Harnessing Regulatory T cells to Suppress Asthma
From Potential to Therapy

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Regulatory T cells (Tregs) play an essential role in maintaining the homeostatic balance of immune responses. Asthma is an inflammatory condition of the airways that is driven by dysregulated immune responses toward normally innocuous antigens. Individuals with asthma have fewer and less functional Tregs, which may lead to uncontrolled effector cell responses and promote proasthmatic responses of T helper type 2, T helper 17, natural killer T, antigen-presenting, and B cells. Tregs have the capacity to either directly or indirectly suppress these responses. Hence, the induced expansion of functional Tregs in predisposed or individuals with asthma is a potential approach for the prevention and treatment of asthma. Infection by a number of micro-organisms has been associated with reduced prevalence of asthma, and many infectious agents have been shown to induce Tregs and reduce allergic airways disease in mouse models. The translation of the regulatory and therapeutic properties of infectious agents for use in asthma requires the identification of key modulatory components and the development and trial of effective immunoregulatory therapies. Further translational and clinical research is required for the induction of Tregs to be harnessed as a therapeutic strategy for asthma.

Keywords: asthma; regulatory T cell; forkhead box p3; immunoregulatory therapy

Asthma has dramatically increased in developed countries over the past 3 decades. It is a common chronic inflammatory disease of the airways characterized by episodes of breathlessness, coughing, wheezing and airway hyperresponsiveness (AHR). The causes are complex and multifactorial, and are therefore difficult to target therapeutically. Current treatment strategies only suppress the symptoms, rather than inhibiting the underlying mechanisms, and fail to control the disease in a significant proportion of individuals with asthma.

A variety of different cell types are involved in promoting the inflammatory component of asthma. The prevailing paradigm is that T helper (Th) type 2 lymphocytes drive inflammation through the secretion of cytokines, such as IL-4, IL-5, and IL-13, which induce the recruitment and activation of eosinophils, pulmonary inflammation, mucus hypersecretion, B cell isotype switching, and AHR. Over time, lung function declines as a result of airway remodeling, which leads to increased susceptibility to exacerbations of disease.

Th17 cells are a recently recognized member of the T cell family, and are important in modifying immune responses in the airways. Bronchial biopsies from patients during acute episodes of severe asthma are infiltrated with Th17 cells (1). Furthermore, studies using animal models have established that Th17 cells and their cytokines are major inducers of neutrophilic, eosinophilic, and steroid-resistant airway inflammation (2, 3).

Natural killer T (NKT) cells are another unique subset of T cells, which respond to glycolipids and secrete large amounts of Th2 cytokines (4). NKT cells have been detected at higher levels in the sinus mucosa and sputum of individuals with asthma compared with healthy individuals (5, 6). Furthermore, animal studies have identified a potential requirement for NKT cells in asthma, particularly in the induction of AHR that is independent of Th2 cell responses (7).

Antigen-presenting cells (APCs), such as dendritic cells (DCs), have crucial roles in antigen presentation, initiation, and maintenance of allergic disease. Both myeloid and plasmacytoid DCs are increased in the airways of patients with asthma after allergen challenge, highlighting their role in allergic inflammation (8).

Th2 cell–driven B cell secretion of IgE, subsequent cross-linking on mast cells, and release of inflammatory mediators also contributes substantially to the allergic response. Anti-IgE therapy (Omalizumab) has proven effective for allergic disease when administered in conjunction with steroids (9).

Together, the dysregulation of these cellular aspects in asthma highlights the multifactorial processes that contribute to the development and maintenance of disease. Although there have been many attempts, the targeting of individual factors by direct therapeutic intervention has not led to effective therapies to date. This highlights the need for the development of therapeutic strategies that have multifactorial suppressive effects on the causes of asthma.

CLINICAL RELEVANCE

This review highlights a central role for regulatory T cells (Tregs) in suppressing the dysregulated immune responses involved in the pathogenesis of asthma. The development of an immunoregulatory therapy that induces Tregs offers a novel therapeutic strategy for asthma.

ASTHMA: THE REGULATORY T CELL DEFICIENCY

In healthy individuals, regulatory T cells (Tregs) play an essential role in modulating and regulating immune responses by promoting tolerance, counterbalancing aggressive inflammatory

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reactions, and maintaining homeostasis. Several independent studies have shown that the number and function of Tregs is impaired or altered in allergic patients compared with healthy individuals.

