The Role of Cytokines in Atopic Asthma

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Abstract: Atopic asthma results from airway inflammation triggered by an environmental allergen. Symptoms include wheezing, dyspnea and cough, airway narrowing and/or hyperresponsiveness to several inhaled stimuli. Inflammation develops in a two-phase fashion. The first phase after exposure to the allergen consists of degranulation and release of both histamine and other stored preformed inflammatory mediators as well as newly synthesized ones, including cytokines, all of which increase mucus secretion and smooth muscle contraction. The second phase occurs later and lasts longer; it is due to different molecules: several cytokines and chemokines, arachidonic acid derivatives, enzymes such as metalloproteinases and cell adhesion molecules. Cytokines are key players in the chronic inflammation in asthma patients, but details on their role and interactions still remain undetermined. Recent evidence suggests that allergic asthma is a multifaceted condition actively controlled by effector as well as regulatory T cells (Tregs). T helper (Th) 2 cells and Th17 cells increase airway inflammation, while Tregs are anti- inflammatory. Cytokines are involved in the development and activation of all T cell subpopulations. They are also involved directly or indirectly in most approaches to asthma treatment. Several cytokines have been tested as therapeutic targets and some of the currently used therapies like corticosteroids, beta agonists and allergen immunotherapy affect cytokine production. The increased knowledge on cytokine interplay and lymphocyte subsets should generate new therapeutic strategies in the near future.

Keywords: Airway hyperresponsiveness, asthma therapy, atopic asthma, CD4+ Tcell subsets, cytokines, Th2 cells.

ATOPIC ASTHMA

Asthma is a serious and sometimes life-threatening condition that affects an estimated 300 million individuals worldwide [1]. It is a chronic inflammatory disorder of the airways in which many cells and molecular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning [2]. Acute exacerbations take place in asthma patients, associated to an increase of airway inflammation, increased levels of inflammatory factors and higher numbers of infiltrating cells. All symptoms such as dyspnea, sibilance, cough and chest oppression [3] are exacerbated. More than 100 genes have been associated to asthma but none of them contributes more than 5% to the observed pattern [4]. Atopic asthma is the most common form of the disease with airway hyperresponsiveness to a number of inhaled stimuli. The inflammation pattern in the airways both in allergic asthma and in non-allergic asthma presents a similar expression of inflammatory mediators, including Th2 cytokines and eosinophilotactic chemokines and increased T helper (Th) 2 cells. In atopic asthmatic patients the inflammatory process presents itself in a two- phase fashion. When allergen induces cross-linking of IgE- FcεR1 complexes on the tissue resident mast cell surface, the early phase of allergic reaction occurs within minutes. It involves mast cell triggering and degranulation, with release of histamine, synthesis of lipid mediators, and release of a variety of granule-stored and newly formed mediators which provoke an increase in mucus secretion and smooth muscle contraction. After this activation, mast cells secrete cytokines and chemokines (cytokines with chemotactic properties), thus inducing the recruitment and activation of inflammatory cells, in particular eosinophils, into the inflamed tissue [5-7]. This results in the second or late phase which lasts over time. Some authors have described different phenotypes of asthma [8, 9], defining two groups of patients: those with Th2-high and those with Th2-low asthma based on epithelial cell gene signatures for the activity of Th2 cytokines and according to the Th2 inflammation degree [8]. A significant percentage of patients with the Th2-low phenotype present clinical features of asthma: airway obstruction, airway hyperresponsiveness, and bronchodilator reversibility despite a paucity of Th2-driven inflammation. This may be caused by neutrophilic inflammation, IL-17–driven inflammation, intrinsic defects in barrier function and chronic subclinical infection by viruses, and atypical intracellular bacteria [8].

Bronchial hyperresponsiveness (BHR), typical of asthma, results from increased sensitivity of the airways to physical or chemical stimuli. A novel susceptibility gene for BHR, Protocadherin-1 (PCDH1), expressed as an adhesion molecule in airway epithelial cells and macrophages, has been recently described, suggesting that loss of integrity in the airway epithelium, the barrier for inhaled substances, contributes to development of BHR [10]. Mechanisms related to the function of this gene contribute to susceptibility to BHR, considered by itself as an intermediate phenotype of asthma. This condition may evolve to asthma and assigns to PCDH1 a possible role in asthma pathogenesis [10]. Therefore, different forms of asthma may be produced by different mechanisms, some involving the epithelium [11].

