T regulatory cells during clinical manifestations of Asthma: A therapeutic standpoint

AUTHOR: Mohammad Afzal Khan

RUNNING TITLE: T regulatory cells in asthma.

KEYWORDS: T regulatory cells, Airway inflammation, inflammatory cells.

CORRESPONDING AUTHOR:

Dr. Mohammad Afzal Khan (M.Sc, PhD)

Organ Transplant Research Section

Comparative Medicine Department

King Faisal Specialist Hospital and Research Centre

MBC 03, P.O. Box 3354, Riyadh, 11211, Saudi Arabia

Phone: +966-112162919 Extn: 32894

Email: mkhan26@kfshrc.edu.sa

Abstract:

Asthma is a chronic inflammatory disease of the airways, which is considered to be mediated by the allergen-specific $CD4^+$ T cells, Th2 cytokines, and allergen-specific IgE antibodies to play a key role in the initiation and perpetuation of chronic airway inflammation. The most common clinical manifestations of asthma are characterized by airway inflammation, airway obstruction, airway hyperresponsiveness, and airway microvascular remodeling.

In addition to inflammatory cells, a tiny population of T regulatory cells (Tregs) control immune homeostasis, suppress allergic responses and participate in the resolution of inflammation-associated tissue injuries. Preclinical studies from animal models have demonstrated the huge therapeutic potential of Tregs in asthma conditions. Increasing evidence indicates that Tregs could be used to inhibit pathogenic asthma inflammation, and airway microvascular remodeling during the progression of asthma. This review addresses the relationship between locally accumulated Tregs and the development of asthmatic inflammation, and associated airway remodeling during the disease progression.

ASTHMA AND INFLAMMATION:

Asthma is a chronic inflammatory disease of the bronchi arising because of inappropriate immunological responses and globally as many as 334 million people have been affected due to asthma pathogenesis [1-3]. The immune system guards the host against a broad range of pathogenic microorganisms and foreign tissue antigens while preventing unwarranted immune reactions that would be deleterious to the host tissue [4-7]. However, both protective and harmful immune reactions are principally mediated by T cells, B cells, which possess vast diversity in antigenic recognition, high antigenic specificity, potent effector activity, and long-lasting immunologic memory [6]. Because of this killing potency, serious damage to the tissue may ensue if aberrant immune responses during airway inflammation, hyper-responsiveness are triggered. Activated Th1 cells produce IL-2 and IFN-γ; they are important in immune responses in allergic inflammation while Th2 cells are essential in the allergic inflammation through IL-4, IL-5, IL-9 and IL-13 [1, 8-10]. Th1 cells secreted INF-γ has inhibitory effects on Th2 cells, and during allergic inflammation, it suppresses isotype switching of IgE and IgE production of Th cells and it can also stimulate cell-mediated cytotoxic effects[1]. Furthermore, Th17 cells are a distinct lineage of Th cells expressing IL-17 and mediate neutrophilic type inflammation and exacerbate Th2 mediated allergic inflammation [11, 12]. Immunoregulatory therapies that balance from Th2 to Th1 paradigm have also been investigated but with very limited success in clinical trials[13]. Numerous mouse models have been used to investigate the immunological mechanism of asthma pathogenesis [14, 15].

Clinical manifestations of asthma are characterized by airway inflammation, airway obstruction, airway hyperresponsiveness and massive infiltration of eosinophils, neutrophils, T lymphocytes and mast cells in the airway walls, and T helper lymphocyte subsets, defined by the cytokines they secrete, are thought to play a key role in the initiation and perpetuation of chronic airway inflammation and airway remodeling [10, 16-21]. Airway remodeling mainly represents structural changes associated with a reduction in lung functions, which includes sub-epithelial fibrosis, airway smooth muscle hypertrophy, and hyperplasia, tissue eosinophilia, epithelial injuries[22].

REGULATORY T CELLS: Tregs play a vital role in maintaining immunological unresponsiveness to self-antigens, and in suppressing heightened immune responses destructive to the tissue during asthma inflammation [18, 23-30]. Tregs are generated in the thymus as a functionally mature T cell subset and in the periphery of naive T cells [31, 32]. Tregs are a unique $CD4^+$ T-cell subpopulation, which in mice is characterized by the surface expression of CD25, nuclear expression of FOXP3, and secrete IL-10, TGF-β to suppress heightened immune responses, and also trigger iTreg expansion [33]. $CD4^+FOXP3^+$ natural Tregs and peripheral induced Tregs are key in maintaining immunotolerance against mucosal injury, pathogenic alloimmunity, diabetes, and facilitate tolerance induction in murine models of organ transplantations [26, 34, 35]. In general, Tregs are known to be crucial in the maintenance of peripheral immune tolerance and are the key modulators of the immune reaction during asthma inflammations [30].