Reduced numbers of Tregs are observed in blood and/or induced sputum from patients with severe eczema, elevated IgE levels, eosinophilia, food allergy, and asthma, and, during exacerbations, individuals with asthma have an even greater deficiency of Tregs (10, 11). By contrast, some studies have detected an increase in the number of Tregs in severe disease (12, 13). It is likely that, in severe cases, Tregs are induced to moderate inflammation; however, they are not induced to a sufficient extent to overcome the aggressive inflammatory responses involved. However, the interpretation of these studies may be confounded by the characterization of Tregs with CD4 and CD25 positivity alone, as some of these cells may represent activated effector T cells. Furthermore, the assessment of Treg number in blood does not account for Tregs that may have migrated to the site of inflammation.

As well as reductions in numbers, Tregs from individual atopy have a significantly reduced capacity to suppress effector T cells and Th2 cytokines (14, 15). This may be due to differences in the proportions of Treg subsets in healthy and allergic individuals (16). Chemotactic signals for Tregs, such as those in the CCL-1 pathway, may also be defective in individuals with asthma (17). Furthermore, the forkhead box (Fox) p3 locus is subject to epigenetic modification, which may result in alterations in the suppressive capacity of Tregs (18). The role of epigenetic modifications in Treg function in individuals with asthma requires further investigation.

Evidence that supports the requirement for Tregs in the control of asthma has been provided by the use of mouse models of allergic airways disease. Adoptive transfer of $CD4^+CD25^+$ Tregs into sensitized mice before antigen challenge suppresses the development of allergic disease (19, 20). In addition, adoptive transfer of Tregs after the onset of disease attenuates established inflammation (21).

Glucocorticoid treatment of asthma is effective in suppressing inflammation and symptoms. These agents induce a short-term up-regulation of Foxp3 expression and Tregs in patients with asthma (22). However, animal models suggest that, in the long term, corticosteroids may also prevent the development of Tregs and exacerbate Th2 immune responses (23).

Together, these observations provide strong evidence that an effective regulatory response, which is controlled by Tregs, is required to prevent the development and progression of asthma.

TREG CHARACTERIZATION

Tregs are characterized by many phenotypic and functional markers that distinguish them from conventional T cells. CD4, CD25, and Foxp3 are the three markers that have been classically used to characterize Tregs. The transcription factor, Foxp3, is essential for the suppressive activity, survival, and stability of Tregs, and CD25 is found on the vast majority of $Foxp3^+$ T cells.

Tregs may develop as two distinct populations, termed natural or induced Tregs. Lineage commitment into natural Tregs is instructed by self-antigens in the thymus. Induced Tregs have a more "plastic" phenotype. They are derived from the naive CD4 precursor pool in peripheral lymphoid tissue after foreign antigen encounter and are generated under the influence of IL-2 and transforming growth factor (TGF)–β (24). Notably, induced Tregs comprise both $Foxp3^+$ and $Foxp3^-$ populations (25).

Two subtypes of Tregs that release soluble factors have also been identified, which are Treg type (Tr) 1 cells that secrete high levels of IL-10 with or without TGF-β production, and Tr3 cells that release TGF-β (26). However, the continual reporting of additional suppressive mechanisms and markers, which are associated with Tregs, indicates that numerous other subtypes are likely to exist.

TREG-SUPPRESSIVE MECHANISMS

The mechanisms of suppression of immune responses that are employed by Tregs remain controversial, which largely stems from discrepancies between *in vivo* and *in vitro* studies. The widely recognized mechanisms of suppression include the secretion of suppressive soluble factors, cell contact–mediated suppression, and competition for growth factors (Figure 1). The regulatory effects of Tregs on effector cell responses include: cell cycle arrest and inhibition of proliferation; induction of apoptosis; and suppression of cytokine release, DC maturation, or antigen presentation and costimulation.

