

The Role of B Regulatory Cells and Semaphorin3A in Atopic Diseases

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Allergic inflammatory disease · Semaphorin3A · B and T regulatory cells

Abstract

When the pathogenesis of allergic inflammatory diseases such as asthma, allergic rhinitis and atopic dermatitis is discussed, one should take into consideration the involvement of regulatory cells/molecules whose role is to prevent the induction and/or deterioration of such diseases. The involvement of T regulatory cells and FoxP3 is well established in asthma, but only little is known about the involvement of B regulatory cells (Bregs) and the soluble regulatory molecule semaphorin3A (sema3A) in atopic diseases. During the last decade, research has sought to better define the various subtypes of Breg cells and how similar they are to their parallel subtypes of Tregs. In this review, we focus on the newly reported role of Bregs in both experimental and human models of asthma. Bregs are also involved in the pathophysiology of food allergy. We also show how sema3A plays a role in the pathogenesis of allergic rhinitis and atopic dermatitis. Determining the above processes could facilitate the use of regulatory molecules as therapeutic tools in treating these diseases.

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The Emergence of B Regulatory Cells' Role in Immune Response

The issue of immune-mediated inflammation in general, and allergy in particular, being partially due to insufficient regulatory responses, has been under continuous investigation for more than 2 decades. Regulatory T cells (Tregs) have been shown to be the leading regulatory cells in many inflammatory diseases. In one of many recent studies, the percentage of Tregs in allergic patients, such as those with asthma, atopic dermatitis (AD) and/or food allergy (FA), was found to be significantly decreased in comparison to in healthy controls [1]. In this respect, allergen-specific immunotherapy has been shown to be highly effective by enhancing the frequency and function of IL-10-secreting type I Tregs [2]. Tolerogenic dendritic cells are also recognized to be key regulators of peripheral tolerance in various inflammatory responses. It has been proven that the role of the microenvironment is crucial in generating tolerogenic dendritic cells [3].

More than a decade ago, in experimental animal models, researchers began to notice that B cell depletion could paradoxically contribute to the development of experimental autoimmune encephalomyelitis, which resolved following the administration of syngeneic B cells. This observation highlighted the possibility that B cells also play a regulatory role in the repertoire of immune responses [4].

Table 1. Similarities and differences in regulatory cells

Treg		Breg
Similarities		
Tr1	IL-10 ^{pos}	Br1, B10
Tr3	TGF-β ^{pos}	Br3
Treg	CD5 ^{pos}	Breg
Differences		
nTreg	Foxp3 ^{pos}	?
nTreg	CTLA-4 ^{pos}	?
Stimulators		
IL-2		CpG ODN
TGF-β		CD40L

CD40L = CD40 ligand; CpG ODN = CpG oligodeoxynucleotide; IL = interleukin; TGF-β = transforming growth factor.

It was later shown that the insufficient beneficial effect of B cell depletion in some patients with systemic lupus erythematosus (SLE) was attributable to the enhanced depletion of B regulatory cells (Bregs), which are basically decreased in these patients [5]. This insight led the way to understanding the important role of Bregs in maintaining self-tolerance in autoimmunity.

In early reports, Br1 (B10) cells were defined by their ability to express and produce high amounts of IL-10 (a potent inhibitory cytokine). The regulatory properties were assessed by showing that these Bregs inhibited the differentiation and proliferation of Th1 and Th2 in an IL-10-dependent way. It was also shown that IL-10 production by Bregs was enhanced when T cell CD40L binds CD40 on B cells and/or when B cell Toll-like receptors are signaled [6]. We previously demonstrated that B cells defined as CD19⁺CD25^{high}/IL-10^{high}/TGF-β^{high} have regulatory properties, and when activated by CD40L and CpG-ODN, could effectively suppress CD4 T cell proliferation. In addition, the coculture of these activated Bregs with autologous Tregs was shown to enhance Treg properties, namely by increasing Foxp3 and cytotoxic T lymphocyte antigen 4 (CTLA-4) expression on Tregs [7].

