT is usually accompanied by a decreased IgE/IgG4 ratio. Besides this common feature, AIT-induced changes in allergen-specific IgE levels seem to vary greatly among studies. Depending on treatment duration, many studies report an early increase in

allergen-specific IgE levels followed by a later decline (Shamji et al. 2019b; Huber et al. 2018; Vizzardelli et al. 2018). The increase in the concentrations of specific IgG1 and IgG4 antibodies mainly results in a competition with IgE for the allergen and, consequently, in a decreased degranulation and activity of mast cells and basophils. Besides the competitive function of IgG4, it has other remarkable features rendering it an anti-inflammatory antibody such as a low binding affinity to Fc γ receptors on immune cells and an incapability to induce complement activation (van der Neut Kolfsochten et al. 2007). However, the question how antibody subclass levels are correlating with clinical outcome, as determined by provocation tests or the scoring of symptoms, remains a matter of discussion. An indicator and broadly accepted biomarker for treatment efficacy is the blocking capacity of serum-derived antibodies able to inhibit IgE-facilitated allergen binding (Kouser et al. 2017; Shamji and Durham 2017). In addition to serological blocking effects, recently, nasal fluids of SCIT-treated grass pollen allergic patients were found to possess an increased IgG-associated inhibitory activity compared to untreated patients. The magnitude of this local blocking activity even exceeded by 27% the serological inhibitory capacity (Shamji et al. 2019b). Accordingly, the nasal Phl p 1- and Phl p 5-specific IgG4 levels were increased in the SCIT group. A not negligible aspect when summarizing AIT-induced alterations of the humoral antigen-specific immune response is the potential of de novo sensitizations induced as a side effect of extract-based vaccines serving as source for multiple other allergenic proteins (e.g., minor allergens), which can induce allergen-specific IgE within the patients.

4.2 Allergen-Specific Breg Cells

When discussing the role of B cells in allergen tolerance, either induced by AIT or pre-existing in a non-allergic/healthy subject, allergen-specific Breg cells seem to exert an extraordinary function. By secreting anti-inflammatory cytokines (mainly IL-10) and the direct as well as indirect suppression of effector T cells, Breg cells are able to skew the inflammatory allergic immune response towards an immunosuppressive, tolerant response. Although several review articles discussing the role of Breg cells in allergic diseases are available, studies addressing these aspects are rather limited and urgently needed.

4.2.1 Phenotype of Breg Cells

By definition, Breg cells are classified either upon their functional properties or by their phenotype, based on the expression of distinct cell surface molecules and signal transduction-associated proteins such as CD19, CD62L, MHC-II, Fas ligand, T cell immunoglobulin and mucin domain, and programmed death ligand 1 (van de Veen 2017). Functional-based classification mainly refers to the secretion of immunomodulatory cytokines such as IL-10, TGF- β , and IL-35 (Layhadi et al. 2019; Shamji et al. 2019a). An involvement of Breg cells was described for autoimmune and infectious diseases, as well as for malignancies and organ transplantations (Wortel and Heidt 2017), where their main function, in general, is to suppress effector CD4+

and CD8⁺ T cells and, in parallel, to promote the induction of immune-regulatory Tregs. In allergy research, Breg cells were mostly investigated in the context of allergies to bee venom, foods, grass pollen, and house dust mite (Berthelot et al. 2013; Boonpiyathad et al. 2017, 2019; Noh et al. 2010). Allergen tolerance was primarily described to be associated with IL-10-producing Bregs of either the B regulatory 1 (Br1) or B10 subset (Noh et al. 2010; Shamji et al. 2019b; van de Veen 2017). Therefore the focus herein will be on these two subtypes. In human, IL-10-secreting Br1 cells were identified to be CD19⁺ CD25^{hi} CD71^{hi} CD73[−], whereas B10 cells have a CD19⁺ CD24^{hi}, CD27⁺ cell phenotype (Wortel and Heidt 2017; Berthelot et al. 2013; Iwata et al. 2011; van de Veen et al. 2013). The general abundance of Br1-like cells in human is relatively low and they encompass approximately 0.6% of peripheral blood B cells, although this number can increase up to 5% since, especially, CD24^{hi} CD27⁺ cells were found to occasionally differentiate into Br1 cells (Berthelot et al. 2013). However, until now, no Breg-specific transcription factor has been identified.

