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Review Article

Regulatory T cells and regulation of allergic airway disease

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Abstract: Diseases like asthma have dramatically increased in the last decades. The reasons for the rising prevalence are still controversially discussed. Besides the genetic predisposition a number of different causes are thought to affect the increase of allergies. These include the hygiene hypothesis as well as changes in intestinal microbiota. Allergic airway inflammation is driven by T cells but it has become clear that tolerance and also suppression of allergic inflammation are mediated by so called regulatory T cells (Tregs). Indeed, naturally occurring Treg as well as induced Tregs have been shown to suppress allergic airway disease. In addition, the effectiveness of different therapeutic strategies (e.g. allergen immunotherapy) are mediated via Tregs. In addition, several Treg based approaches have been shown to effectively suppress allergic airway disease in different models. However, more research is needed to explore these potentially interesting approaches for the treatment of human disease.

Keywords: Allergy, asthma, inflammation, regulatory T cell, suppression

Introduction

Allergic diseases have an increasing prevalence worldwide, but especially in industrialized countries an increase in allergic diseases has been noticed in the last decades. Besides hay fever and allergic rhinitis also the number of patients with allergic asthma has grown dramatically. For example, asthma is now the most prevalent chronic disease in childhood in developed countries and approximately 300 million people suffer from this disease worldwide. Different reasons for this increment in incidence are still under debate. Genetic predisposition is for sure an important risk factor for the development of allergic disease and many different susceptibility genes have been identified. However, genetic changes are unlikely to account for the increase in allergic disease within a relatively short time period and simple genetic explanations for increased disease susceptibility is very unlikely. Other potential causes for increased prevalence of allergic diseases are the improved hygienic standards in the civilized society. One theory is the so-called "hygiene hypothesis", which postulates that the increase of allergic disease is a result of decreased exposure to microbial antigens, especially during early childhood [1]. In the last decades also the understanding of the underlying pathogensis of asthma has steadily increased. Indeed, asthma is characterized by different features, including airway hyperresponsiveness (AHR), airway inflammation and bronchoconstriction (GINA Report, Global Strategy for Asthma Management and Prevention, www.ginasthma.org 2011). In recent years the perception of asthma has altered and it is apparent now that asthma is not a single disease but rather an umbrella term for different heterogeneous phenotypes. The diverse asthma phenotypes can be distinguished based on differing etiologies, immunological parameters, or the clinical course of the disease [2, 3]. Indeed the clinical phenotype allergic asthma is one of the most common in children and adults. Development of allergic asthma often starts in childhood after sensitization of the airways to widespread allergens like house dust mite, animal dander or tree pol-

lens. Allergic asthma was regarded for a long time as a characteristic example for T helper 2 cell (Th2)-mediated disease. Indeed, several studies have elegantly shown that numbers of Th2 cells are increased in the lung of patients with asthma [4] and animal models have further demonstrated that $CD4^+$ Th2 cells play a pivotal role in the development of the disease [5, 6]. Still, many different inflammatory cell types are involved in the pathogenesis of asthma including mast cells, dendritic cells, B lymphocytes and eosinophilic and neutrophilic granulocytes. Identification of predominant cell types in sputum samples of atopic patients allows discriminating different phenotypes of asthma. Besides eosinophilic inflammation, types with neutrophilic pattern or paucigranulocytic asthma with both, eosinophil and neutrophil granulocytes occur. In allergic asthma eosinophilic airway inflammation is often present, which is thought to be a result of Th2-induced inflammation. The Th2-cytokines Interleukin (IL)-4, IL-5 and IL-13 directly influence the allergic airway disease. IL-4 is an important factor for the polarization of T cells to become Th2 cells, which is thought to be mediated by activation of transcription factors signal transducer and activator of transcription 6 (STAT6) and GATA-binding protein 3 (GATA3) [7]. IL-4 competency in T helper cells enables them to mature into IL-4-secreting T follicular helper cells that induce istotype switch and Immunglobulin (Ig)E production in B cells [8]. IL-5 is critically involved in the differentiation of eosinophils from precursors in the bone marrow and participates on survival and attraction of eosinophils into the airways [9, 10]. IL-13 directly acts on epithelial cells promoting goblet-cell metaplasia, myofibroblast differentiation, IgE production in B cells and development of AHR [10-13]. However, also other Th cell subgroups have been linked to the development of allergic airway disease. Recent studies suggest the involvement of IL-9 producing Th9 cells in the development of eosinophilic airway inflammation and AHR [14, 15] but also Th1 and Th17 cells can trigger AHR and pulmonary inflammation. Not surprising, Th1 and Th17 induced allergic airway disease is more associated with recruitment of neutrophilic granulocytes into the airways [16, 17]. Interestingly, studies also show that IL-17 is directly involved in promoting contraction of airway smooth muscle cells [18].