The role of Tregs in asthma is scanty, very few studies reported their clinical benefits which shows that depletion of $CD4^+CD25^+FOXP3^+$ Tregs augments, whereas reconstitution of Tregs subdue lung allergic responses and in some studies of AHR[36-38]. On the other hand, Treg depletion before sensitization is proven sufficient to augments the severity of inflammation, and AHR in the lung [39]. These studies emphasized that the reconstitution of antigen-specific $CD4^+CD25^+$FOXP3+ Treg was found to subdue allergic inflammatory response and hyperreactivity via IL-10 dependent pathway [30, 40, 41], and further downregulates established inflammation and prevent airway remodeling when injected after the disease onset [42]. In subsequent studies, Joetham and colleagues examined the function of nTregs ($CD4^+CD25^+FOXP3^+$) isolated from the lungs of naive mice [43, 44]. Although it is difficult to conclude from animal models to the clinic there is increasing evidence from preclinical models that highlights the therapeutic significance of $CD4^+CD25^+FOXP3^+$ Tregs in the control of allergic diseases, including asthma. The therapeutic benefits of Tregs have been investigated both in clinical and preclinical studies, and based on previous research outcomes, Treg-mediated immunosuppression has been an increasing area of cell-based immunotherapies to rescue asthma inflammation[42, 45-48]. Several types of T cells with immunosuppressive properties have been identified, but $FOXP3^+$ Treg have

emerged as a dominant cell type; they are vitally involved in the tolerance induction, and maintenance of immune tolerance to alloantigens during asthma inflammation[49]. Recent research investigations highlighted the cellular and molecular basis of Tregs development and functions and implicate dysregulation of Tregs in major pulmonary diseases including asthma[50]. Naturally occurring $CD4^+CD25^+FOXP3^+$ Tregs (nTregs) are thymic-derived, and a second population of $CD4^+CD25^+FOXP3^+$ Tregs can be induced in vitro and in vivo through antigen stimulation, where both subsets regulate immune responses through the production of IL-10 and TGF-β[45, 51]. Both of these subsets appear to play an important role in regulating the development or expression of allergic diseases [49].

MODE OF ACTION: Tregs have been reported in suppressing Th2 mediated immune responses to allergens and subdue allergic inflammatory conditions, and numerous preclinical studies have shown that the adoptive transfer of antigen-specific Tregs subdue the onset and progression of asthma in mice [38, 41, 52, 53]. In general, Tregs prevent the generation of immune responses to self-antigens and other foreign antigens, including allergens, also limit immune responses to pathogens, protecting tissue from severe injuries[30]. Tregs modulate Th2-mediated lung inflammation, and their therapeutic potential is best described by evidence that therapies with Treg in allergic and asthma disease are associated with the induction or restoration of Treg function, e.g. glucocorticoids, allergen immunotherapy[54]. Tregs mediated immunosuppression have the potential to protect against allergic inflammation and asthma pathogenesis [52, 55, 56]. The primary immunosuppressive and regulatory function of Tregs is to control immune responsiveness and regulate hyper-airway response [56, 57]. Tregs also play a crucial role in maintaining immunological unresponsiveness to self-antigens, inhibit antigen-specific inflammatory responses [28], prevent pathological self-reactivity in the immune system, neutralizing killer T cells during inflammation [58], and more specifically participate in suppressing heightened immune responses destructive to the airway epithelium and normal physiological outcomes. Treg operates through a variety of immunosuppressive functions that regulate T lymphocyte, antigen-

presenting cell, and innate cell functions through cell- contact, competition for essential growth factors, cytotoxicity [50, 56].

During allergic inflammation, Tregs suppress inflammation through the secretion of inhibitory cytokine IL-10, transforming growth factor (TGF) β or by cell surface molecules [57, 59]. IL-10 reduces the effects of pro-inflammatory cytokines, maintains epithelial layer integrity, tissue healing, and inhibits eosinophil survival and migration during allergic inflammation, also down-regulate IL-4 induced isotype switching of activated B-cells [52, 60, 61]. These modulatory effects have been mainly associated with the release of IL-10 and TGF-beta, thus, harnessing the therapeutic power of Tregs, their induction and activation may provide an important strategy in controlling Th2-mediated allergic inflammation [62]. In addition, Tregs have been associated with the maintenance of immune responses and secreted immunosuppressive cytokines such as TGF-β, IL-10, and IL-35 are involved in immune responses following antigens/ allergen exposure [63, 64]. **Figure 1**

In addition to cytokine-mediated suppressive activity, Tregs are also mediate suppressive functions through the release of perforin and granzymes B and the release of cAMP [62]. However, some clinical studies also validated these roles, when the treatment with glucocorticosteroids in asthmatics might increase this FOXP3 protein expression within Tregs in human, and revealed suppression of Tregs number as reported from lung tissues in a model of asthma[54] while asthmatic patients have been reported to show decreased FOXP3 protein expression within their $CD4^{+}CD25^{high}$ T regulatory cells repertoire[65]. Data collected from asthmatic patients further highlighted the crucial role of Treg, which reported lower Tregs ratio and FOXP3 mRNA expression, and lower levels in peripheral blood mononuclear cells may be associated with the asthma pathogenesis in human[66]. A significant number of murine models of allergic inflammation/asthma have been adopted, although none replicates all pathological parameters of human asthma conditions [14, 67]. However, studies in animal models of allergic airway inflammation have investigated a fair number of preclinical and clinical research which included the key roles of $CD4^{+}CD25^{+}$ Treg, IL-10, and TGF-β in asthma prevention[52]. In other clinical studies, adoptive transfer of purified antigen-specific $CD4^{+}CD25^{+}FOXP3^{+}$Treg cells in pre-sensitized