4.2.2 Function of Breg Cells

With regard to allergic diseases, five major functions can be attributed to Breg cells in mediating allergen tolerance, which are (1) the cytokine-mediated independent or (2) the direct suppression of effector T cells (including T follicular helper cells), (3) the suppression of dendritic cell maturation, (4) the induction of Treg cells, as well as (5) the secretion of anti-inflammatory antibodies (Lin et al. 2019; Achour et al. 2017; Palomares et al. 2017; Samitas et al. 2010). Most of these functions can be allocated to the immunosuppressive activity of the cytokine IL-10, a key regulator of inflammatory immune responses able to restrict and terminate the responsiveness of effector T cells. Via induction of a signaling cascade that involves the associated IL-10 receptor and the transcription factor STAT3, T cell proliferation and cytokine secretion are downregulated (Schulke 2018; Saraiva and O'Garra 2010; van de Veen et al. 2013). Breg-secreted IL-10 also affects other critical effector cells involved in the acute and late-phase allergic reactions. Mast cell degranulation and cytokine release induced by cross-linking of antigen by FcεRI-bound IgE were inhibited by IL-10-secreting Breg both, *in vitro* and *in vivo* conditions (Kim et al. 2015). In addition to the IL-10 secretion, which decreases the activity of tyrosine kinases in mast cells by activating the JAK/STAT3 pathway, a direct cell-to-cell contact through CD40/CD40L interaction was required to facilitate this suppressive function. Noteworthy, the direct cell-to-cell contact further enhanced the secretion of IL-10 by Breg. This study also demonstrated the suppression of IgE-mediated anaphylactic reactions by Breg cells in an IL-10-dependent manner (Kim et al. 2015). Furthermore, IL-10 prevents antigen-presenting cells such as DCs from maturation resulting in a diminished signal transduction to Th cells and thus, an intensification of the suppressive effect on effector T cells (Schulke 2018). In general, the most important function of IL-10 is to initiate a crucial regulatory feedback-loop for mediation of anti-inflammatory responses via differentiation of naïve T cells into Treg cells. B10 and other IL-10-producing Breg cells can induce the differentiation of IL-10-secreting type 1 regulatory T cells (Tr1) and Foxp3⁺

Treg cells (Pennati et al. 2016; Mielle et al. 2018). Besides the undeniable crucial role of IL-10 secreted by Breg cells, it should be stated that Breg cells secreting other anti-inflammatory cytokines such as IL-35, TGF- β , TSP-1, and IDO exist as well (van de Veen 2017). However, their involvement in allergic diseases has not been fully investigated yet.

In contrast to the IL-10-mediated indirect suppression of effector T cells, by expressing Fas ligand Breg cells have the potential to induce apoptosis in effector T cells, thus mitigating allergic Th2 response by direct cell contact (Tian et al. 2001; Lundy and Klinker 2014). Finally, the secretion of anti-inflammatory IgG4 blocking antibodies by Breg cells able to compete with IgE for the allergen is an exceptionally important aspect in mediating allergen tolerance. Noteworthy, human IL-10-secreting Br1 cells were found to represent the main source for the production of phospholipase A₂-specific IgG4 antibodies in bee venom-tolerant subjects (van de Veen et al. 2013).

4.2.3 The Role of IL-10-Producing Breg in Natural Tolerance

When discussing the role of Breg cells in the context of allergic diseases and the onset and maintenance of natural tolerance, the comparison between tolerant subjects and allergic patients becomes evident. Noh et al. investigated the frequency of allergen-specific Br1 cells in milk-allergic and milk-tolerant subjects, which were selected by double-blind, placebo-controlled food challenges (Noh et al. 2010). The study showed that the abundance of Br1 cells increased upon allergen stimulation in the milk-tolerant group (around 9%) and decreased in the allergic group (10%), implying a superordinate regulatory role of Br1 in the maintenance of tolerance in healthy individuals. In parallel, the level of non-IL-10-producing Breg undergoing apoptosis increased upon allergen stimulation in the milk allergy group, whereas remained unaltered in the tolerant group, indicating the presence of at least a second functional-active Breg subset relevant for allergen tolerance that is not secreting IL-10. Noteworthy, in this study the frequency of IL-10-producing Breg cells exceeded the number of IL-10-secreting T cells (Noh et al. 2010). In a study comparing bee venom-allergic patients with tolerant beekeepers, the authors observed that allergen-specific IgG4 was mainly produced by Br1 cells, secreting high levels of IL-10, and subsequently resulted in the suppression of CD4⁺ T cell proliferation. This suppressive effect could be reversed by blockage of the IL-10 receptor, implying an essential role for IL-10 in the suppression of effector T cells. Beekeepers, possessing natural tolerance towards bee venom, showed an increased IL-10 and IgG4 expression by Br1 cells, whereas in allergic patients the frequency of IL-10⁺ B cells was in general very low (van de Veen et al. 2013).