Current studies focus on epithelial cells and their role in induction of allergic airway disease. Epithelial cells of the airways are the first border that interacts with allergens. Asthmatic patients have constrictions in the barrier function of epithelial cells and therefore tend to develop hypersensitivity to harmless environmental antigens [19]. Airborne allergens from mites, fungi and pollens exert proteolytic activities that can promote the disruption of epithelial tight junctions and activation of protease-activated receptors [20]. Epithelial cells are further equipped with pattern recognition receptors that can mediate activation of the cells. After contact with the allergen in association with danger signals activation of epithelial cells occurs and leads to release of different cytokines and chemokines like thymic stromal lymphopoietin (TSLP) and IL-33 that alter function of innate immune cells [21]. Upon secretion of those cytokines inflammatory cells are recruited into the lung and dendritic cells (DCs) are activated. IL-33 orchestrates both innate and adaptive immunity and promotes inflammation in the lung. It activates primary human mast cells and basophils but also integrates eosinophils, natural killer (NK) and natural killer T (NKT) cells and Th2 lymphocytes [22, 23]. Furthermore recently discovered innate lymphoid cells (ILCs) in the lung can be activated by IL-33 and IL-25 to increase production of IL-13 resulting in development of AHR and atopy [24, 25]. ILCs includes cells that besides IL-13 produce IL-5, IL-17 and IL-22, have a lack of specific lineage markers and requires expression of the transcriptional repressor Id2 [25]. They can be differentiated by their expression of RORgt. Whereas RORγt-positive ILCs secrete IL-17A and IL-22 ILCs that are RORgt-negative express the Th2-associated cytokines IL-4, IL-5 and IL-13 and comprise nuocytes, natural helper cells, innate helper type 2 cells and multipotent progenitor type 2 cells [26]. They are activated through epithelial released cytokines IL-25 and IL-33 and promote Th2-mediated immunity.

As many of the described pathways are proinflammatory, also different important regulatory mechanisms, which limit the development of inflammation, have been described. Important for immune homeostasis are regulatory T cells (Tregs), which are key players in the regulation of immune responses. Accumulating evidence

suggests that they play important regulatory roles also in the development of an allergic disease. Indeed, allergic and healthy individuals exhibit certain amounts of allergen-specific effector cells in their blood and it seems that development of a healthy or an allergic immune response strongly depends on the ratio of those oppositional cell types [27]. Furthermore, Tregs from allergic patients are constricted in quantity and function. In comparison to healthy individuals Tregs from allergic patients have a reduced potential to suppress proliferation of allergen-specific effector cells after exposure to allergens. Whereas in non-allergic individuals Tregs are induced after contact with common environmental allergens, IL-4-secreting T cells are the dominant fraction in allergic individuals with sensitization against Birch pollen [28]. A further study exposed diminished numbers of Tregs in patients with hay fever during pollen season [29]. As Treg function seems to be impaired in patients with asthma they display an appealing target for the treatment of allergic disease.