Another phenotypic Br1 cell in humans (CD24^{high}CD38^{high}CD27^{high}) was shown – when assessed in normal individuals – to alter TNF expression in effector CD4 T cells in an IL-10-dependent manner. However, these so-called Bregs failed to do so in SLE patients, indicating the defective function of Bregs in SLE [8]. Positing that it was expressed in Bregs as well, Foxp3 (a transcription factor of Tregs) was analyzed in CD19⁺CD5⁺ and was indeed found to be expressed. This finding suggested that

CD19⁺CD5⁺Foxp3⁺ are a subset of Bregs that should be looked for and the role of which should be evaluated carefully [9]. All the above are evidence of the many similarities and differences between Tregs and Bregs (table 1).

Apart from being contributory in the pathogenesis of autoimmune diseases such as SLE and also being modulated following biological therapies for such diseases, B cells, namely Bregs, have been shown to be involved in the regulation of atopic-related immune responses such as asthma. In this context, a role for Bregs has been described in several helminthic infection models which showed helminth-induced Bregs to strongly suppress allergic inflammation such as allergic asthma in experimental models [10]. It was also shown that Bregs are actively involved in other allergic responses such as bee-venom allergy [11].

Bregs and Semaphorin3A

Semaphorin3A (sema3A; a soluble molecule of the semaphorin family) was recently shown to be involved in the regulation of the immune system, and was therefore denoted as an ‘immune semaphorin’. The expression of sema3A has also been found on differentiating macrophages and activated T cells, highlighting their role in modulating inflammatory conditions. Moreover, the addition of sema3A to dendritic cell/T cell cocultures significantly inhibits T cell proliferation [12]. These findings demonstrate that sema3A is a frontline player in the regulation of immune responses and the maintenance of self-tolerance, and, therefore, can be expected to be involved in the pathogenesis of many immune-mediated inflammatory diseases including atopic diseases. The expression of sema3A is especially visible on Tregs as a suppressor marker, contributing to the regulatory properties of these cells [13]. Looking for additional markers for better identification of Bregs, we analyzed the expression of sema3A on Bregs, seeking to confirm its higher expression on these cells. Indeed, it was found that sema3A expression as well as the expression of its receptor NP-1 was significantly higher on CD19⁺CD25^{high} (which we considered to be Bregs) from normal individuals but was lower on Bregs from SLE patients, correlated with disease severity. This observation suggests sema3A could become a useful marker for this subset of cells. We also demonstrated a decrease in TLR9 expression in B cells after the incubation of B cells from SLE patients with sema3A; this reduced the inflammatory process. Our finding of decreased NP-1 expression, along with decreased sema3A expression on B cells in SLE, further suggests that this

defect may play an important role in the pathogenesis of immune-mediated diseases. CD72, another regulatory molecule on B cells, was found to be upregulated following incubation with recombinant sema3A. This molecule conveys its inhibitory signals via the ITIM motif, and thereby inhibits activation signals from BCR. Thus, sema3A appears to be an important B cell regulator [14].

In this review, we summarize the state of the art on how Bregs are involved in experimental and human models of asthma and in other allergic diseases such as food allergies. We also summarize the status of how sema3A is involved in allergic rhinitis (AR) and AD. We postulate that targeting Bregs or using soluble sema3A are likely therapeutic tools for the treatment of all the above disorders.

Bregs in Asthma

Bronchial asthma is a chronic inflammatory disease in which Th2-dysregulated immunity is characterized by the increased secretion of related cytokines such as IL-4, IL-5 and IL-13. Recurrent exposure to environmental allergens induces the expansion of allergen-specific IgEs, mast cell degranulation and the release of histamine, leukotrienes and other inflammatory cytokines. This is followed by airway hyperresponsiveness (AHR), wheezing, coughing and recurrent attacks of breathlessness [15]. The involvement of immune-mediated inflammation in allergic asthma is complex and includes many factors, notably: a strong genetic susceptibility, the timing and extent of allergen exposure, coexposure to infectious agents and defects in regulatory mechanisms such as a reduction in the amount and function of Tregs during the inflammation process.