Soluble Factors

The immunosuppressive cytokines, IL-10 and TGF-β, were the first factors considered to be involved in mediating suppression by Tregs, and the roles of these cytokines in suppression have been discussed extensively elsewhere (27, 28). In summary, IL-10 release from Tregs prevents the synthesis of proinflammatory cytokines, and down-regulates the expression of effector T cell cytokines and antigen presentation and costimulatory properties of APCs. TGF-β directly prevents T cell proliferation and differentiation by inhibiting the release of many cytokines, including IL-1 and IL-2, and their receptors. TGF-β also inhibits B cell proliferation and apoptosis, and macrophage proliferation and function, including the release of reactive oxygen species. Furthermore, TGF-β maintains Treg function and promotes the differentiation of adaptive Tregs. Nevertheless, the roles and contribution of IL-10 and TGF-β to Treg-mediated immunosuppression are controversial. *In vivo* studies have demonstrated that both IL-10– and TGF-β–dependent mechanisms exist (29–31). By contrast, neutralizing antibodies against IL-10 and TGF-β fail to abrogate suppression *in vitro* and *in vivo*, and supernatants from cell-suppression assays do not attenuate effector T cell responses (19, 32, 33). In addition, the suppressive function of Tregs from mice deficient in these cytokines is not affected (34, 35).

More recently, IL-35 has been identified as an important cytokine released by Tregs to target effector cells directly (36). Epstein-Barr virus–induced gene 3 and IL-12α form the heterodimeric structure of IL-35, which is highly expressed by $Foxp3^+$ cells, but not resting $CD4^+$ cells. Furthermore, Epstein-Barr virus–induced gene $3^{-/-}$ and IL-$12a^{-/-}$ mice have Tregs with reduced suppressive capacity, which confirms the importance of IL-35 in Treg-mediated suppression.

Fibrinogen-like protein 2 (FGL2) is also highly expressed by Tregs (37). FGL2 down-regulates DC function, limits activation of naive T cells, and induces apoptosis of B cells. Subsequently, a role for this suppressive factor has been confirmed, because anti-FGL2 blocks the suppressive activity of Tregs, and Tregs from $Fgl2^{-/-}$ mice are less effective.

The release of cytotoxic molecules, in close proximity of target cells to induce their apoptosis, has also been implicated as a mechanism of suppression. Tregs can express granzyme A and/or granzyme B and apoptosis may be mediated in a perforin-dependent or -independent manner (38–40). In addition, granzyme B–deficient Tregs have reduced suppressive function.

Tregs preferentially express CD39 and CD73, which convert ADP and ATP to AMP, which is rapidly degraded to adenosine. Adenosine binds the A2A receptor on effector cells to