mice suppressed airway hyper-responsiveness (AHR), eosinophil recruitment and Th2 cytokine release through the release of IL-10 and TGF-β, while depletion of $CD25^+$ Tregs before allergen challenge shifted Th2 cytokine upregulation, IgE levels, eosinophilia and AHR in allergy-resistant mice (C3H strain), concluded that Treg control disease resolution[38, 39, 68]. Altogether these previous investigations proved the therapeutic value of Treg to resolve established allergen-induced pulmonary inflammation (eosinophilia, Th2 infiltration, IL-5, IL-13, and TGF-β), but also prevent the progression of airway remodeling, and reduce mucus hypersecretion and peribronchial collagen deposition [38, 39]. IL-10 is a key anti-inflammatory and immunoregulatory cytokine that has distinct pleiotropic effects on both innate and adaptive immunity [69]. Primarily, it restrains inflammation and immune response and extensively participates in immunity activities by regulating cell proliferation, differentiation and the function of T cells, B cells, macrophages and endothelial cells [52]. IL-10 is produced by $CD4^+CD25^+FOXP3^+$ Tregs and also secreted by B cells, natural killer cells, antigen-presenting cells (APCs), mast cells, granulocytes. IL-10 can subdue the release of major pro-inflammatory cytokines such as IFN-γ, IL-2, IL-3, and TNF-α produced by Th1 cells, activated T helper cells, mast cells, NK cells, endothelium, eosinophils, and macrophages[40, 52]. Further, IL-10 can modulate Tregs cells to conserve the intracellular expression of FOXP3 and also suppressive functions [70, 71]. IL-10 has wide immunosuppressive and anti-inflammatory properties suitable to attenuate asthma pathology [72, 73]. It is a powerful inhibitor of major proinflammatory cytokines and acts on antigen-presenting cells to subdue T lymphocyte activation (Th2), suppresses effector cells, mast cells, and eosinophils [40, 52, 63]. In addition, IL-10 augments IgG4 release, which plays a key protective in allergic responses but inhibits IgE [74]. A number of clinical studies have reported higher IL-10 in allergic and asthmatics as compared to healthy individuals[75]. IL-10 has been involved in effective immunosuppression of allergic immune reactions in the lung [41, 44, 76], which signifies dependence on IL-10 and further highlights the T regulatory cell-mediated modulation of pulmonary immune responses. These preclinical reports validated the key role of Tregs during airway remolding and disease progression, and key secreted anti-inflammatory cytokine-IL-10 play a vital role in airway allergic immunomodulation to maintenance

pulmonary physiological functions, and as reported, IL-10 suppresses Th1- and Th2-type immune responses, inhibits mast cells, eosinophils mediators, and pro-inflammatory cytokines [40, 52, 77]. In addition, decreased IL-10 has been observed in allergic and asthmatic diseases compared with healthy control subjects[40].

Most of the ongoing therapies target the suppression of inflammatory response without modulating the actual pathogenic mechanism. Although glucocorticoids are the first drug choice to subdue airway inflammation, glucocorticoids treatment is also associated with the expression of IL-10, FOXP3 mRNA, and induction of Tregs in bronchoalveolar lavage of asthmatic patients [54, 78, 79]. These observations speculated that the presence of Tregs in BAL is crucial to play as an immunoregulatory role in mediating the suppressive effect of corticosteroids [80-83]. In the last decade, several therapeutic alternatives for asthma cure have been acquired; however, their selectivity limits their success because asthma pathology is a multifactorial event.

AIRWAY MICROVASCULAR REMODELLING:

In healthy lungs, the airway microvasculature supplies key vital functions necessary for maintaining a normal physiological process[84]. In particular, it delivers oxygen and nutrients, and act as a primary site for most of the humoral immune response to foreign antigens, which confers the first line of immunity before the onset of disease. Microvascular remodeling during airway inflammation mainly triggers though the pro-angiogenic action of growth factors and inflammatory mediators, and as seen in both human asthma and allergic reaction that the airway microvasculature affected during the progression of the disease, and affected during treatment further signifies the key involvement of microvasculature during asthma and airway remodeling phase and also in the pathology of a other chronic inflammatory, and ischemic pulmonary malfunctions[85]. Previous investigations on airway microvascular remodeling in chronic airway inflammation demonstrated that microvascular components of airway remodeling are the vital contributors to the alteration of the airway wall in asthma and COPD progression [20, 86]. Tregs adoptive transfer has been proven sufficient to subdue inflammation before the start of tissue inflammation and microvascular repair [26, 28].

Airway microvascular alterations as seen in asthmatic patients are accompanied by a rise in airway blood flow and diminished β2- adrenergic vasodilator responsiveness, suggesting the presence of endothelial dysfunction. Increased microvascular permeability and edema are common features during vascular remodeling in bronchial asthma [87]. Clinical studies have shown the role of Tregs in human asthma but these studies have been hampered by the lack of a clear correlation between Tregs and airway microvascular remodeling, which is the main pathological symptom of asthma. Whilst most studies identify the immunosuppressive properties of $CD4^{+}CD25^{+}$ Tregs to control allergic airway inflammation, these studies do not explain any impact of Tregs in microvascular changes and associated remodeling as reported in clinical condition. The majority of the previous investigations on airway microvascular remodeling in chronic airway inflammation extracted clinical outcomes of asthma patients, and these data demonstrated that microvascular components of airway remodeling are the vital contributors to the alteration of the airway wall in asthma progression[20]. Interestingly, these

airway microvascular perturbations are also seen during the development of COPDs [86]. Altogether, these airway microvascular changes in asthma and COPD are strongly associated with airway inflammation and may contribute to an increase in airway wall thickness which may be associated with disease progression[88]. Increased microvascular permeability and edema are common features during vascular remodeling in bronchial asthma [87].