The overactivity of Th2 and the enhanced secretion of related cytokines and airway inflammation have been shown to be controlled, in part, by subsets of Tregs like IL-10-producing Tregs and/or CD25^{high}Foxp3⁺ Tregs, the function of which is closely related to the clinical status of asthma. In uncontrolled allergic asthma, these subsets are functionally and/or numerically deficient and are even associated with disease severity. In addition, it has been reported that in animal models of allergic airway diseases, enrichment with Tregs suppresses established airway inflammation and AHR [16–18].

Bregs have also been shown to play a regulatory role in bronchial asthma. Traditionally, B cells are known for their capacity to produce antibodies, contributing to pathogen clearance through humoral immunity. They play a central role in allergic responses, being the source

of allergen-specific antibodies and proinflammatory cytokines [19, 20]. The role of Bregs also in controlling allergic responses was recently assessed by many in vitro and in vivo studies. In one study, ovalbumin (OVA)-sensitized mice were acutely exposed to OVA aerosol, afterwards developing allergic airway disease. When continually exposed to this antigen, however, these mice have been shown to develop local inhalational tolerance (LIT). Using this model, Singh et al. [21] and Natarajan et al. [22] reported that the adoptive transfer of hilar lymph node (HLN) B cells from LIT mice to OVA-sensitized mice leads to reduced tissue inflammation, decreased bronchoalveolar lavage leukocytosis and eosinophilia and a decrease in AHR, suggesting that LIT HLN B cells protected OVA-sensitized mice from allergic airway disease. This Breg protection is associated with an increase in the number of Foxp3⁺ Tregs in bronchoalveolar lavage and HLNs but not in the spleen. They also showed that LIT HLN B cells induce the formation of functional CD4⁺CD25⁺Foxp3⁺ Tregs from CD4⁺CD25[−] effector T cells via the secretion of TGF- β . They also reported that these LIT HLN B cells mainly expressed TGF- β and CD5⁺, able to enhance Treg induction [21, 22]. In support of this, it has been found that antigen-loaded B cells isolated from Peyer's patches are more tolerogenic, and have the potential to generate more suppressive Treg-of-Breg cells via the production of IL-10 and cell-cell contacts. Treg-of-Breg cells express CTLA-4 and other suppressive molecules, and produce lower concentrations of IL-2 and higher concentrations of IL-10. Thus, B cells isolated from gut-associated lymphoid tissues can generate Tregs that may be applicable to the alleviation of allergic symptoms [23]. In another experimental model, *Schistosoma mansoni* worm infection induced the expansion of a spleen IL-10-producing B cell population characterized by high expression of CD1d, and defined as CD5⁺CD21^{high}CD23⁺IgD⁺IgM^{high} B cells. These CD1d^{high} IL-10-producing Bregs prevent the development of OVA-induced allergic airway inflammation through the induction of pulmonary infiltration of CD4⁺CD25⁺Foxp3⁺ Tregs in an IL-10- and CD1d-dependent way. In addition, it has been reported that these Bregs could reverse airway inflammation in mice with established AHR [24]. Furthermore, it has been shown that IL-10-producing Bregs, which are also CD1d^{high} Bregs, are increased in helminth/schistosome-infected children compared to uninfected children. Notably, after antischistosome treatment, the number of IL-10-producing CD1d^{high} Bregs is reduced. Helminth infections drive the development of CD1d^{high} Bregs in both mice

and humans, and according to murine models, helminth infection-induced Bregs are involved in the inhibition of allergic inflammation by IL-10- and Treg-dependent mechanisms [25]. The frequency and function of IL-10-producing B cells in human asthma was recently evaluated. Lower numbers of IL-10-producing B cells were recorded in asthma patients when these cells were cocultured with LPS. When they were added to CD4⁺ T cells following their activation with the dust-mite allergen, they demonstrated diminished IL-10 production compared to similar cocultures with such Bregs from controls, pointing to their defective function in asthma patients [26]. Further studies are required in order to better understand the various mechanisms by which Bregs regulate inflammation in asthma. We also need to assess the conditions under which Bregs increase and determine which stimuli promote their growth. We also should assess whether all subtypes of Bregs are equally involved in asthma. The answers to these questions may suggest that bronchial asthma is a disease that evolves due to the failure in Breg functioning.