4.2.4 Induction of IL-10-Producing Breg During AIT

For allergic patients, who are suffering from symptoms resulting from the exposure to an allergen that is not tolerated by their immune system, the most meaningful therapeutic action would be the reconstitution of allergen tolerance by AIT. Therefore, the contribution of Breg in the induction of allergen tolerance during AIT, and the question to what extent the thereby induced state of tolerance would reflect

natural tolerance need to be further assessed and discussed. In this respect, van de Veen et al. investigated the induction of circulating allergen-specific IL-10+ Breg in bee venom-allergic patients during AIT. The authors observed a steady increase in the frequency of allergen-specific IL-10+ and IgG4+ B cells, which were presumably Br1 cells, in both, treatment-induced tolerant (from 1.8–2.9% to 5.5–13.9%) and naturally tolerant subjects (non-allergic beekeepers), when compared to the untreated group (van de Veen et al. 2013). Although the IgE/IgG4 ratio in patients' sera was significantly reduced upon AIT (100-fold), mainly because of the increase of IgG4, still a highly significant difference remained between the IgE/IgG4 ratio of patients undergoing AIT compared to tolerant beekeepers (tenfold), which can mostly be ascribed to the higher IgE baseline levels that remained unaltered during the patients' therapy. In a follow-up study by Boonpiyathad et al. a striking similarity regarding the allergen-specific B cell response, including IL-10+ Br1 cells, IgG4+ memory cells and plasmablasts, was observed between AIT patients before or after treatment and beekeepers before or during beekeeping season, respectively (Boonpiyathad et al. 2017). In summary, although in both groups a high similarity was achieved, AIT-induced allergen-specific tolerance seems not to completely restore the immunological conditions observed in natural tolerance. Whether high dose exposure to bee venom, as it is the case in non-allergic beekeepers, can be interpreted as natural "inherited" tolerance rather than an altered variant of immunotherapy is also a matter of debate.

In a recent study, increased frequencies of two distinct populations of IL-10+ Breg cells were observed by monitoring the allergic immune response in and out of pollen season in grass pollen allergic patients undergoing SCIT. In contrast, untreated patients had a significantly lower number of Breg that did not change in and out of season (Shamji et al. 2019b). In another study, a correlation between Breg number and the improvement of clinical symptoms was also observed during grass pollen AIT (Zissler et al. 2018). These findings strongly support a beneficial regulatory effect of treatment-induced Breg in grass pollen allergic patients.

In the context of another respiratory allergy, the frequency of IL-10+ Breg was significantly increased in house dust mite-allergic patients who responded well to the treatment, as shown by the treatment-induced improvement of clinical symptoms, compared to non-responders (Boonpiyathad et al. 2019). Noteworthy, the induction of allergen-specific B10 during AIT and the associated improvement of symptoms in asthmatic, house dust mite-allergic patients were observed to be significantly enhanced by the concomitant daily administration of oral probiotics (Liu et al. 2016). Taken together, Breg cells could potentially be regarded as biomarkers for monitoring treatment efficacy in respiratory allergies.

4.2.5 The New Breg-Treg Paradigm in AIT

The present paradigm indicates that tolerogenic antigen-specific Breg cells are being induced early during AIT (early treatment/up-dosing phase) and functionally active prior to the onset of allergen-specific Treg cells. In course of the proceeding AIT (late-treatment/tolerance-mounting phase), allergen tolerance is maintained by Treg

cells, which seem to completely take over this function from Breg cells (Zissler et al. 2018; Berthelot et al. 2013).

Acknowledgements The authors declare no conflict of interest.