Classification and phenotype of regulatory T cells

Tregs have the potential to inhibit the activation, proliferation and effector functions of other immune cells like $CD4^+$ or $CD8^+$ effector cells. They were firstly described in the early 1970s as thymocytes with immunosuppressive effects [30, 31]. Because of the difficulties to recognize and expand these suppressor cells no new insights were evaluated in the following 15 years. Today it is generally accepted that Tregs have a key role in the prevention of autoimmune disease, immune homeostasis and the modulation of immune responses during infections. Sakagushi and coworkers renewed the interest in Tregs by a closer determination of Tregs as $CD4^+$ T cells that coexpress the interleukin-2 receptor (IL-2R) α-chain (CD25) [32]. These cells emerged from the thymus and comprise 5-10% of whole $CD4^+$ T cells. Furthermore a depletion of the cells prior to transfer into T cell-deficient mice leads to autoimmune disease in the recipients [33]. These $CD4^+CD25^+$ T cells with regulatory potential were initially described into the mouse but several studies identified similar cell populations in humans [34-38]. Thereby expression of CD25 is not restricted to Tregs, but also activated T cells upregulate this surface molecule. However, human CD4 regulatory function was only observable with a high expression of CD25, whereas in the murine system Tregs can be isolated from all $CD4^+CD25^+$ cells independent of their CD25 expression level [38].

A breakthrough in our understanding of Tregs occurred in the year 2003 with the discovery of the intracellular transcription factor forkhead box P3 (Foxp3) [39, 40]. Foxp3 was exposed to be a master control gene in the development and function of Tregs that acts as a repressor of transcription and regulator of T cell activation [41]. The transcription factor Foxp3 can inhibit the expression of effector cytokines including IL-4, TNF-α, IFN-γ, IL-17 and IL-21 [42]. Foxp3 expressing naturally occurring Tregs (nTregs) develop in the thymus after high-affinity interactions between their T cell receptor and autoantigen-presenting major histocompatibility complex (MHC) molecules on thymic stromal cells [43]. Tregs that occur in the thymus have an essential role in maintaining self-tolerance and further in the control of immune responses. Mice that have a mutation in the gene coding the transcription factor Foxp3 exhibit a hyperactive $CD4^+$ T cell phenotype with fatal production of high amounts of proinflammatory cytokines. This phenotype is named scurfy. Hemizygous males that possess this X-linked mutation die within a few months after birth. Equivalent in humans, mutation of the Foxp3 gene causes the autoimmune disease IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) [44].

However, T cells with regulatory functions can not only develop in the thymus. Generation of inducible or adaptive Tregs (iTregs) occurs in the periphery after antigenic stimulation. iTregs mediate tolerance for antigens that are not expressed in the thymus like food antigens, commensal microorganisms and self-antigens. Their induction in the periphery occurs under specific cytokine conditions or via tolerogenic DCs. Indeed, one example are IL-10 treated DCs, which are able to induce tolerance [45]. Addition of IL-10 to DC cultures results in a diminished expression of co-stimulatory molecules and human leukocyte antigen (HLA)-DR, reduces the potential of these cells to stimulate T effector cells and further promotes the suppressive activity of both $CD4^+$ and $CD8^+$ T cells

[46, 47]. Also TGF-β plays an important role in the generation of iTregs and enables peripheral T cells to become suppressor cells [48]. Tolerance induction through TGF-β is thereby mediated by expression of Foxp3 that promotes the transition of naïve T cells toward a Treg phenotype with immunosuppressive potential.

Interestingly, iTregs show variable amounts of Foxp3 expression. Furthermore studies in humans also revealed that, similar to CD25, the Foxp3 gene is also expressed in activated T cells [49], making the differentiation between activated T cells and Tregs more difficult. However, a stable and high expression of Foxp3 has been reported to be necessary for the suppressive function in human Tregs [50]. Identification of Tregs via their surface molecules remains a crucial goal, because identification by Foxp3 expression levels requires permeabilization that affects viability of the cell. Maintenance of Treg viability would be advantageous for therapeutic approaches that target their expansion. But the identification of Treg-specific surface molecules remains difficult. To date differentiation of Tregs from T effector cells more relates distinct expression patterns of a common surface molecule. In contrast to T effector cells Tregs show a diminished expression of CD127, the IL-7 receptor α-chain [51]. Furthermore Tregs exert a constitutive expression of cytotoxic T lymphocyte antigen 4 (CTLA-4) whereas T effector cells upregulate CTLA-4 only upon activation. CTLA-4 expression contributes to immune suppression and loss of CTLA-4 expression in Tregs resulted in the accumulation of $CD4^+Foxp3^+$-effector T cells with autoimmunity and early death [52]. GITR (glucocorticoid-induced tumor-necrosis-factor-receptor-related protein) is another molecule on Tregs and may affects their antigen-nonspecific proliferation and activation during the progress of an immune response [53]. Recently focused was the receptor molecule glycoprotein A repetitions predominant (GARP). GARP is expressed on Tregs but lacks on conventional T cells [54]. Ectopic expression of GARP in human naïve T cells abrogates their proliferation and cytokine secretion upon T cell receptor stimulation and induces expression of Foxp3 [55]. In contrast to T effector cells Tregs can produce active TGF-β. Therefore GARP probably acts as receptor for latency-associated peptide (LAP) in complex with latent TGF-β and takes part in the transformation of latent TGF-β into its bioactive form. In association with LAP TGF-β is not able to bind to its receptor. Association of latent TGF-β on the surface of activated Tregs may leads to activation of the cytokine [56].