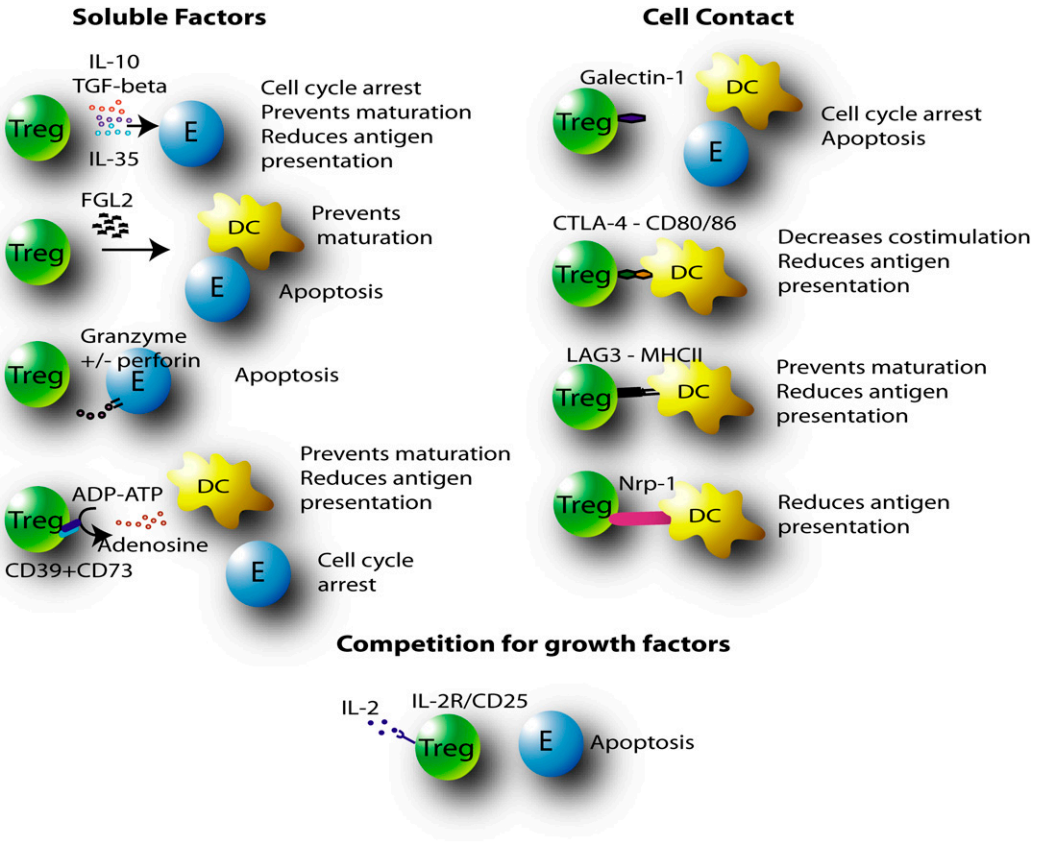

Figure 1. Regulatory T cell (Treg)–mediated suppression of immune responses may occur through soluble factors (IL-10, transforming growth factor [TGF]–β, IL-35, fibrinogen-like protein (FGL) 2, granzyme$^{+/-}$ perforin, and adenosine), cell contact–dependent mechanisms (galectin-1, cytotoxic T-lymphocyte–associated protein [CTLA]–4, lymphocyte-activation gene (LAG)-3, neuropilin [Nrp]-1), or competition for growth factors (IL-2). Suppression may be the result of cell cycle arrest, apoptosis, prevention of dendritic cell (DC) maturation or antigen presentation, or reduced costimulation of effector cells (E: T or B cells).

suppress their function, and may reduce DC function and the expression of costimulatory markers (41). Indeed, CD39-deficient Tregs are dysfunctional and less effective at suppressing effector T cell responses.

Cell Contact–Mediated Suppression

The existence and importance of cell contact–mediated suppression is controversial. Transwell experiments have shown that, in some instances, Tregs require cell contact to suppress target cells, and, in others, cell contact is not required (42, 43).

Galectin-1 is a β-galactoside–binding protein that is preferentially expressed on Tregs, and binds glycoproteins (44). Galactin-1 binding to effector cells leads to cell cycle arrest or apoptosis. Furthermore, blocking of Galactin-1 reduces the inhibitory effects of Treg cells and Galactin-1$^{-/-}$ mice have reduced Treg function.

To inhibit the priming and differentiation of effector T cells, Tregs are known to target APCs. Tregs are the only lymphocytes that express cytotoxic T-lymphocyte–associated protein (CTLA)–4, which closely resembles the T cell costimulatory molecule, CD28, but has higher ligand-binding affinity. CD28 ligation with CD80/86 on DCs is essential for T cell activation, and CTLA-4 ligation inhibits CD28 ligation and results in a higher proportion of anergic T cells. CTLA-4 may also block or down-regulate the expression of CD80/86, resulting in reduced priming of naive T cells. Furthermore, Treg CTLA-4–mediated ligation of CD80/CD86 can stimulate the production of indoleamine 2,3-dioxygenase and therefore condition DCs to become more immuno-suppressive (45). Indoleamine 2,3-dioxygenase is the rate-limiting enzyme for the degradation of tryptophan. Tryptophan depletion results in APC immunosuppressive activity by inducing the production of proapoptotic factors. Anti–CTLA-4 treatment reverses Treg suppression of effector T cell responses, and CTLA-4–deficient mice have defective Tregs (46, 47).

Tregs may also express lymphocyte-activation gene (LAG)-3 (CD223), a homolog of the major histocompatibility complex (MHC) II coreceptor, CD4, but with higher binding affinity. The direct interaction of LAG-3 with MHCII maintains the immaturity of DCs by reducing MHCII–peptide presentation to naive T cells (48). The control of APCs by Tregs at different stages of the immune response provides fine modulatory control.

Neuropilin-1 (Nrp-1) is also expressed by Tregs, and prolongs the interaction with DCs and reduces antigen presentation to naive T cells. The role of murine Nrp-1 in suppression was confirmed when anti–Nrp-1 was used to abrogate Treg-mediated suppressive activity (49). However, Nrp-1 cannot be used as a marker of human Foxp3^{+} Tregs, because Nrp-1 is not only expressed on human Foxp3^{+} Tregs, and occurs on other CD4^{+} cells (50). This study also demonstrated that Nrp-1 expression can be induced by stimulation of peripheral blood T cells, and may, in fact, be a novel marker of T cell activation. This suggests that anti–Nrp-1 may abrogate Treg-mediated suppression by interfering with cell activation rather than Treg function. The identification of factors that are important in initiating Treg suppression and are separate from contact-dependent suppression events requires further study.