Airway remodeling is one of the features of chronic asthma. It involves structural changes like peribronchial fibrosis, epithelial mucus metaplasia, enlargement of airway smooth muscle mass and angiogenesis, caused by cytokines and growth factors.

Angiogenesis, the formation of new blood vessels from pre existing ones, plays an important role in tissue remodeling in asthma [12]. The increase in number and size of vessels can contribute to narrowing of the bronchial lumen leading to an amplification of BHR [13]. Th2 cytokines modulate the synthesis and release of vascular endothelial growth factor (VEGF) secreted by different structural and inflammatory cells of the airways. Broncheoalveolar lavage fluid (BALF) from asthmatic individuals has increased angiogenic activity *in vitro* and contains angiogenin, fibroblast growth factor 2 and VEGF. An imbalance in favor of pro-angiogenic factors generates an abnormal growth of new vessels in asthma [14].

CELLS INVOLVED IN ASTHMA

The immunological pathogenesis of asthma is complex. Both innate and adaptive immune responses are involved. Epithelial cells are disrupted and activated, cytokines are produced, dendritic cells (DCs) and other antigen presenting cells (APC) are activated. Upon activation Th2 cells proliferate and secrete cytokines; mast cells are also activated and other inflammatory cells, such as eosinophils, basophils, neutrophils and lymphocytes, are recruited from periph-

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eral blood. All of them produce several cytokines, chemokines or release granule contents such as histamine or compounds with enzymatic activity [5].

The characteristic pattern of inflammation present in allergic asthma results from IgE triggered resident mast cells and the influx of basophils and eosinophils, cells that have an important role in allergies and asthma, into the inflamed airways. Basophils are a minor component of peripheral blood leukocytes that express the high affinity receptor for IgE-FcεR1 on their surface. They are recruited to the sites of allergic inflammation [15, 16]. It was recently described that, besides releasing histamine and leukotriene C4 in response to stimulation, they can release Th2 cytokines such as interleukin (IL)-4 and IL-13 in large quantities [17]. Basophils are regulators of Th2 responses in helminth-infected hosts and in allergen-injected animals. Recently, it has been shown that basophils are antigen-presenting cells (APC), which induce Th2 cells both *in vitro* and *in vivo* [18]. They also stimulate IgE synthesis *in vitro* [19, 20], while mast cells participate as part of the immune response to bacteria and virus, and can also have regulatory activity [17]. Both mast cells and basophils synthesize and release VEGF [13] (Fig. **1**).

Infiltration and subsequent activation of eosinophils in the inflamed airways is a feature of allergic asthma [21]. Eosinophils cause tissue damage through the release of granule-associated cationic protein major basic protein (MBP) and eosinophil derived neurotoxin [22]. These cells also produce transforming growth factor (TGF) β and hence modulate fibrosis in asthma [7]. Eosinophils are considered responsible for immune regulation and remodeling in the lung of asthmatic subjects. Besides the liberation of active proteins from their granules, lipid mediators, cytokines, growth factors, and reactive oxygen species, they elicit chronic Th2 inflammation in the lung by cellular interactions [22].

Recent evidence suggests the participation of several cell types in airway inflammation, including smooth muscle cells and epithelial cells. Airway smooth muscle cells can originate airway constriction and inflammation, and are able to respond to a variety of stimuli like histamine, inflammatory cytokines, bradykinin, acetylcholine and cyclic stretch, responding by secreting inflammatory cytokines, chemokines and other bioactive mediators [23]. Epithelial cells have a central role in the pathogenesis of asthma [24]. Receptors of the innate immune system such as Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors and retinoic acid –inducible gene receptors are expressed on airway epithelial cells and on DC. These receptors recognize surface characteristics of pathogens. Activation of airway epithelial cells with TLR agonists induces the expression of cytokines, chemokines, complement proteins and signaling molecules [25]. Asthma has been defined as "a chronic wound of the airways" [24] where an abnormally functioning epithelium is the source of a variety of cytokines, chemokines and autacoid mediators.