Sema3A and Allergic Rhinitis

AR is a common allergic disease that is characterized by sneezing, rhinorrhea and nasal obstruction, frequently accompanied by itching of the nasal mucosa. Recently, it has been shown that hyperinnervation of sensory neurons in the nasal turbinate might be associated with the itching and sneezing. Since AR goes along with neural hypersensitivity, Sawaki et al. [27] immunohistochemically examined the distribution of nerve fibers in the nasal mucosa and found that nerve-fiber density in the lamina propria was significantly increased in AR mice compared to control mice, which may correlate with the increased allergic symptoms. Since sema3A restricts innervation of sensory neurons by inhibiting the sprouting of nerve fibers, the researchers examined its contribution to the neural hypersensitivity in the nasal mucosa. They reported a decreased expression of sema3A in the nasal epithelium of a murine AR model, accompanied by increased nerve-fiber density in the lamina propria of the turbinate, leading to nasal neurologic hypersensitivity including itch. Furthermore, they revealed that treating AR mice with recombinant sema3A decreases the density of nerve fibers in the lamina propria of turbinate compared to untreated AR mice (fig. 1a). These results suggest that sema3A alleviates allergic symptoms such as sneezing and nasal rubbing behavior by suppressing hyperinnervations [27].

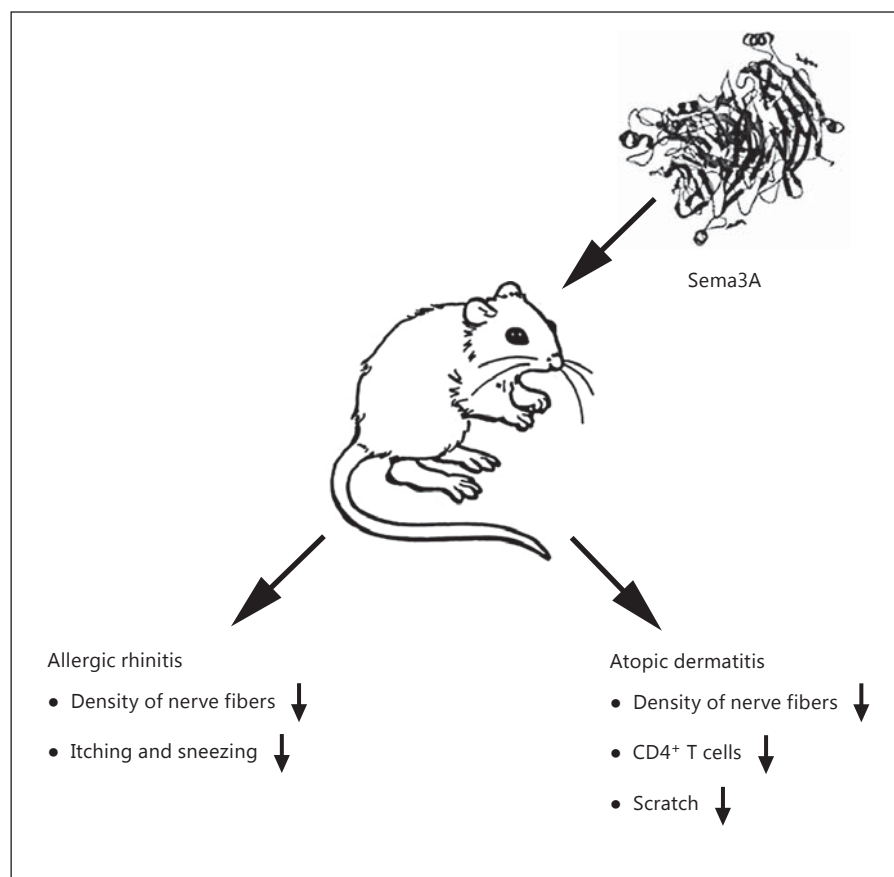
Bregs and Food Allergies

Food-allergic reactions are immunological disorders induced by certain food(s) in susceptible individuals. These are serious disorders that can have life-threatening implications for afflicted individuals and their families. FA affects up to 5–10% of children and 3–4% of adults in westernized countries [28–30], and the incidence appears to be increasing along with other atopic disorders [31, 32]. Clearly, FA is a growing healthcare concern, yet effective treatment other than strict dietary avoidance remains elusive [29]. FA can be classified into one of the following two subtypes according to the underlying immunological mechanism: IgE-mediated type and non-IgE-mediated type [33]. In particular, FA involving a late eczematous reaction is considered to be non-IgE-mediated. A failure in the establishment and maintenance of oral tolerance in infancy may result in FA. Recent reports have supported the hypothesized role for Tregs in the development of normal tolerance or the spontaneous resolution of milk allergy [29–31]. Indirect evidence exists suggesting that the development of FA may be controlled by CD4⁺CD25⁺ Tregs [34, 35].

Recently, the clinical significance of Bregs in non-IgE-mediated FA has been investigated. Twelve patients with a milk allergy were compared to 12 milk-tolerant patients with regard to their response to allergen exposure (casein). Br1 cells (IL-10-producing B cells) proliferate in response to casein stimulation in milk-tolerant subjects, but not in milk-allergy patients. Br3 (TGF- β -producing B cells) cells showed similar responses in non-IgE-mediated FA and proliferated in response to allergen stimulation in milk-tolerant subjects. Here, Br1 and Br3 cells appeared to be critical in the induction of immune tolerance in non-IgE-mediated FA that was related to AD, while Treg responses were not reported to be decisive in the development of either allergy or tolerance [36].