Mechanims of suppression

Initially, the underlying mechanism of Treg mediated suppression has been associated to competition for IL-2 with T effector cells, leading to cytokine deprivation-mediated apoptosis of effector T cells [34]. However, several different suppressive mechanisms have been described. Indeed, suppression can be mediated by direct cell contact, cytokine milieu and antigen-presenting cells and the role of different surface molecules that are involved in suppression still has to be elucidated, especially in the human system. It was described that suppression mediated via nTregs occurs in a contact dependent manner. A recent study identified cyclic adenosine monophosphate (cAMP) as an important molecule in the cell contact-dependent Treg-mediated suppression [57]. Thereby cAMP diffuses through gap junctions from the Treg into the T effector cell where it activates proteinkinase A that mediates inhibition of proliferation and IL-2 synthesis by induction of inducible cAMP early repressor (ICER) [58].

Besides mediating immunosuppression via direct cell contact between Tregs and T effector cells indirect mechanisms of suppression were described. An indirect suppression mechanism takes place when Tregs interact with antigen presenting cells (APCs). This interaction leads to suppression of APC activation following reduced upregulation of CD80/CD86, CD54, CD40 and MHC class II costimulatory molecules. The insufficient costimulation further leads to the suppression of IL-2 production and proliferation by $CD4^+CD25^+$ cells. Also CTLA-4 could play a role in the contact-dependent suppression of Tregs. CTLA-4 is involved in the reduction of immunostimulatory activity of DCs. Several groups have demonstrated that the interaction of CTLA-4 on Tregs with CD80 and CD86 on DCs leads to expression of indoleamine 2, 3-dioxygenase (IDO) that can promote pro-apoptotic metabolites that mediate suppression of T effector cells [59]. A role of CTLA-4 on Tregs in humans is still under investigation. Experiments that tried to block the action

of CTLA-4 revealed divergent results [37, 60]. A further molecule that was described to be involved in contact-dependent suppression of Tregs is the lymphocyte activation gene-3 (LAG-3) that is expressed on Tregs upon activation. LAG-3 is associated with CD4 and binds to MHC class II molecules. Antibodies against LAG-3 abrogate Treg-mediated suppression in vitro and in vivo. Furthermore Tregs from LAG-3-deficient mice have a reduced capacity to suppress [61].

In contrary to nTregs, iTregs are thought to mainly mediate suppression via inhibitory cytokines. iTregs are further subdivided into type 1 IL-10-secreting Tregs (Tr1 cells) and TGF-β-secreting Th3 cells [62, 63]. IL-10 and TGF-β function as immunosuppressive cytokines. IL-10 inhibits proliferation and cytokine responses in T cells [64] and recent findings suggest that it plays a pivotal role in suppression of inflammatory Th17 cell responses [65]. TGF-β could induce naïve $CD4^+$ T cells to become suppressive cells that inhibit T cell cytotoxic activity [66] and further influences proliferation and function of a wide range of lymphocytes and antigen presenting cells [64]. Another cytokine with inhibitory capacity, IL-35, a member of the IL-12 cytokine family was described. IL-35 is presumably secreted from cells with regulatory potential and further required for maximal Treg activity [67].