In asthma, activated epithelial and other tissue cells secrete cytokines, chemokines and other mediators with activity on cells of

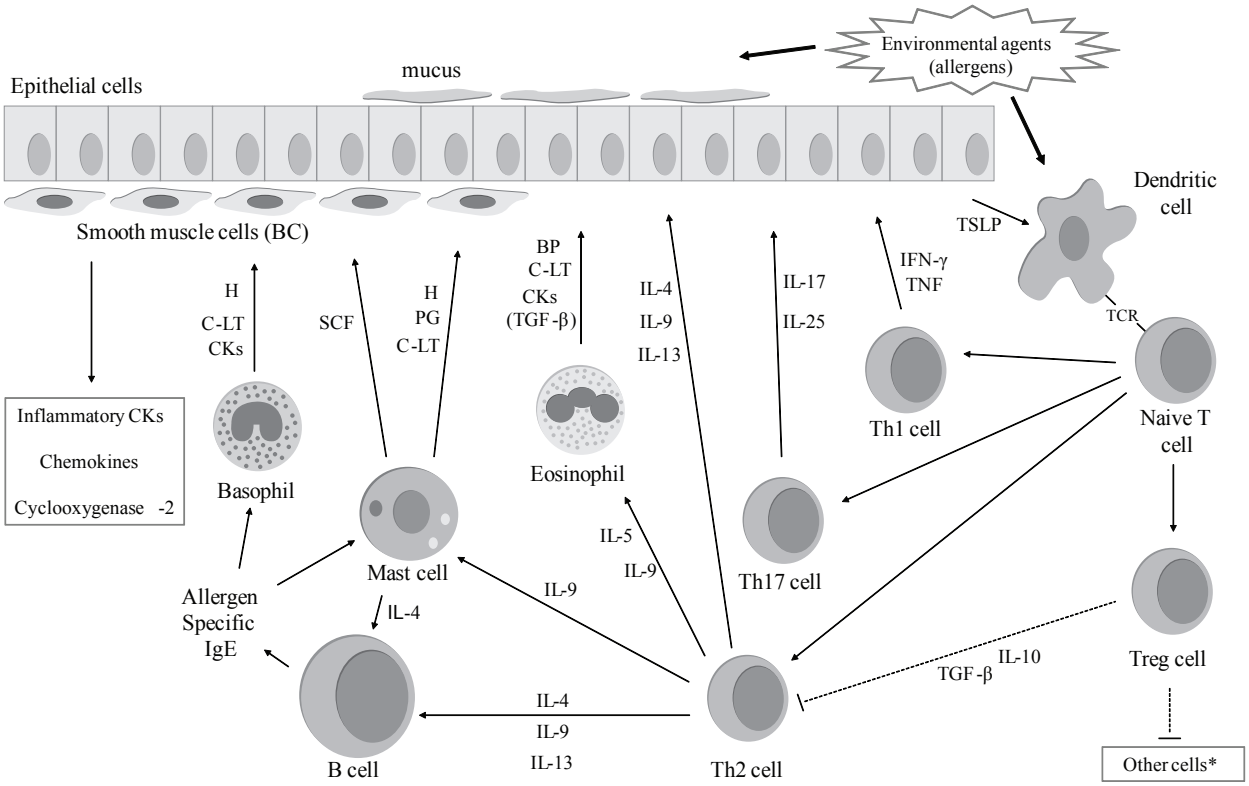

Fig. (1). Cells involved in asthma.