Recently, Collison and colleagues (51) showed that Treg/effector cell contact increased the expression of IL-35. They also found that conventional T cell activation was required for heightened Treg function. They proposed that the function of Tregs is not contact dependent; however, the induction of suppression by T cell receptor (TCR) activation is. Elucidation of the requirements for the initiation of suppression will further

the understanding of the contextual importance of each mechanism.

Deprivation of Growth Factors

Tregs may also attenuate effector responses by competing with effector cells for essential growth factors. IL-2 is essential for both Treg and effector cell function (52). Tregs compete with effector T cells for secreted IL-2; subsequently, effector T cells are deprived of stimulation, and this leads to B cell lymphoma-2-interacting mediator (Bim)-mediated apoptosis (53).

Other Mechanisms of Suppression

Immune modulation by Tregs may also occur via nonspecific "bystander suppression" or outgrowth of a new population of Tregs, known as "infectious tolerance." This is supported by data showing that Tregs do not require TCR recognition to suppress effector T cells (54). In this study, Tregs were shown to require activation via their TCR to become suppressive; however, their function was antigen nonspecific. This was demonstrated *in vitro* by culture of transgenic Tregs, which have TCRs specific for one antigen, with transgenic T cells in the presence of either specific or nonspecific antigen. Transgenic Tregs suppressed proliferation of transgenic T cells, regardless of the antigen used. Hence, Tregs possess constitutive activity, and suppression can occur in the absence of MHC–peptide recognition and in the absence of APCs.

It is possible that, depending on the nature of immune response, eliciting agent, immunological make-up of the host, and site of suppression, certain mechanisms of Treg-mediated suppression prevail. Furthermore, in the absence of one suppressive mechanism, Tregs may employ alternative suppressor functions. Therefore, multiple Treg cell functions may act alone or synergistically, directly or indirectly, at the site of antigen presentation to suppress immune responses.

OTHER MARKERS OF TREGS

A number of additional markers have been associated with Tregs (Table 1). These markers have enabled the further delineation of the subtypes, activation, and function of Tregs that may be important in different disease states.

TABLE 1. ADDITIONAL MARKERS AND THEIR ASSOCIATION WITH REGULATORY T CELLS

Marker	Associated with:	Ref. No.
CD69	A unique subset	(55)
CD103	Increased activation status	(56)
TNFR2	Increased activation status	(57)
CD101	Increased activation status	(58)
CD45RB	Increased activation status	(59)
GITR	Clonal expansion	(60)
ICOS	Clonal expansion	(61)
Activin A	Clonal expansion	(62)
IL-9	Enhanced suppressive function	(63)
HO-1	Generation of Tregs	(64)
GPR83	Generation of Tregs	(65)
Retinoic acid	Homing and differentiation	(66)
CD62L	Homing state	(67)
LFA-1	Induction and function	(68)
OX40/CD134	Inhibition of suppression	(69)
PD-1	Inhibition of suppression	(70)
CD127	Low expression on Tregs	(71)
CD137/4-1BB	Survival	(72)

Definition of abbreviations: GITR, glucocorticoid-induced TNFR-related protein; GPR, G-protein coupled receptor; HO, heme oxygenase; ICOS, inducible T-cell co-stimulator; LFA, lymphocyte function-associated antigen; PD, programmed death; TNFR, tumour necrosis factor receptor; Tregs, regulatory T cells.

TREG SUPPRESSION OF EFFECTOR TARGETS IN ASTHMA

Defective Treg responses may play central roles in mediating dysregulated cellular responses and have been implicated at different stages in the development, progression and exacerbation of asthma (Figure 2). Both natural and induced Tregs are key players in the maintenance of immune homeostasis.

Recently, antigen presentation by alveolar epithelial cells has been shown to promote TGF-β–dependent induction of Foxp3 expression (73). In addition, alveolar macrophages may direct the induction of Treg differentiation, and a role for these cells in the suppression of allergic airways disease has been proposed (74). These observations highlight the important interplay between Tregs and APCs in mediating the control of immune responses in allergic airways disease.

During the initiation stage of an immune response, Tregs may attenuate the establishment of stable contacts between APCs and naive T cells, inhibit APC activity, or promote suppressive factors that prevent effector T cell development.