References

- Acharya M, Borland G, Edkins AL, Maclellan LM, Matheson J, Ozanne BW, Cushley W (2010) CD23/FcepsilonRII: molecular multi-tasking. *Clin Exp Immunol* 162:12–23
- Achour A, Simon Q, Mohr A, Seite JF, Youinou P, Bendaoud B, Ghedira I, Pers JO, Jamin C (2017) Human regulatory B cells control the TFH cell response. *J Allergy Clin Immunol* 140:215–222
- Akiba H, Oshima H, Takeda K, Atsuta M, Nakano H, Nakajima A, Nohara C, Yagita H, Okumura K (1999) CD28-independent costimulation of T cells by OX40 ligand and CD70 on activated B cells. *J Immunol* 162:7058–7066
- Berthelot JM, Jamin C, Amrouche K, Le Goff B, Maugars Y, Youinou P (2013) Regulatory B cells play a key role in immune system balance. *Joint Bone Spine* 80:18–22
- Boonpiyathad T, Meyer N, Moniuszko M, Sokolowska M, Eljaszewicz A, Wirz OF, Tomasiak-Lozowska MM, Bodzenta-Lukaszyk A, Ruxrungtham K, van de Veen W (2017) High-dose bee venom exposure induces similar tolerogenic B-cell responses in allergic patients and healthy beekeepers. *Allergy* 72:407–415
- Boonpiyathad T, van de Veen W, Wirz O, Sokolowska M, Ruckert B, Tan G, Sangasapaviliya A, Pradubpongsa P, Fuengthong R, Thantiworasit P, Sirivichayakul S, Ruxrungtham K, Akdis CA, Akdis M (2019) Role of Der p 1-specific B cells in immune tolerance during 2 years of house dust mite-specific immunotherapy. *J Allergy Clin Immunol* 143:1077–86 e10
- Cyster JG, Allen CDC (2019) B cell responses: cell interaction dynamics and decisions. *Cell* 177:524–540
- Dullaers M, De Bruyne R, Ramadani F, Gould HJ, Gevaert P, Lambrecht BN (2012) The who, where, and when of IgE in allergic airway disease. *J Allergy Clin Immunol* 129:635–645
- Erazo A, Kutchukhidze N, Leung M, Christ AP, Urban JF Jr, Curotto de Lafaille MA, Lafaille JJ (2007) Unique maturation program of the IgE response in vivo. *Immunity* 26:191–203
- Geha RS, Jabara HH, Brodeur SR (2003) The regulation of immunoglobulin E class-switch recombination. *Nat Rev Immunol* 3:721–732
- Gould HJ, Sutton BJ (2008) IgE in allergy and asthma today. *Nat Rev Immunol* 8:205–217
- Gould HJ, Wu YB (2018) IgE repertoire and immunological memory: compartmental regulation and antibody function. *Int Immunol* 30:403–412
- He JS, Meyer-Hermann M, Xiangying D, Zuan LY, Jones LA, Ramakrishna L, de Vries VC, Dolpady J, Aina H, Joseph S, Narayanan S, Subramaniam S, Puthia M, Wong G, Xiong H, Poidinger M, Urban JF, Lafaille JJ, Curotto de Lafaille MA (2013) The distinctive germinal center phase of IgE+ B lymphocytes limits their contribution to the classical memory response. *J Exp Med* 210:2755–2771
- He JS, Subramaniam S, Narang V, Srinivasan K, Saunders SP, Carbajo D, Wen-Shan T, Hidayah Hamadee N, Lum J, Lee A, Chen J, Poidinger M, Zolezzi F, Lafaille JJ, Curotto de Lafaille MA (2017) IgG1 memory B cells keep the memory of IgE responses. *Nat Commun* 8:641
- Huber S, Lang R, Steiner M, Aglas L, Ferreira F, Wallner M, Hawranek T, Gadermaier G (2018) Does clinical outcome of birch pollen immunotherapy relate to induction of blocking antibodies preventing IgE from allergen binding? A pilot study monitoring responses during first year of AIT. *Clin Transl Allergy* 8:39
- Iwata Y, Matsushita T, Horikawa M, Dilillo DJ, Yanaba K, Venturi GM, Szabolcs PM, Bernstein SH, Magro CM, Williams AD, Hall RP, Clair EWS, Tedder TF (2011) Characterization of a rare

- IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood* 117:530–541
- Janeway CA Jr, Travers P, Walport M, Shlomchik MJ (2001) B-cell activation by armed helper T cells. In: *Immunobiology: the immune system in health and disease*, 5th edn. Garland Science, New York
- Jimenez-Saiz R, Chu DK, Mandur TS, Walker TD, Gordon ME, Chaudhary R, Koenig J, Saliba S, Galipeau HJ, Utley A, King IL, Lee K, Ettinger R, Waserman S, Kolbeck R, Jordana M (2017) Lifelong memory responses perpetuate humoral TH2 immunity and anaphylaxis in food allergy. *J Allergy Clin Immunol* 140:1604–1615.e5
- Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O (2005) Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol* 116:608–613
- Kim HS, Kim AR, Kim DK, Kim HW, Park YH, Jang GH, Kim B, Park YM, You JS, Kim HS, Beaven MA, Kim YM, Choi WS (2015) Interleukin-10-producing CD5+ B cells inhibit mast cells during immunoglobulin E-mediated allergic responses. *Sci Signal* 8:ra28
- Klein U, Dalla-Favera R (2008) Germinal centres: role in B-cell physiology and malignancy. *Nat Rev Immunol* 8:22–33
- Kouser L, Kappen J, Walton RP, Shamji MH (2017) Update on biomarkers to monitor clinical efficacy response during and post treatment in allergen immunotherapy. *Curr Treat Options Allergy* 4:43–53
- Layhadi JA, Eguiluz-Gracia I, Shamji MH (2019) Role of IL-35 in sublingual allergen immunotherapy. *Curr Opin Allergy Clin Immunol* 19:12–17
- LeBien TW, Tedder TF (2008) B lymphocytes: how they develop and function. *Blood* 112:1570–1580
- Lin X, Wang X, Xiao F, Ma K, Liu L, Wang X, Xu D, Wang F, Shi X, Liu D, Zhao Y, Lu L (2019) IL-10-producing regulatory B cells restrain the T follicular helper cell response in primary Sjogren's syndrome. *Cell Mol Immunol* 16:921–931
- Liu J, Chen FH, Qiu SQ, Yang LT, Zhang HP, Liu JQ, Geng XR, Yang G, Liu ZQ, Li J, Liu ZG, Li HB, Yang PC (2016) Probiotics enhance the effect of allergy immunotherapy on regulating antigen specific B cell activity in asthma patients. *Am J Transl Res* 8:5256–5270
- Lundy SK, Klinker MW (2014) Characterization and activity of Fas ligand producing CD5(+) B cells. *Methods Mol Biol* 1190:81–102
- Medina F, Segundo C, Campos-Caro A, Gonzalez-Garcia I, Brieva JA (2002) The heterogeneity shown by human plasma cells from tonsil, blood, and bone marrow reveals graded stages of increasing maturity, but local profiles of adhesion molecule expression. *Blood* 99:2154–2161
- Mielle J, Audo R, Hahne M, Macia L, Combe B, Morel J, Daien C (2018) IL-10 producing B cells ability to induce regulatory T cells is maintained in rheumatoid arthritis. *Front Immunol* 9:961
- Moutsoglou DM, Dreskin SC (2016) B cells establish, but do not maintain, long-lived murine anti-peanut IgE(a). *Clin Exp Allergy* 46:640–653
- Noh J, Lee JH, Noh G, Bang SY, Kim HS, Choi WS, Cho S, Lee SS (2010) Characterisation of allergen-specific responses of IL-10-producing regulatory B cells (Br1) in cow milk allergy. *Cell Immunol* 264:143–149
- Oracki SA, Walker JA, Hibbs ML, Corcoran LM, Tarlinton DM (2010) Plasma cell development and survival. *Immunol Rev* 237:140–159
- Palomares O, Akdis M, Martin-Fontecha M, Akdis CA (2017) Mechanisms of immune regulation in allergic diseases: the role of regulatory T and B cells. *Immunol Rev* 278:219–236
- Pennati A, Ng S, Wu Y, Murphy JR, Deng J, Rangaraju S, Asress S, Blanchfield JL, Evavold B, Galipeau J (2016) Regulatory B cells induce formation of IL-10-expressing T cells in mice with autoimmune neuroinflammation. *J Neurosci* 36:12598–12610
- Pieper K, Grimbacher B, Eibel H (2013) B-cell biology and development. *J Allergy Clin Immunol* 131:959–971
- Reisinger J, Horak F, Pauli G, van Hage M, Cromwell O, Konig F, Valenta R, Niederberger V (2005) Allergen-specific nasal IgG antibodies induced by vaccination with genetically modified