In another study, the antigen-specific responses of B cell subsets were examined by using in vitro allergen stimulation in milk-allergy patients with late eczematous reactions. Eight milk-allergy subjects and 13 milk-tolerant subjects were enrolled in this study. PBMCs were stimulated by casein in vitro and were analyzed for B cell subsets. CD19⁺ B cells were unchanged in the milk-allergy group and had decreased in the milk-tolerant group after the stimulation. The fraction of apoptotic B cells significantly decreased in the allergy group. Of interest was the finding of a decreased CD5⁺ Breg population in the milk-allergy subjects, with no change in the milk-tolerant subjects. In addition, IL-10-producing Bregs were found to

Fig. 1. The induction of sema3A in animal models of AR decreased the infiltration of eosinophils, itching and sneezing and improved symptoms of AD, decreasing the nerve fiber density and CD4 T cell infiltration.



have significantly decreased following casein stimulation in the milk-allergy group, but were unchanged in the milk-tolerant group [37]. Another study examined the specific response of TGF- β -producing Bregs (Br3) following casein stimulation. Peripheral blood mononuclear cells from 10 subjects with a milk allergy and from 13 milk-tolerant controls were enrolled in this study. The cells were stimulated in vitro with casein. Following allergen stimulation, the percentage of Br3 cells decreased in the milk-allergy group and increased in the healthy group. Using carboxyfluorescein succinimidyl ester labeling as a marker of proliferation, it was demonstrated that the percentage of proliferating Br3 among CD5⁺ B cells was unchanged in the milk-allergy group and had increased in the milk-tolerant controls. The conclusion of this study was that Br3 cells proliferated in response to allergen (casein) stimulation in the milk-tolerant controls and not in the milk-allergy patients. This particular study suggested that Br3 cells may be involved in allergy tolerance by negatively regulating the immune system with TGF- β [38]. Taking all the above results together, it seems that Bregs are of great importance in maintaining normal immune

tolerance to food allergens. The interruption of this normal tolerance contributes to the development of FA. Further studies are still needed to be able to specifically verify the importance of Bregs in this immune homeostasis.

Atopic Dermatitis and the Role of Sema3A

AD is frequently the first manifestation of atopic disease in infancy and early childhood. It is a heterogeneous clinical phenotype characterized by spongiosis, keratinocyte apoptosis and dermal immune infiltrate in its acute phase. Several factors favor the onset of AD, e.g. genetically determined alterations either of the epidermal physical barrier or of the immune system regarding a type-2 response. Both these alterations may also occur in response to environmental stimuli regardless of the genetic background. It is theorized that when the sum effect of genetic factors and environmental stimuli exceeds the immune threshold, a cutaneous inflammation is initiated, mainly as a Th2-dominant immunity (i.e. increased IgE levels). The heterogeneity of the disease, with its variable clinical course and ther-

apy response, might be a result of the mosaicism that reflects divergent response rates toward different stimuli [39].