CONCLUSIONS:

Clinical studies have shown the role of Tregs in human asthma but these studies have been hampered by the lack of a clear correlation between Tregs and airway microvascular remodeling, which is the main pathological symptom of asthma. The clinical demand of Treg cell-based immunotherapy is rapidly rising, and different Treg subsets have been described, including natural Tregs, induced Tregs, $CD8^+$ Treg cells, and regulatory cells has been described [34, 56, 74]. Tregs mediated immunotherapy is relatively a new addition in the modern drug development repertoire and therapeutics. Treg-mediated immunotherapy is expanding, and tend to replace conventional immunotherapy without negligible side effects in asthma patients. In this review, we highlighted the key molecular mechanism of Treg mediated protection to airway inflammation, and implications for the development of therapeutic strategies for major life-threatening disease including asthma. Modern drug discovery plan is quickly drifting toward a biological mode of therapeutic agents, which involve cells and their unique products to rescue the disease with minimum side effects, and global research is now in a new era with the introduction of clinical trials investigating the safety and potential therapeutic role of Treg therapy to rescue asthma exacerbations. Treg cell-mediated immunotherapy showing superiority over the current immunosuppressive regimen makes this concept of cure more of therapeutic value and will significantly minimize the cost of current immunosuppression for future medicine. The conventional therapeutic formulations to control asthma have focused on the use of potent anti-inflammatory drugs, particularly steroids, which have broad-spectrum suppressive activity against effector cells and their mediators[89]. Most popular glucocorticoid regimens are potent in the

majority of asthma patients but ineffective to support continuous respite of disease without repeated long term administration, which can be associated with serious toxic side-effects, and fail to control the disease in a large number of asthma patients, which are most at risk of hospitalization and death from their asthma[90]. These disturbing facts inspire the need for more specific therapies with the potential to support long-term recovery without side effects. The multi-regulatory action of Tregs recognized them as a potential candidate to rescue the occurrence of progressive inflammatory modulations. Therefore, the addition of Tregs to target effector responses may be the key approach to modulate the underlying cause of asthma disease. New immunotherapeutic, based on our understanding of Treg response to the pathophysiology of asthma, could have overwhelming benefits for the cure of asthma patients. Therefore, it is not surprising that the potential to utilize the immunoregulatory potency of Tregs as a therapeutic is of utmost requirement in asthma.