Emerging evidence suggests that there is also an important interplay between Tregs and Th17 cells during the early stage of naive T cell differentiation, which is currently the subject of intense research (75). Differentiation of naive T cells in the presence of IL-6 and TGF-β results in the development of Th17 cells. However, in the absence of IL-6, Tregs arise. IL-21 is also known to contribute to the induction of Th17 differentiation and suppresses Foxp3. Given their role in asthma, the prevention or suppression of Th17 cells by Tregs facilitates the maintenance of immune homeostasis.

In addition to modulating the priming of immune responses, Tregs also directly suppress fully differentiated effector cells. Indeed, the investigation of the suppressive effects of Tregs on Th2 cells has identified an array of suppressive mechanisms. Direct suppression of Th2 cells results in attenuated Th2 cytokine release, leading to reduced cellular inflammation, B cell isotype switching, and hallmark features of asthma.

The emerging involvement of multiple effector cell types in asthma pathogenesis, in addition to Th2 cells, indicates a much broader role for Tregs in counteracting dysregulated immune response in asthma.

Human Tregs suppress the proliferation, cytokine release, and cytotoxic effects of NKT cells in a cell contact–dependent manner (76). Interestingly, however, NKT cells from individuals with asthma, but not healthy control subjects, have the ability to be cytotoxic toward Tregs (39). This supports studies that show a reduced number of Tregs in individuals with asthma, and suggests that Tregs in individuals with asthma may be more vulnerable to destruction. Conversely, NKT cells may also provide proliferative help to Tregs through the secretion of IL-2 (77). This interplay highlights an important relationship between Tregs and NKT cells in individuals with asthma, which is not completely understood.

Effector B cells may also be directly suppressed by Tregs, which provides a secondary mechanism of immune attenuation after the suppression of Th2 function. Activated $CD4^+CD25^+$ T cells selectively kill B lymphocytes through close contact–mediated release of granzyme and perforin, in the absence of suppression of Th2 cells (78, 79). Hence, Tregs can specifically prevent IgE release and subsequent mast cell–mediated inflammation. Recently, immunosuppressive IL-10–producing B regulatory cells have been identified, and these cells may also control T cell–mediated inflammation (80). Furthermore, IL-10 induces IgG4 isotypes that are protective against the development of IgE and allergic disease in healthy individuals (81).