Several cell types participate in airway inflammation, including epithelial cells and involving both innate and adaptive immune responses. Inhaled allergens disrupt and activate epithelial cells, cytokines (CKs) are produced, DCs and other APC are also activated, then Th2 cells are activated, they proliferate and secrete cytokines. Mast cells are activated in turn, they secrete histamine (H) and lipid mediators such as cysteinyl-leukotrienes (C-LT) and prostaglandins (PG), and other inflammatory cells, such as eosinophils, basophils, neutrophils and lymphocytes, are recruited from peripheral blood. Histamine, cysteinyl-leukotrienes and several cytokines induce bronchoconstriction (BC) in the smooth muscle cells underlying the airways. Th2 cells secrete IL-4, IL-5, IL-9, and IL-13; inducing eosinophilia, the production of allergen-specific IgE, mucus hypersecretion and permissiveness of endothelium for the recruitment of inflammatory cells. Th1 cells secrete IFNγ and TNFα and induce epithelial apoptosis generating shedding of these cells. Th17 cells have been shown to increase airway inflammation, while Treg cells have anti-inflammatory activity. They inhibit Th2 cells and other cells*. Other cells*: Th1 cells, Th17 cells, B cells, dendritic cells, eosinophils, basophils and mast cells. B cells produce allergen-specific IgE that binds to the surface of mast cells and basophils.

the innate immune system: DCs, basophils, eosinophils and mast cells as well as on B and T cells (adaptive immune system).

In asthma and other allergic conditions, B cells are important because they are a source of allergen-specific IgE, which may be produced locally in the airways [26]. This immunoglobulin then binds to the high affinity receptor FcεRI present on the surface of mast cells and basophils, and also to the low affinity receptor FcεRI expressed by other inflammatory cells such as B cells, macrophages and possibly eosinophils [27]. In healthy individuals the allergen specific antibody response may be not detectable or may be composed of IgG4, IgG1 and IgA, with low or no IgE.

DCs regulate Th2 cells and present processed peptides from inhaled allergens to them [28, 29]. They are associated to the chronic inflammation of the lungs in asthma patients. Epithelial cells and mast cells of these patients secrete large quantities of thymic stromal lymphopoietin (TSLP), which stimulates maturation of myeloid DCs and induces the release of TARC (CCL17) and MDC (CCL22), which bind the receptor CCR4 expressed by Th2 cells, and hence recruits these cells to the airways [30].

It has been considered that the immune system has inducible peripheral mechanisms of tolerance to allergens. DCs act as antigen presenting cells but also have a tolerogenic function. Several reports support a role of DCs in the induction of different T regulatory (Treg) cell subsets, resulting in the maintenance of immunotolerance to tumor antigens, microbial persistence and limitation of collateral tissue damage [31, 32]. Immature DCs control peripheral tolerance by the induction of Treg cells [31]. Airway DCs control the pulmonary immune response, and decide immunity as well as tolerance to newly encountered antigens. Immature DC can be found throughout the lungs, they capture allergens and migrate to lymph nodes where T cells are, inducing T-cell tolerance [33].

An increase in CD8+ T cells in the airways after allergen challenge has been reported by several authors, but their role is still unknown. CD8+ T cells are present in patients with the most severe form of asthma, and their phenotype may be Th1 as well as Th2. There are also contradicting reports on the percentage of natural killer (NK)T cells in the broncheoalveolar lavage of asthmatic patients, and their role has not been elucidated [34, 35].

CYTOKINES IN ASTHMA

Cytokines are key players in the chronic inflammation observed in asthma patients. While allergic airway responses have been associated to Th2 cytokines long time ago, their relationship was demonstrated by using animal models: gene knock-out mice, antibody neutralization and experiments involving T cell depletion and adoptive transfer [36]. IL-4 and IL -13 are Th2 cytokines overproduced in clinical asthma and are essential for allergic airway inflammation in mouse models. IL-4 displays an important role in Th2 differentiation and acts, together with IL-13, on B cells promoting isotype switching from IgM to IgE, while regulating the FcεR expression on mast cells and basophils. It can also contribute to goblet cell hyperplasia [5]. IL-4 is responsible for optimal stimulation of B cells by antigen and also stimulates the expression of MHC Class II molecules and the low affinity IgE receptor among other surface antigens in these cells, increasing the antigen presenting capacity of B cells. It also promotes Th2 responses by modulating the differentiation of T cells. Activation of the IL-4 receptor regulates STAT-6, this factor then regulates GATA-3 expression in T cells. The number of GATA-3 + T cells in the airways is increased in asthma patients. When antigen stimulation occurs, mitogen activated protein kinase (MAPK) p38 phosphorylates and activates the T cell GATA-3, which translocates to the nucleus and activates gene transcription [37].