Role of Tregs in models of allergic airway disease

Animal models of allergic airway disease have improved the understanding of basic mechanisms of allergic sensitization and disease development and were further used to evaluate the effects and functions of Tregs in the induction of tolerance. Tregs can influence both, the sensitization towards a certain antigen and the development of an allergic disease after sensitization. *In vivo* depletion of Tregs before antigen challenge increased airway hyperresponsiveness (AHR), airway eosinophilia, IgE synthesis and the production of Th2-related cytokines in strains of mice, which are usually less prone to develop allergic disease. The enhanced allergic phenotype was thereby accompanied by an increased expression of costimulatory molecules like MHC class II along with an elevated ability of pulmonary DCs to stimulate T effector functions [68]. Hence, it seems that Tregs are able to induce tolerance during sensitization phase via suppression of DC activation.

Furthermore the role of Tregs for initiation and development of allergic airway disease after sensitization was analyzed. In early studies transfer of $CD4^+CD25^+$ depleted allergen-specific T cells into T and B cell deficient hosts lead to a reduction of Th2-polarized airway inflammation but promoted a Th1-mediated response [69]. In further studies depletion of $CD4^+CD25^+$ Tregs in mice sensitized to Ovalbumin (OVA) prior to allergen challenge impaired allergen specific tolerance that was induced by nasal application of OVA. Additionally, depletion of Tregs enhanced airway inflammation indicated by increased eosinophil recruitment into the lung and increased T cell proliferation [70]. On the other hand the adoptive transfer of isolated allergen-specific Tregs was effective for the reduction of AHR, lung eosinophilia and cytokine production in an OVA-dependent mouse model of asthma [71]. Also transfer of non allergen-specific Tregs could reduce AHR and inflammation in dependency of IL-10 and TGF-β [72]. However, in another study the additional transfer of $CD4^+CD25^+$ cells was not sufficient to diminish the development of AHR despite the reduced Th2 immunity in the airways [73]. Taken together these studies demonstrate that Tregs are regulators of allergic airway disease, however with different effectiveness in different models.

Tregs and environmental exposure – lessons from hygiene hypothesis

Results of urbanization are reduction of environmental biodiversity associated with the use of biocides, antibiotics and disinfection. The heightened hygiene standards could explain the increasing prevalence of allergic diseases as a result of modified nutrition and environment. Several studies demonstrated that exposure to environmental microbes during early childhood for example in a farm environment diminished the risk to develop allergic diseases later in life [74-76]. Interestingly, these environmental changes have been associated also with Treg responses. Continuous stimulation of the immune system following contact with pathogens is essential for tolerance induction. Tregs seems to be involved in mediating tolerance to harmless antigens. Also Tregs are