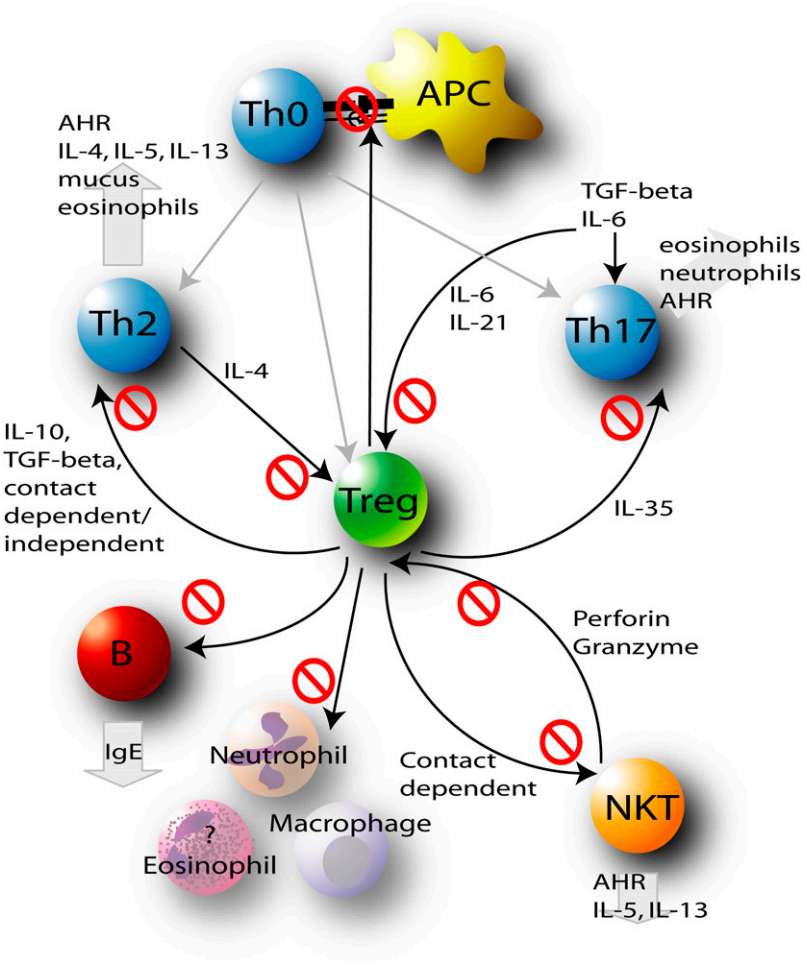

Figure 2. Treg-mediated immunoregulation is crucial in preventing the dysregulated immune responses that drive the initiation, progression, and exacerbation of asthma. By regulating Th2, Th17, and natural killer T (NKT) cells, antigen-presenting cells (APCs), B cells, and inflammatory cells, Tregs prevent the development of allergic inflammation, IgE release, mucus hypersecretion, and airway hyperresponsiveness (AHR).

Interestingly, B cells may contribute to the control of peripheral development of $CD4^+CD25^+$ cells, possibly by inducing the expansion or prolonging the survival of these cells (82).

Innate inflammatory cells in the lung are also potential targets for Tregs. Neutrophil function and survival are inhibited by Tregs in response to LPS exposure *in vitro* (83). Macrophage function and proinflammatory cytokine release are also attenuated *in vitro* and *in vivo* (84). These and other studies suggest that Tregs have the capacity to target innate immune responses directly. The potential for Tregs to suppress neutrophil, macrophage, and eosinophil responses directly, in the context of asthma, has not been assessed.

The capacity of Tregs to suppress the initial priming of an allergic response, and the multiple effector cells involved in the pathogenesis of asthma, indicates the potential for the induction of Tregs as a multifactorial immunoregulatory therapeutic approach for asthma.

INDUCTION OF TREGS BY INFECTIOUS AGENTS AND THEIR COMPONENTS

It has been widely proposed that a lack of infection-induced tolerance promotes the development of asthma, and may be responsible for the current asthma epidemic. This lack of tol-

TABLE 2. INFECTIOUS MICRO-ORGANISMS THAT MAY INDUCE REGULATORY T CELLS–MEDIATED SUPPRESSION OF ALLERGIC AIRWAYS DISEASE

Organism	Suppressive Mechanism	Ref. No.
Bifidobacterium lactis	Associated increase in TGF-β	(85)
Heligmosomoides polygyrus	Involves/dependent on IL-10	(86, 87)
Lactobacillus reuteri	Unknown, conflicting results; no change or increased IL-10	(88)
Lactobacilus rhamnosus	Associated increase in TGF-β	(85)
Litomosoides sigmodontis	Associated increase in TGF-β, however, blocking had no effect suppression of IL-10	(89)
Mycobacterium vaccae	Dependent on IL-10 and TGF-β	(90)
Nippostrongylus brasiliensis products	Independent of TLR-2, TLR-4, IFN-γ, and IL-10	(91)
Nippostrongylus brasiliensis	Involved IL-10	(92)
Schistosoma japonicum	Associated increase in IL-10	(93)
Schistosoma mansoni	Independent of IL-10, likely to be cell contact mediated	(94)
Streptococcus pneumoniae	Unknown, not IL-10 or TGF-β mediated, likely to be cell contact mediated	(95) and unpublished data
Toxiplasma gondii	Associated increase in IL-10	(96)

Definition of abbreviations: TGF, transforming growth factor; TLR, Toll-like receptor.

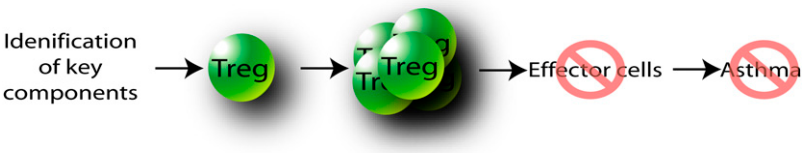

Figure 3. Identification of key components that expand the Treg pool and suppress effector cells involved in the pathogenesis of asthma have the potential to guide the development of successful immunoregulatory therapies.

erance may be mediated by reductions in infection-induced Tregs, which results in maladaptive immune responses that drive the development of allergy and asthma. This indicates the potential of harnessing the induction of Tregs by infectious agents or their components to target the development and effector-phase responses of allergic disease, including those that occur at the site of inflammation.