References

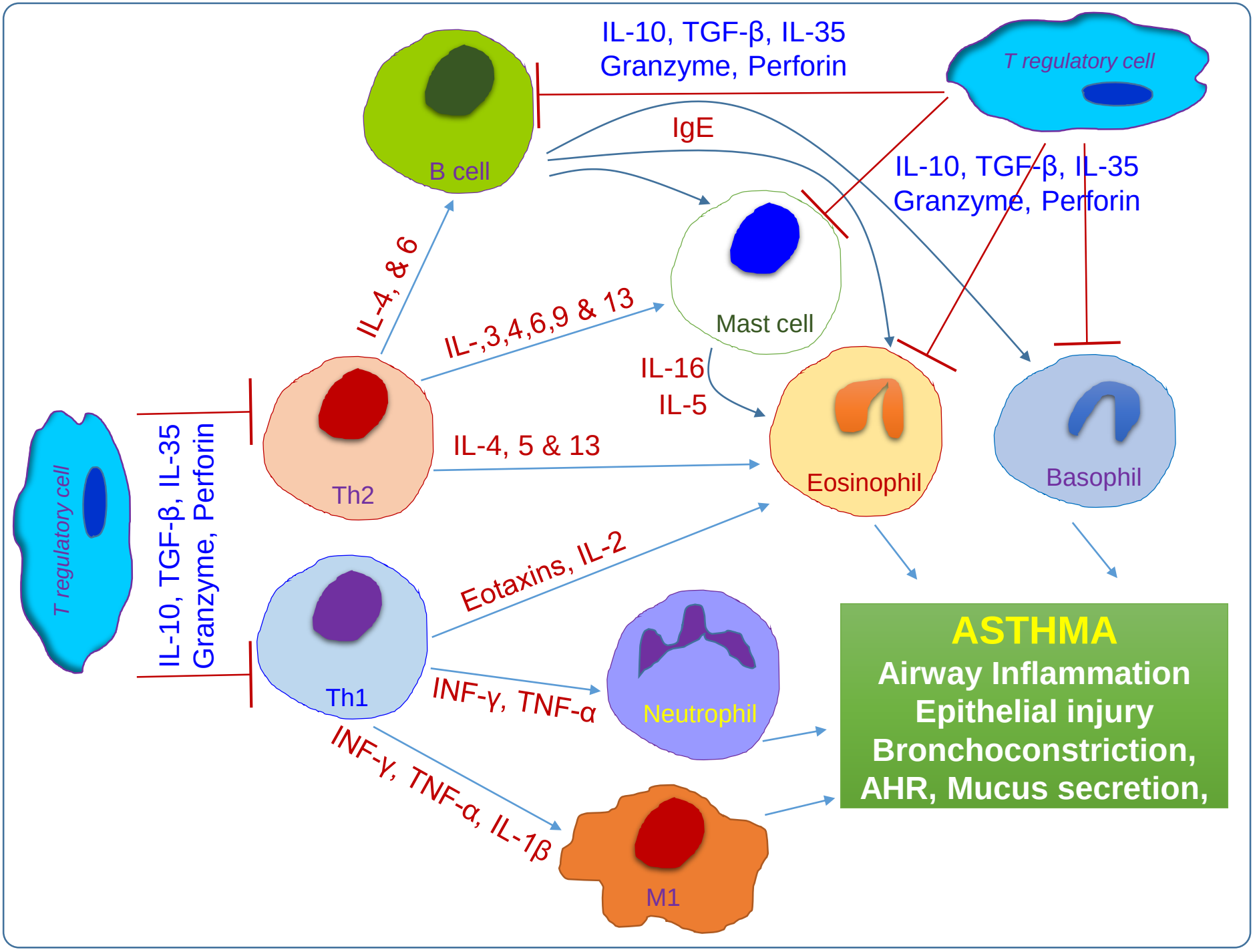
1. Biedermann T, Röcken M, Carballido JM. TH1 and TH2 Lymphocyte Development and Regulation of TH Cell–Mediated Immune Responses of the Skin. Journal of Investigative Dermatology Symposium Proceedings 2004; **9**:5-14.
2. Alotaibi G. Asthma control and self-management: The role of asthma education. Saudi Journal for Health Sciences 2015; **4**:16-22.
3. Alreshidi* NM. Dimensions of Asthma and Its Treatment in the Kingdom of Saudi Arabia: Literature Review. Adv Res Gastroentero Hepatol 2017; **5**:01-7.
4. Khan MA, Nicolls MR. Complement-mediated microvascular injury leads to chronic rejection. Adv Exp Med Biol 2013; **734**:233-46.
5. Janeway CA, Jr., Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002; **20**:197-216.
6. Heiss G, Hofmann KW, Trachtmann N, Walters DM, Rouviere P, Knackmuss HJ. npd gene functions of Rhodococcus (opacus) erythropolis HL PM-1 in the initial steps of 2,4,6-trinitrophenol degradation. Microbiology 2002; **148**:799-806.
7. Kew RR, Ghebrehiwet B, Janoff A. Characterization of the third component of complement (C3) after activation by cigarette smoke. Clin Immunol Immunopathol 1987; **44**:248-58.
8. Durrant DM, Metzger DW. Emerging roles of T helper subsets in the pathogenesis of asthma. Immunological investigations 2010; **39**:526-49.
9. Tulic MK, Christodoulopoulos P, Hamid Q. Small airway inflammation in asthma. Respir Res 2001; **2**:333-9.
10. Kaiko GE, Horvat JC, Beagley KW, Hansbro PM. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? Immunology 2008; **123**:326-38.
11. Jin W, Dong C. IL-17 cytokines in immunity and inflammation. Emerging Microbes & Infections 2013; **2**:e60.
12. McKinley L, Alcorn JF, Peterson A, DuPont RB, Kapadia S, Logar A, Henry A, Irvin CG, Piganelli JD, Ray A, Kolls JK. T(H)17 Cells Mediate Steroid-Resistant Airway Inflammation and Airway Hyperresponsiveness in Mice(). Journal of immunology (Baltimore, Md : 1950) 2008; **181**:4089-97.
13. Maggi E. T cell responses induced by allergen-specific immunotherapy. Clinical and Experimental Immunology 2010; **161**:10-8.
14. Nials AT, Uddin S. Mouse models of allergic asthma: acute and chronic allergen challenge. Disease Models & Mechanisms 2008; **1**:213-20.
15. Lloyd CM. Building better mouse models of asthma. Current Allergy and Asthma Reports 2007; **7**:231-6.
16. Boulet LP, Turcotte H, Laviolette M, Naud F, Bernier MC, Martel S, Chakir J. Airway hyperresponsiveness, inflammation, and subepithelial collagen deposition in recently diagnosed versus long-standing mild asthma. Influence of inhaled corticosteroids. American journal of respiratory and critical care medicine 2000; **162**:1308-13.
17. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. American journal of respiratory and critical care medicine 2000; **161**:1720-45.
18. Khan MA. Inflammation signals airway smooth muscle cell proliferation in asthma pathogenesis. Multidiscip Respir Med 2013; **8**:11.
19. Khan MA, Assiri AM, Broering DC. Complement mediators: key regulators of airway tissue remodeling in asthma. J Transl Med 2015; **13**:272.
20. Shifren A, Witt C, Christie C, Castro M. Mechanisms of remodeling in asthmatic airways. J Allergy 2012; **2012**.
21. An SS, Bai TR, Bates JH, Black JL, Brown RH, Brusasco V, Chitano P, Deng L, Dowell M, Eidelman DH, Fabry B, Fairbank NJ, Ford LE, Fredberg JJ, Gerthoffer WT, Gilbert SH, Gosens R, Gunst SJ, Halayko AJ, Ingram RH, Irvin CG, James AL, Janssen LJ, King GG, Knight DA, Lauzon AM, Lakser OJ, Ludwig MS, Lutchen KR, Maksym GN, Martin JG, Mauad T, McParland BE, Mijailovich SM, Mitchell HW, Mitchell RW, Mitzner W, Murphy TM, Pare PD, Pellegrino R, Sanderson MJ, Schellenberg RR, Seow CY, Silveira PS, Smith PG, Solway J, Stephens NL, Sterk PJ, Stewart AG, Tang DD, Tepper RS, Tran T, Wang L. Airway smooth muscle dynamics: a common pathway of airway obstruction in asthma. Eur Respir J 2007; **29**:834-60.
22. Bergeron C, Tulic MK, Hamid Q. Airway remodelling in asthma: from benchside to clinical practice. Can Respir J 2010; **17**:e85-93.