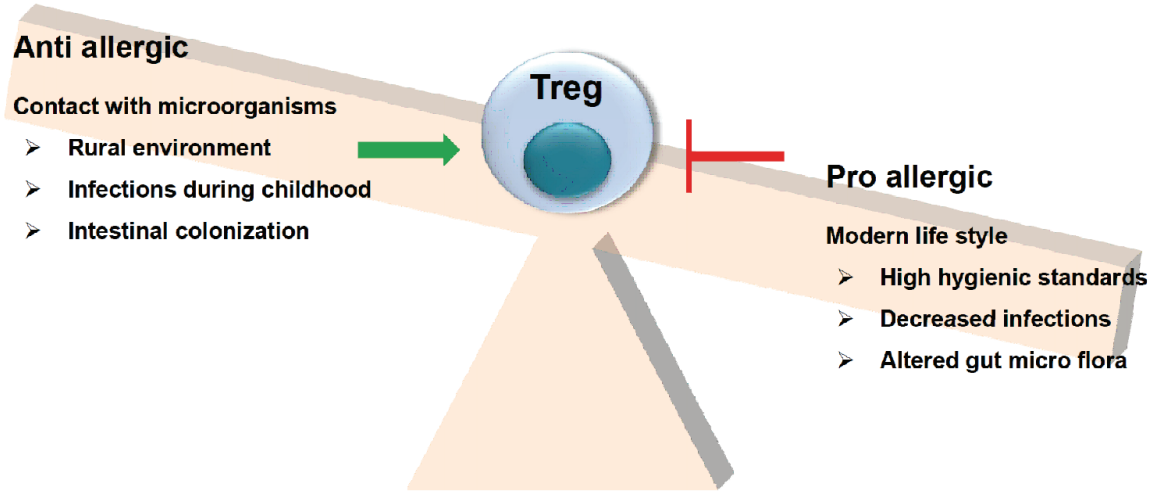

Figure 1. Tregs influence allergic disease development. Evidences accumulate that contact with microorganisms, especially during childhood impairs the composition of the intestinal microbiota and is critically involved in tolerance induction. The modern life style together with high hygienic standards and decreased infections may participate on alteration of the intestinal microbiota and development of allergy.

known to take part in the regulation of an immune response and infection induced immunopathology.

The intestinal mucosa essential participate on regulation of peripheral tolerance and the composition of the gut flora affects the development of atopic diseases [77]. Commensal bacteria in the microbiota of the gut seem to be important factors for the induction of Tregs through activation of tolerogenic DCs that produce anti-inflammatory cytokines like IL-10 and TGF-β [78]. In addition, the intestinal flora is thought to antagonize the postnatal Th2-polarized immune system via driving Th1 cell differentiation [78]. Symbiotic colonization of the gut with environmental microorganisms begins immediately after birth and is controlled by specialized mucosal DCs. DCs in the gut encounter antigens and promote T effector cells or suppressive Tregs. Although the DCs in the lamina propria express high levels of Toll-like receptors (TLRs) and costimulatory molecules in response to TLR ligands they produce IL-10 constitutively instead of pro-inflammatory cytokines [79]. It can be speculated that this selective regulation of TLR responsiveness is one mechanism to prevent unnecessary inflammatory reactions. Furthermore, intestinal Macrophages and DCs extend the Treg repertoire through secretion of IL-10, TGF-β and retinoic acid that induce Foxp3 expression in Tregs [80, 81].

Interestingly also infection with a certain bacteria has been associated with Treg induction. Indeed, neonatal mice infection with helicobacter pylori (H.p.) protects adult animals from the development of allergic airway disease and is associated with elevated numbers of Tregs in the lung [82]. DCs that were exposed to H.p. are constricted in their maturation upon lipopolysaccharide stimulation and induce the expression of Foxp3 in naïve T cells. The tolerogenic function of DCs requires the secretion of IL-18 that acts directly on T cells driving their conversion into Tregs [83]. Further studies underscore that especially DCs could facilitate Treg induction. Particularly IL-10-producing or immature DCs participate on the induction of Tregs. It was shown that Tregs can be indirectly induced during infection after stimulation of TLRs on DCs that produce IL-10 upon activation. Infection of TLR-4 deficient mice with the respiratory pathogen Bordetella pertussis exhibit in comparison to wild type animals diminished production of IL-10 and furthermore enhanced inflammation based on reduction of Treg numbers [84]. Hence, the heightened hygiene standards are correlated to alterations in the composition of the gut microbiota caused by modern lifestyle and probably influences the development of allergic diseases by affecting tolerance induc-