An inverse association between a number of infectious agents and the prevalence of asthma has been reported. These observations have initiated the elucidation of the mechanisms of induction and protection against asthma with animal models. As a result, a number of infections have been shown to induce Tregs and suppress allergic responses in mouse models of allergic airways disease (Table 2). The majority of infections appear to promote IL-10 or TGF-β–mediated suppression of effector responses; however, additional mechanisms of suppression have not been widely explored. Identification of the microbial components that are involved in the induction of Tregs and subsequent suppression of allergic airways disease is necessary before these effects can be harnessed for therapeutic application.

FUTURE DIRECTIONS

Numerous therapeutic strategies for asthma have been developed (97); however, their specificity toward particular factors limits their success. This is not surprising, because asthma is a multifactorial disease, and allergic airways disease continues to develop in the absence of Th2 cells, IgE, or eosinophilic inflammation in mouse models (98, 99). Immunoregulatory therapies that initiate a shift from Th2 to Th1 responses have also been explored; however, these approaches have had limited success in clinical trials (100, 101).

The multitargeting nature of Tregs allows for the regulation of a number of different effector arms of the immune response involved in asthma. The induction of Tregs to target effector responses may be the most holistic approach to modulate the underlying cause of disease. Although this would seem a straightforward approach, there are numerous issues that need to be addressed. Key components of infectious agents need to be identified and developed into immunoregulatory therapies, and administration regimes (dose, timing route) would need to be optimized (Figure 3). Furthermore, a successful immunoregulatory therapy needs to overcome the existing Treg pool within an individual with asthma, and the expansion of Tregs with normal function and chemotactic properties is required. Nevertheless, this approach of inducing Tregs to suppress the numerous allergic inflammatory responses in asthma would seem to be the most logical approach for the development of effective therapies.

Current and ongoing studies of harnessing the induction of Tregs by infectious agents for application to allergic disease have been based on the conversion of empirical data into effective therapies. Helminth infection with *Litomosoides sigmodontis*, *Nippostrongylus brasiliensis*, *Schistosoma japonicum*, and *Schistosoma mansoni* has been shown to induce Tregs and suppress allergic airways disease (89, 92–94). One study has extended these observations and identified helminth-derived products that inhibit allergic responses (91). However, these helminth products have not yet been extensively tested in animal models of allergic disease or in clinical trials. Treatment with probiotic bacteria has been shown to prevent the development of allergic airways disease in both adult and neonatal mouse models (85, 88). Numerous studies have investigated the potential of probiotic treatment for asthma and allergic rhinitis in humans, but have produced conflicting results, and are inconclusive at this stage. These studies have been recently reviewed (102). *Mycobacterium vaccae* administration has protective effects on allergic airways disease in mouse models, and there have been several attempts to translate these observations in clinical trials. Early studies showed that treatment with heat-killed *M. vaccae* reduced allergen-induced responses in atopic dermatitis; however, more recent studies that assessed the effect on asthma and atopic dermatitis showed a lack of efficacy (100, 103–105). The full potential of *M. vaccae*–based therapy for allergic disease remains to be determined, and is the subject of ongoing clinical trials.

Other promising therapeutic strategies for allergic disease, which involve increasing the numbers or function of Tregs, have been recently reviewed (106). Allergen immunotherapy is particularly effective, and involves the administration of increasing doses of a specific allergen. Therapy promotes the development of antigen-specific Tregs that release IL-10 or TGF-β and inhibit allergen-specific Th2 responses (107, 108). However, this strategy requires treatment that is tailored to the specific allergen, constant patient monitoring, and has been associated with serious side effects, including anaphylaxis. Further investigations are underway to improve safety and efficacy of this approach (109). Glucocorticoid administration in conjunction with the active form of vitamin D (1α,25-dihydroxyvitamin D3 or calcitrol) has also been shown to promote the induction of Tregs that release IL-10 (110). Importantly, this strategy is effective in patients that are refractory to steroid treatment, and further studies are refining this strategy (111).

In addition to the development of an immunoregulatory therapy for asthma, *in vivo* models that involve the induction of Tregs and suppression of allergic airways disease may provide valuable tools to further our understanding of the characteristics, mechanisms, and function of Tregs. These models may facilitate the delineation of "real–time" events that are important in the induction and enhanced suppressive function of Tregs.

New therapeutics, based on our understanding of Treg function and the pathophysiology of asthma, could have profound benefits for the care of individuals with asthma. Hence, it is not surprising that the potential to harness the power of Tregs as an immunoregulatory therapeutic is of great interest. Through Tregs, we have a multifactorial approach to a multifactorial disease, if only we can develop their potential into therapy.

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