23. Cobbold SP, Graca L, Lin CY, Adams E, Waldmann H. Regulatory T cells in the induction and maintenance of peripheral transplantation tolerance. Transplant international : official journal of the European Society for Organ Transplantation 2003; **16**:66-75.
24. Fehervari Z, Sakaguchi S. CD4+ Tregs and immune control. The Journal of clinical investigation 2004; **114**:1209-17.
25. Khan MA. T regulatory cell mediated immunotherapy for solid organ transplantation: A clinical perspective. Mol Med 2016; **22**.
26. Khan MA, Alanazi F, Ahmed HA, Al-Mohanna FH, Assiri AM, Broering DC. FOXP3+ regulatory T cell ameliorates microvasculature in the rejection of mouse orthotopic tracheal transplants. Clin Immunol 2017; **174**:84-98.
27. Khan MA, Alanazi F, Ahmed HA, Vater A, Assiri AM, Broering DC. C5a Blockade Increases Regulatory T Cell Numbers and Protects Against Microvascular Loss and Epithelial Damage in Mouse Airway Allografts. Frontiers in Immunology 2018; **9**.
28. Khan MA, Moeez S, Akhtar S. T-regulatory cell-mediated immune tolerance as a potential immunotherapeutic strategy to facilitate graft survival. Blood Transfus 2013; **11**:357-63.
29. Khan MA, Shamma T. Complement factor and T-cell interactions during alloimmune inflammation in transplantation. J Leukoc Biol 2018.
30. Lloyd CM, Hawrylowicz CM. Regulatory T Cells in Asthma. Immunity 2009; **31**:438-49.
31. Bilate AM, Lafaille JJ. Induced CD4+Foxp3+ regulatory T cells in immune tolerance. Annu Rev Immunol 2012; **30**:733-58.
32. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol 2012; **30**:531-64.
33. Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol 2005; **6**:345-52.
34. Marek-Trzonkowska N, Mysliwiec M, Dobyszuk A, Grabowska M, Techmanska I, Juscinska J, Wujtewicz MA, Witkowski P, Mlynarski W, Balcerska A, Mysliwska J, Trzonkowski P. Administration of CD4+CD25highCD127-regulatory T cells preserves beta-cell function in type 1 diabetes in children. Diabetes Care 2012; **35**:1817-20.
35. Lundgren A, Stromberg E, Sjoling A, Lindholm C, Enarsson K, Edebo A, Johnsson E, Suri-Payer E, Larsson P, Rudin A, Svennerholm AM, Lundin BS. Mucosal FOXP3-expressing CD4+ CD25high regulatory T cells in Helicobacter pylori-infected patients. Infect Immun 2005; **73**:523-31.
36. Baru AM, Hartl A, Lahl K, Krishnaswamy JK, Fehrenbach H, Yildirim AO, Garn H, Renz H, Behrens GM, Sparwasser T. Selective depletion of Foxp3+ Treg during sensitization phase aggravates experimental allergic airway inflammation. European journal of immunology 2010; **40**.
37. Baru AM, Ganesh V, Krishnaswamy JK, Hesse C, Untucht C, Glage S, Behrens G, Mayer CT, Puttur F, Sparwasser T. Absence of Foxp3(+) Regulatory T Cells during Allergen Provocation Does Not Exacerbate Murine Allergic Airway Inflammation. PLoS ONE 2012; **7**:e47102.
38. Xu W, Lan Q, Chen M, Chen H, Zhu N, Zhou X, Wang J, Fan H, Yan C-S, Kuang J-L, Warburton D, Togbe D, Ryffel B, Zheng S-G, Shi W. Adoptive Transfer of Induced-Treg Cells Effectively Attenuates Murine Airway Allergic Inflammation. PLOS ONE 2012; **7**:e40314.
39. Lewkowich IP, Herman NS, Schleifer KW, Dance MP, Chen BL, Dienger KM, Sproles AA, Shah JS, Kohl J, Belkaid Y, Wills-Karp M. CD4+CD25+ T cells protect against experimentally induced asthma and alter pulmonary dendritic cell phenotype and function. J Exp Med 2005; **202**:1549-61.
40. Hawrylowicz CM. Regulatory T cells and IL-10 in allergic inflammation. The Journal of Experimental Medicine 2005; **202**:1459-63.
41. Kearley J, Barker JE, Robinson DS, Lloyd CM. Resolution of airway inflammation and hyperreactivity after in vivo transfer of CD4+CD25+ regulatory T cells is interleukin 10 dependent. J Exp Med 2005; **202**:1539-47.
42. Kearley J, Robinson DS, Lloyd CM. CD4+CD25+ regulatory T cells reverse established allergic airway inflammation and prevent airway remodeling. The Journal of allergy and clinical immunology 2008; **122**:617-24 e6.
43. Joetham A, Takeda K, Okamoto M, Taube C, Matsuda H, Dakhama A, Gelfand EW. Antigen specificity is not required for modulation of lung allergic responses by naturally occurring regulatory T cells. Journal of immunology (Baltimore, Md : 1950) 2009; **183**:1821-7.
44. Joetham A, Takeda K, Taube C, Miyahara N, Matsubara S, Koya T, Rha YH, Dakhama A, Gelfand EW. Naturally occurring lung CD4(+)CD25(+) T cell regulation of airway allergic responses depends on IL-10 induction of TGF-beta. Journal of immunology (Baltimore, Md : 1950) 2007; **178**:1433-42.