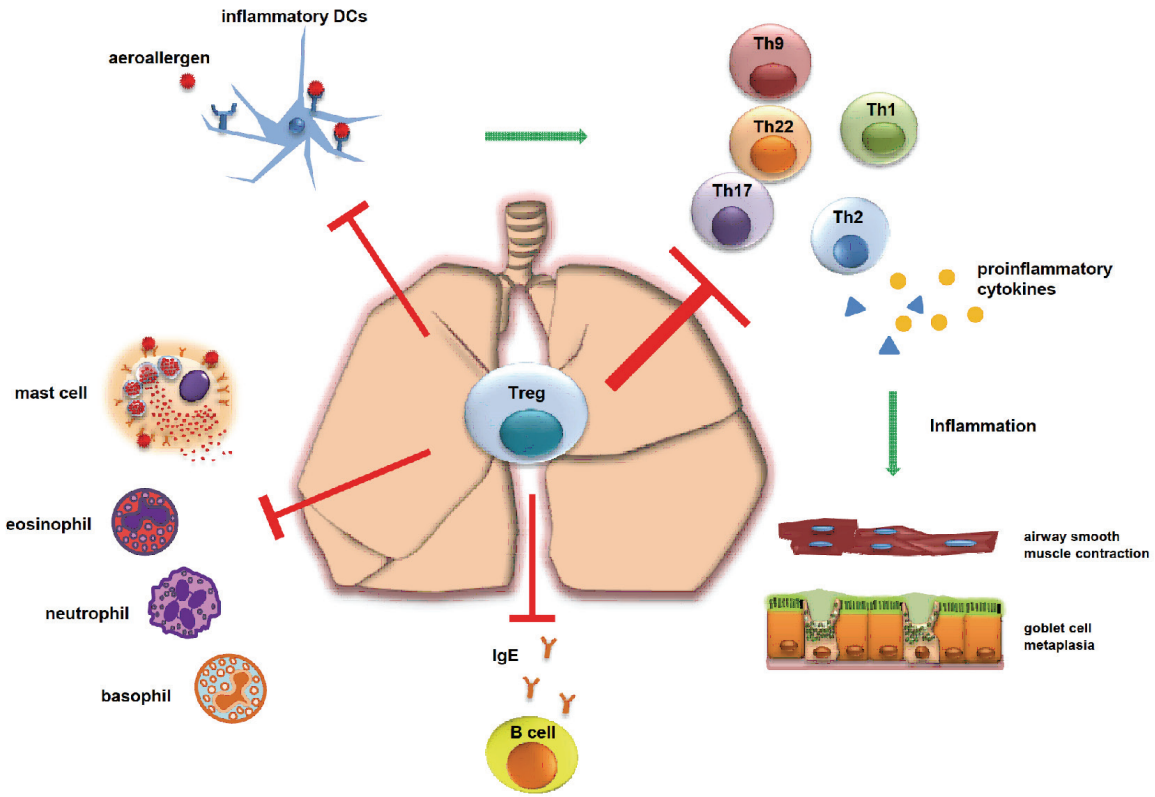

Figure 2. Effects of Tregs in asthma. Tregs can counteract the allergic inflammation in asthma in different ways. During sensitization Tregs are able to suppress the activation of inflammatory DCs that promote the allergic inflammation. Furthermore they can reduce the survival of mast cells and eosinophil, neutrophil and basophil granulocytes. In addition they can inhibit the degranulation of mast cells and basophils. Besides the reduction of B cells isotype switch to IgE production Tregs can further abrogate the activation and recruitment of different Th subsets that otherwise would enhance the inflammation in the airways.

tion that occurs in the intestinal microbiota (**Figure 1**).

Induction and modulation of Tregs as a potential target in asthma therapy

Asthma consists of different heterogenic phenotypes with a large variability of distinct disease features, e.g. airway inflammation. In addition, substantially variations in treatment responses are present and some of these variations can be explained to differences in inflammatory phenotype of patients. Current therapies target the down-regulation of inflammation without affecting the underlying disease mechanism. Glucocorticoids are most frequently used for the treatment of airway inflammation, whereas bronchodilators target airway obstruction. Indeed, the effectiveness of steroid treatment has been linked to type of airway inflammation, with a better response in patients with eosinophilic inflammation [85]. In addition to steroids and bronchodilators, also other treatment options are available, including leukotriene receptor antagonists and monoclonal antibodies against IgE. Different new treatment options are currently under investigation in clinical trials. However, it becomes apparent that the effectiveness of these new drugs depends largely on the phenotype of patients they are tested in. Indeed, anti-IL-5 antibody seems to be mainly effective in patients with a robust eosinophic inflammation [86, 87], whereas anti-IL-13 antibody seems to work most efficiently in patients with evidence for Th2 driven airway inflammation [88].