AD is also defined as 'an itchy disease'. Because itch is an unavoidable symptom and markedly reduces the quality of life in AD patients, controlling itch is an important issue in the treatment of AD. The itch-scratch cycle has attracted attention as a factor in the aggravation and chronicity of AD [40]. Itch is conducted by afferent C-fibers, which are unmyelinated nerve fibers originating from dorsal root ganglia neurons. Normally, C-fibers possess free nerve endings at the boundary between the epidermis and the dermis, and some nerve fibers invade the epidermis. In AD, the C-fibers in the epidermis sprout, probably caused by nerve growth factor, which is released from keratinocytes or fibroblasts due to stimulations including scratching. Sema3A, which has been shown to be a potent immune regulator, also potently induces the retraction of nerve growth factor-sensitive neurons among dorsal root ganglia neurons [41]. It was therefore assumed that using sema3A topically might interrupt the itch-scratch cycle – a potentially novel strategy for the treatment of AD. In order to investigate the effects of sema3A, NC/Nga mice, commonly used as an AD model, were treated topically with sema3A. The results of these experiments demonstrated that sema3A had a marked effect, leading to a reduction in the itch-scratch cycle behavior and an improvement in skin lesions, both macroscopically and histologically in the NC/Nga mice. Histological examination also showed a reduction of the invasive nerve fibers in the epidermis of the lesions area and a reduction in the infiltration of inflammatory cells and IL-4 production in the dermis. The study researchers postulated that sema3A might exert its therapeutic effects through both neurological and immunological mechanisms [42]. In another study, the efficacy of a sema3A ointment in the AD NC/Nga mouse model was assessed in comparison to Vaseline, heparinoid, betamethasone and tacrolimus. These ointments were applied to skin lesions once a day for 4 days. Transepidermal water loss was measured before and after each treatment, the degree of dermatitis was clinically scored and videos were made to observe scratching behavior. Skin samples from these mice were collected for histological analyses. It was found that topical application of sema3A, betamethasone and tacrolimus ointments significantly inhibited scratching behavior and improved dermatitis scores. A significant improvement of transepidermal water loss was observed only in sema3A-ointment-treated mice, as was a reduction in the densities of nerve fibers in the epidermis and the numbers of inflam-

matory cells, such as CD4 immunoreactive T cells and eosinophils. Here again, it was suggested that sema3A ointment may have therapeutic efficacy in patients with pruritus and AD [43] (fig. 1b). The importance of sema3A in the pathogenesis of AD was also assessed in patients and controls. Biopsies from normal abdominal skin lesions of 9 healthy male controls and abdominal skin lesions of 11 male patients with clinical appearances of chronic AD were used in a study by Tominaga et al. [44]. The expression pattern of sema3A was examined immunohistochemically in the epidermis of patients with AD. The expression level of sema3A was remarkably decreased in the epidermis of patients with AD compared with that in healthy controls. A significant reduction of the sema3A mRNA level in the skin of patients with AD was also confirmed by quantitative RT-PCR analysis. While nerve fibers were occasionally present in the normal epidermis, they were observed at higher densities in patients with AD. These findings were in line with the above results in mice models of AD.

In conclusion, the role of both Tregs and Bregs and the regulatory molecule sema3A in maintaining self-tolerance and in preventing immune-mediated diseases, namely atopic diseases, has been the subject of increasing focus during the last decade. We tried to show how Br1 regulatory cells are involved in both experimental and human asthma, whereas Br3 are the main subtype involved in FA. The regulatory function of sema3A manifests mainly in AR and AD. Here, the injection of sema3A has been demonstrated to be highly beneficial in diminishing allergic inflammation in experimental models of both diseases. Future studies are needed to establish the role of both Bregs and sema3A in human asthma, AR and AD. Targeting these cells or injecting sema3A might become therapeutic options worth considering.

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