IL-5 drives the differentiation, maturation and passage to peripheral blood of eosinophils, as well as their activation and survival [38, 39]. These cells secrete TGFβ among other mediators, favoring remodeling of the airways. Animal model studies have shown that neither IL-4 nor IL-5 is required for airway hyperresponsiveness [36].

IL-9 has T cell growth factor activity, it supports antigen independent T cell proliferation, it induces in B cells an increase in IgE and IgG production, enhancing the IL-4 mediated IgE production, and increases expression of FcεR [40], it promotes mast cell proliferation, survival and release of inflammatory cytokines and eosinophil maturation, activation and survival [41], it contributes to goblet cell hyperplasia and to other aspects of airway remodeling [42]. IL-9 also plays a key role in the development of virus-specific Th2 responses [43]. Levels of IL-9 mRNA and IL-9R are elevated in allergic asthma [44]. IL-9 is increased in the BALF of asthmatic subjects after allergen challenge and plays a role in airway remodeling and in epithelial mucus production [45]. This cytokine can derive from T cells, eosinophils, mast cells and structural cells, probably from Th2 cells at the initiation of the process. It can increase the release of IL-8 from neutrophils and smooth muscle cells, and together with IL-17 it induces neutrophil accumulation in the airways. Hence IL-9 plays a role in the chronicity of asthma [41].

A significantly lower level of IL-10 has been observed in the lungs of asthma patients than in those of control subjects [46], and polymorphisms in IL-10 have been associated to more severe asthma [47]. When partially mature airway DCs expressing IL-10 present antigen, inducible type 1 Treg (Tr1)-like cells are induced, inhibiting inflammatory responses thereafter [48]. IL-10 producing antigen-presenting cells, such as DCs and B cells and clonally expanded IL-10 producing allergen-specific Tr1 cells all contribute to IL-10 mediated suppression [31]. IL-10 is a fundamental suppressive cytokine that inhibits T-cell proliferation in response to several allergens, without suppressing proliferation induced with anti-CD3. This suppression is exerted by blocking CD2, CD28 and inducible costimulator (ICOS) in the signal transduction cascade [49]. This suppressive effect is not observed in other IL-10 family cytokines like IL-19, IL-20, IL-22 and IL-24 [50]. Besides T cells, other cells such as monocytes and macrophages are susceptible of inhibition by IL-10. This cytokine suppresses costimulatory molecules and downregulates MHC class II molecules and APC capacity.

Taking into account the fundamental role of IL-10 in the regulation of asthma, we evaluated IL-10 in the sera of pediatric atopic asthmatic patients (Fig. **2**), both in steady state and crisis or exacerbation, and normal controls. No difference was observed in the serum concentration of IL-10 when patients were compared in crisis and steady state. This can be due to the fact that the serum concentration represents the accumulation of cytokine in the period previous to the collection of sample and patients in crisis are evaluated at the beginning of it, before medication is provided. A tendency to higher values, although non significant, is observed when asthma patients are compared to healthy controls. Other authors have observed similar serum levels in children in crisis and controls, but much lower levels in patients in stable condition [51]. The difference with our data might be explained because the group of children they studied has a much lower mean age.

IL-13 shares with IL-4 the α chain of its receptor, having some redundant activities. It promotes isotype switching to IgE, but it also acts on epithelial cell maturation, mucus production and goblet cell hyperplasia, it enhances contractility of airway smooth muscle cells and is involved in the recruitment of monocytes, macrophages, T cells and eosinophils [52]. The IL-4/IL-13 system is involved in the development of the allergic phenotype, but these cytokines seem to play a minor role in established allergy in a mouse model of allergic asthma [53].

Elevated concentrations of IL-17 have been reported in the sputum of asthmatic patients [54] and in severe asthma this CK induces neutrophil inflammation. IL-17 as well as IL-17F, another member

of the IL-17 family, induce the release of the chemokines CXCL1 and CXCL8 from airways epithelial cells and hence have been associated to neutrophilic inflammation [27]. IL-17 decreases tissue eosinophil recruitment and bronchial hyperreactivity, while it increases neutrophil infiltration and mucous proteins [55]. This cytokine has also been described as responsible for steroid-resistant phenotype in asthma.