Also Tregs are targeted by currently available therapies. Indeed, studies have shown that treatment of allergic diseases with glucocorticoids and β2-agonists is associated with the induction of Tregs in these patients [89]. Asthmatic patients that received glucocorticoids exhibit elevated numbers of Tregs in the

bronchoalveolar lavage [90] and increased expression of IL-10 and Foxp3 mRNA [91]. It can therefore be speculated that Tregs might play significant role in mediating the suppressive effect of corticosteroids. Glucocorticoid treatment targets human T cells and stimulates them to increase their IL-10 production [92]. Vitamin D3 seems to be involved in the regulation of IL-10 production by T cells. Indeed, stimulation of human $CD4^+$ Tregs with dexamethasone and vitamin D3 strongly enhance their IL-10 production [93] and interestingly administration of Vitamin D3 can overcome impaired IL-10 production by Tregs upon steroid treatment in glucocorticoid-resistant patients [94, 95]. Furthermore, Tregs have been shown to play an important role in the effectiveness of specific immunotherapy (SIT). At this moment SIT is actually the only therapy that influences the mechanism of allergic disease. Repeated exposure to an antigen can induce tolerance and elegant studies in beekeepers have shown that repeated bee stings are associated with the induction of IL-10 producing Tregs [96]. In addition induction of Tregs by subcutaneous or sublingual administration of the allergen has been identified [97]. Indeed, administration of increasing doses of the causative allergen during SIT can reverse an established destructive immune response and enhance tolerance induction. SIT leads to a reduced degranulation of mast cells and basophils and counteracts systemic anaphylaxis [98]. Furthermore also a shift from a Th2-polarized immune response to a Th1-mediated response and diminished production of IgE antibodies and increased IgG antibodies are detectable. Indeed, generation of allergen-specific Tregs during SIT is associated with the suppression of allergen-specific Th2 cells, which as a result leads to reduction in IgE production of B cells and goblet cell metaplasia of epithelial cells and diminished survival and function of mast cells, eosinophils and basophils [98]. Furthermore Tregs are involved in the suppression of Th1, Th9, Th17 and Th22 cells [98]. An overview of effects mediated by Tregs is shown in **Figure 2**.

A different more experimental approach is the modulation of Treg function. In preclinical models it was demonstrated that pharmacological modulation of cAMP, transferred from Tregs into effector T cells by using phosphodiesterase 4 inhibitor did improve the capacity of Tregs to suppress the development of allergic airway disease. Treatment of mice that were adoptively transferred with Tregs with the phosphodiesterase inhibitor rolipram prevents degradation of cAMP in T effector cells and further protects mice to develop allergic airway disease [99]. A second approach could be to try to fully activate their suppressive potential. To exert their suppressive function and further to induce tolerance Tregs have to be activated by antigen-recognition or TCR-stimulation. Thereby stimulation of the CD4 coreceptor on Tregs is sufficient to activate their suppressive capacity also in human Tregs. Treatment of Tregs with antibodies against CD4 enables them to be fully suppressive [100]. Interestingly, activation of the Treg via CD4 is associated with elevated cAMP levels that mediate suppression [101] and studies in humanized models of allergic airway disease have recently show that activation of Tregs via CD4 efficiently suppresses the development of allergic airway disease [102]. In future work it has to be carved out how in vitro expansion and in vivo activation of Tregs could be optimized and translated into the clinic.

Conclusion

Allergic diseases like asthma increase dramatically in the last decades and to date treatment approaches rather affect the symptoms and fail to prevent disease development. Evidence emerged that the increasing occurrence of allergic disorders correlates with the heightened hygienic standards in our civilized society and the closely related disappearing microbiota that presumably influence mechanisms of tolerance induction. Tregs are essential involved in the induction of tolerance mechanisms. Indeed, patients with asthma have diminished frequencies of Tregs or impaired Treg function. Preclinical in vivo studies detected that Tregs control allergic airway inflammation before and after sensitization. Furthermore Tregs are able to reverse an already established allergic inflammation in experimental settings. Contact with pathogens early in life seems to be essential as a protective effect, which also seems to be mediated by Tregs. In addition Tregs are involved in mediating the effects of allergen immunotherapy. Also in experimental settings pharmacological modification of Treg responses have shown first promising effects. However, further research is needed to explore

potential therapeutic options targeting Tregs in patients with asthma.

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