45. Matsuda M, Terada T, Kitatani K, Kawata R, Nabe T. [Analyses of Foxp3(+) Treg cells and Tr1 cells in subcutaneous immunotherapy-treated allergic individuals in humans and mice]. Nihon Yakurigaku Zasshi 2019; **154**:17-22.
46. Tortola L, Pawelski H, Sonar SS, Ampenberger F, Kurrer M, Kopf M. IL-21 promotes allergic airway inflammation by driving apoptosis of FoxP3(+) regulatory T cells. The Journal of allergy and clinical immunology 2019; **143**:2178-89 e5.
47. He YT, Zhou Y, Shao Q, Gan C, Chen M, Bao YL, Gu HY, Zhang SL, Cui Y, Tian M. Immunoregulatory Effects of Subcutaneous Immunotherapy on Lymphocyte Subgroups and Cytokines in Children with Asthma. J Immunol Res 2019; **2019**:7024905.
48. Gorczynski RM, Maqbool T, Hoffmann G. Mechanism(s) of prolonged attenuation of allergic responses after modulation of idiotypic regulatory network. Allergy Asthma Clin Immunol 2019; **15**:79.
49. Zhang H, Kong H, Zeng X, Guo L, Sun X, He S. Subsets of regulatory T cells and their roles in allergy. Journal of Translational Medicine 2014; **12**:125.
50. Larche M. Regulatory T cells in allergy and asthma. Chest 2007; **132**.
51. Zhang H, Kong H, Zeng X, Guo L, Sun X, He S. Subsets of regulatory T cells and their roles in allergy. J Transl Med 2014; **12**:125.
52. Böhm L, Maxeiner J, Meyer-Martin H, Reuter S, Finotto S, Klein M, Schild H, Schmitt E, Bopp T, Taube C. IL-10 and Regulatory T Cells Cooperate in Allergen-Specific Immunotherapy To Ameliorate Allergic Asthma. The Journal of Immunology 2015; **194**:887-97.
53. Schreiber TH, Wolf D, Tsai MS, Chirinos J, Deyev VV, Gonzalez L, Malek TR, Levy RB, Podack ER. Therapeutic Treg expansion in mice by TNFRSF25 prevents allergic lung inflammation. The Journal of clinical investigation 2010; **120**:3629-40.
54. Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Ruckert B, Mantel PY, Menz G, Akdis CA, Blaser K, Schmidt-Weber CB. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. The Journal of allergy and clinical immunology 2004; **114**:1425-33.
55. Kohl J, Baelder R, Lewkowich IP, Pandey MK, Hawlisch H, Wang L, Best J, Herman NS, Sproles AA, Zwirner J, Whitsett JA, Gerard C, Sfyroera G, Lambris JD, Wills-Karp M. A regulatory role for the C5a anaphylatoxin in type 2 immunity in asthma. The Journal of clinical investigation 2006; **116**:783-96.
56. Palomares O, Yaman G, Azkur AK, Akkoc T, Akdis M, Akdis CA. Role of Treg in immune regulation of allergic diseases. European journal of immunology 2010; **40**:1232-40.
57. Verhagen J, Blaser K, Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy: T-regulatory cells and more. Immunology and allergy clinics of North America 2006; **26**:207-31, vi.
58. Issa F, Wood KJ. CD4+ regulatory T cells in solid organ transplantation. Curr Opin Organ Transplant 2010.
59. Taylor A, Verhagen J, Blaser K, Akdis M, Akdis CA. Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: the role of T regulatory cells. Immunology 2006; **117**:433-42.
60. Murdoch JR, Lloyd CM. Chronic inflammation and asthma. Mutation Research 2010; **690**:24-39.
61. Sziksz E, Pap D, Lippai R, Beres NJ, Fekete A, Szabo AJ, Vannay A. Fibrosis Related Inflammatory Mediators: Role of the IL-10 Cytokine Family. Mediators of inflammation 2015; **2015**:764641.
62. Thorburn AN, Hansbro PM. Harnessing Regulatory T cells to Suppress Asthma: From Potential to Therapy. American journal of respiratory cell and molecular biology 2010; **43**:511-9.
63. Chung F. Anti-inflammatory cytokines in asthma and allergy: interleukin-10, interleukin-12, interferon-gamma. Mediators of inflammation 2001; **10**:51-9.
64. Chung KF, Barnes PJ. Cytokines in asthma. Thorax 1999; **54**:825-57.
65. Provoost S, Maes T, van Durme YM, Gevaert P, Bachert C, Schmidt-Weber CB, Brusselle GG, Joos GF, Tournoy KG. Decreased FOXP3 protein expression in patients with asthma. Allergy 2009; **64**:1539-46.
66. Krogulska A, Polakowska E, Wasowska-Krolikowska K, Malachowska B, Mlynarski W, Borowiec M. Decreased FOXP3 mRNA expression in children with atopic asthma and IgE-mediated food allergy. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology 2015; **115**:415-21.
67. Carson WF, Guernsey LA, Singh A, Vella AT, Schramm CM, Thrall RS. Accumulation of regulatory T cells in local draining lymph nodes of the lung correlates with spontaneous resolution of chronic asthma in a murine model. Int Arch Allergy Immunol 2008; **145**.