- allergens are associated with reduced nasal allergen sensitivity. *J Allergy Clin Immunol* 116:347–354
- Roth K, Oehme L, Zehentmeier S, Zhang Y, Niesner R, Hauser AE (2014) Tracking plasma cell differentiation and survival. *Cytometry A* 85:15–24
- Samitas K, Lotvall J, Bossios A (2010) B cells: from early development to regulating allergic diseases. *Arch Immunol Ther Exp* 58:209–225
- Saraiva M, O'Garra A (2010) The regulation of IL-10 production by immune cells. *Nat Rev Immunol* 10:170–181
- Saunders SP, Ma EGM, Aranda CJ, Curotto de Lafaille MA (2019) Non-classical B cell memory of allergic IgE responses. *Front Immunol* 10:715
- Schulke S (2018) Induction of interleukin-10 producing dendritic cells as a tool to suppress allergen-specific T helper 2 responses. *Front Immunol* 9:455
- Shamji MH, Durham SR (2017) Mechanisms of allergen immunotherapy for inhaled allergens and predictive biomarkers. *J Allergy Clin Immunol* 140:1485–1498
- Shamji MH, Layhadi JA, Achkova D, Kouser L, Perera-Webb A, Couto-Francisco NC, Parkin RV, Matsuoka T, Scadding G, Ashton-Rickardt PG, Durham SR (2019a) Role of IL-35 in sublingual allergen immunotherapy. *J Allergy Clin Immunol* 143:1131–1142 e4
- Shamji MH, Kappen J, Abubakar-Waziri H, Zhang J, Steveling E, Watchman S, Kouser L, Eifan A, Switzer A, Varricchi G, Marone G, Couto-Francisco NC, Calderon M, Durham SR (2019b) Nasal allergen-neutralizing IgG4 antibodies block IgE-mediated responses: novel biomarker of subcutaneous grass pollen immunotherapy. *J Allergy Clin Immunol* 143:1067–1076
- Talay O, Yan D, Brightbill HD, Straney EE, Zhou M, Ladi E, Lee WP, Egen JG, Austin CD, Xu M, Wu LC (2012) IgE(+) memory B cells and plasma cells generated through a germinal-center pathway. *Nat Immunol* 13:396–404
- Tian J, Zekzer D, Hanssen L, Lu Y, Olcott A, Kaufman DL (2001) Lipopolysaccharide-activated B cells down-regulate Th1 immunity and prevent autoimmune diabetes in nonobese diabetic mice. *J Immunol* 167:1081–1089
- van de Veen W (2017) The role of regulatory B cells in allergen immunotherapy. *Curr Opin Allergy Clin Immunol* 17:447–452
- van de Veen W, Stanic B, Yaman G, Wawrzyniak M, Sollner S, Akdis DG, Ruckert B, Akdis CA, Akdis M (2013) IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. *J Allergy Clin Immunol* 131:1204–1212
- van der Neut Kolfsoorten M, Schuurman J, Losen M, Bleeker WK, Martinez-Martinez P, Vermeulen E, den Bleker TH, Wiegman L, Vink T, Aarden LA, De Baets MH, van de Winkel JG, Aalberse RC, Parren PW (2007) Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science* 317:1554–1557
- van Ree R, Hummelshoj L, Plantinga M, Poulsen LK, Swindle E (2014) Allergic sensitization: host-immune factors. *Clin Transl Allergy* 4:12
- Vinuesa CG, Linterman MA, Goodnow CC, Randall KL (2010) T cells and follicular dendritic cells in germinal center B-cell formation and selection. *Immunol Rev* 237:72–89
- Vizzardelli C, Gindl M, Roos S, Mobs C, Nagl B, Zimmann F, Sexl V, Kenner L, Neunkirchner A, Zlabinger GJ, Pickl WF, Pflutzner W, Bohle B (2018) Blocking antibodies induced by allergen-specific immunotherapy ameliorate allergic airway disease in a human/mouse chimeric model. *Allergy* 73:851–861
- Wortel CM, Heidt S (2017) Regulatory B cells: phenotype, function and role in transplantation. *Transpl Immunol* 41:1–9
- Wu LC, Zarrin AA (2014) The production and regulation of IgE by the immune system. *Nat Rev Immunol* 14:247–259
- Wypych TP et al (2018) Role of B cells in TH cell responses in a mouse model of asthma. *J Allergy Clin Immunol* 141(4):1395–1410
- Zissler UM, Jakwerth CA, Guerth FM, Pechtold L, Aguilar-Pimentel JA, Dietz K, Suttner K, Piontek G, Haller B, Hajdu Z, Schiemann M, Schmidt-Weber CB, Chaker AM (2018) Early IL-10 producing B-cells and coinciding Th/Tr17 shifts during three year grass-pollen AIT. *EBioMedicine* 36:475–488