68. Burchell JT, Wikstrom ME, Stumbles PA, Sly PD, Turner DJ. Attenuation of allergen-induced airway hyperresponsiveness is mediated by airway regulatory T cells. American Journal of Physiology - Lung Cellular and Molecular Physiology 2009; **296**:L307-L19.
69. Hara M, Kingsley CI, Niimi M, Read S, Turvey SE, Bushell AR, Morris PJ, Powrie F, Wood KJ. IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo. Journal of immunology (Baltimore, Md : 1950) 2001; **166**:3789-96.
70. Bharat A, Fields RC, Steward N, Trulock EP, Patterson GA, Mohanakumar T. CD4+25+ regulatory T cells limit Th1-autoimmunity by inducing IL-10 producing T cells following human lung transplantation. Am J Transplant 2006; **6**:1799-808.
71. Hsu P, Santner-Nanan B, Hu M, Skarratt K, Lee CH, Stormon M, Wong M, Fuller SJ, Nanan R. IL-10 Potentiates Differentiation of Human Induced Regulatory T Cells via STAT3 and Foxo1. Journal of immunology (Baltimore, Md : 1950) 2015; **195**:3665-74.
72. Ng THS, Britton GJ, Hill EV, Verhagen J, Burton BR, Wraith DC. Regulation of Adaptive Immunity; The Role of Interleukin-10. Frontiers in Immunology 2013; **4**:129.
73. O'Garra A, Barrat FJ, Castro AG, Vicari A, Hawrylowicz C. Strategies for use of IL-10 or its antagonists in human disease. Immunological reviews 2008; **223**:114-31.
74. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. The World Allergy Organization Journal 2015; **8**:17.
75. Taams LS, Palmer DB, Akbar AN, Robinson DS, Brown Z, Hawrylowicz CM. Regulatory T cells in human disease and their potential for therapeutic manipulation. Immunology 2006; **118**:1-9.
76. Leech MD, Benson RA, De Vries A, Fitch PM, Howie SE. Resolution of Der p1-induced allergic airway inflammation is dependent on CD4+CD25+Foxp3+ regulatory cells. Journal of immunology (Baltimore, Md : 1950) 2007; **179**:7050-8.
77. Lim S, Caramori G, Tomita K, Jazrawi E, Oates T, Chung KF, Barnes PJ, Adcock IM. Differential expression of IL-10 receptor by epithelial cells and alveolar macrophages. Allergy 2004; **59**:505-14.
78. Peek EJ, Richards DF, Faith A, Lavender P, Lee TH, Corrigan CJ, Hawrylowicz CM. Interleukin-10-secreting "regulatory" T cells induced by glucocorticoids and beta2-agonists. American journal of respiratory cell and molecular biology 2005; **33**:105-11.
79. Hartl D, Koller B, Mehlhorn AT, Reinhardt D, Nicolai T, Schendel DJ, Griese M, Krauss-Etschmann S. Quantitative and functional impairment of pulmonary CD4+CD25hi regulatory T cells in pediatric asthma. The Journal of allergy and clinical immunology 2007; **119**:1258-66.
80. Neujahr DC, Cardona AC, Ulukpo O, Rigby M, Pelaez A, Ramirez A, Gal AA, Force SD, Lawrence EC, Kirk AD, Larsen CP. Dynamics of Human Regulatory T Cells in Lung Lavages of Lung Transplant Recipients. Transplantation 2009; **88**:521-7.
81. Maneechotesuwan K, Kasetsinsombat K, Wamanuttajinda V, Wongkajornsilp A, Barnes PJ. Statins enhance the effects of corticosteroids on the balance between regulatory T cells and Th17 cells. Clinical & Experimental Allergy 2013; **43**:212-22.
82. Sbiera S, Dexneit T, Reichardt SD, Michel KD, van den Brandt J, Schmull S, Kraus L, Beyer M, Mlynski R, Wortmann S, Allolio B, Reichardt HM, Fassnacht M. Influence of Short-Term Glucocorticoid Therapy on Regulatory T Cells In Vivo. PLOS ONE 2011; **6**:e24345.
83. Faustino L, Mucida D, Keller AC, Demengeot J, Bortoluci K, Sardinha LR, Carla Takenaka M, Basso AS, Faria AMC, Russo M. Regulatory T Cells Accumulate in the Lung Allergic Inflammation and Efficiently Suppress T-Cell Proliferation but Not Th2 Cytokine Production. Clinical and Developmental Immunology 2012; **2012**:13.
84. Jacob M, Chappell D, Becker BF. Regulation of blood flow and volume exchange across the microcirculation. Critical Care 2016; **20**:319.
85. Siafakas NM, Antoniou KM, Tzortzaki EG. Role of angiogenesis and vascular remodeling in chronic obstructive pulmonary disease. International Journal of Chronic Obstructive Pulmonary Disease 2007; **2**:453-62.
86. Voelkel NF, Gomez-Arroyo J, Mizuno S. COPD/emphysema: The vascular story. Pulmonary Circulation 2011; **1**:320-6.
87. Orihara K, Matsuda A. Pathophysiological roles of microvascular alterations in pulmonary inflammatory diseases: possible implications of tumor necrosis factor-alpha and CXC chemokines. International Journal of Chronic Obstructive Pulmonary Disease 2008; **3**:619-27.

88. Zanini A, Chetta A, Imperatori AS, Spanevello A, Olivieri D. The role of the bronchial microvasculature in the airway remodelling in asthma and COPD. Respiratory Research 2010; **11**:132-.
89. Kupczyk M, Dahlen B, Dahlen SE. Which anti-inflammatory drug should we use in asthma? Polskie Archiwum Medycyny Wewnetrznej 2011; **121**:455-9.
90. Dahl R. Systemic side effects of inhaled corticosteroids in patients with asthma. Respiratory medicine 2006; **100**:1307-17.


IL-10, TGF-β, IL-35
Granzyme, Perforin
T regulatory cell
B cell
IgE
IL-10, TGF-β, IL-35
Granzyme, Perforin
IL-4, & 6
IL-,3,4,6,9 & 13
Mast cell
IL-16
IL-5
IL-4, 5 & 13
Th2
Eosinophil
Basophil
T regulatory cell
IL-10, TGF-β, IL-35
Granzyme, Perforin
Eotaxins, IL-2
Th1
INF-γ, TNF-α
Neutrophil
INF-γ, TNF-α, IL-1β
M1
ASTHMA
Airway Inflammation
Epithelial injury
Bronchoconstriction,
AHR, Mucus secretion,