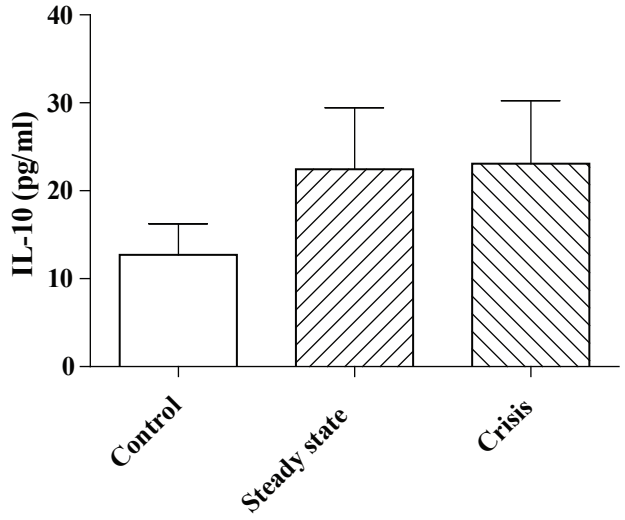

Fig. (2). Serum concentration of IL-10.

The concentration of IL-10 in sera of pediatric atopic asthmatic patients (7-16 years old), both in steady state and in crisis, and of normal controls was determined by a commercial ELISA kit (e-Bioscience, USA, sensitivity: 2pg/ml). No significant differences between asthmatic patients and normal controls were observed. Data are presented as mean $\pm$ standard error of the mean (SEM) and significance was analyzed by unpaired t test.

IL-22 belongs to the IL-10 cytokine family but its signaling is independent of IL-10. Its receptor is expressed exclusively on non-immune cells, so its activity, like that of IL-17, is exerted primarily on tissue cells [56]. IL-22, which levels increase with the severity of asthma [57], stimulates the production of IL-10 and acute phase proteins [56]. It is an important effector molecule of activated Th22, Th1, and Th17 cells, as well as cytotoxic T cell subsets, γδ T cells, NK and NKT cells. It mainly acts on epithelial cells and hepatocytes, where it favors the antimicrobial defense, regeneration, and protection against damage and induces acute phase reactants and some chemokines [56]. It synergizes with IL-17 in the induction of proinflammatory cytokines in human bronchial epithelial cells. On the other hand, it induces the secretion of antimicrobial molecules in lung epithelial cells in Gram-negative bacterial pneumonia, having a protective effect [58].

IL-25 generates Th2 mediated inflammatory responses in animal models. In humans, it is produced mainly by eosinophils and basophils, and acts on Th2 memory cells [55]. Taking all these data into account, both IL-17 and IL-25 may be potential therapeutic targets in asthma.

IL-33, a new member of the IL-1 cytokine family, is also involved in allergic inflammation [5]: it activates basophils to secrete IL-4, IL-13 and IL-8, and enhances FcεR1 mediator release and cytokine production. IL-33 expression is elevated in bronchial biopsies of asthmatic patients. This cytokine has been proposed as a novel inflammatory marker of severe and refractory asthma [59].

TSLP affects the expression of OX-40 Ligand, a Th2 polarizing signal, in an animal model [5]. It induces the secretion of IL-5, IL-13 and several chemokines in cord blood derived mast cells and also activates human eosinophils.

It has been described that TGFβ has a role in the pathogenesis of asthma in the remodeling of lung tissue because of its profibrotic activity [42, 60]. Evidence to support this has been obtained with mice deficient in Smad-3. Smad signal transducers are required for TGFβ-mediated developmental events in many organisms including humans, through ligand-induced activation of TGFβ receptor kinases. Smad-independent mechanisms comprising MAPKs, and phosphoinositide 3-kinase may also be activated through TGFβ receptors. This cytokine is expressed in the airways of asthmatic subjects and stimulates the production of collagen and fibronectin by fibroblasts, but it also reduces the production of collagenase, and augments the production of inhibitors of degrading enzymes, resulting in an increment of extracellular matrix proteins [31]. TGFβ expression has been shown to correlate with the severity of subepithelial fibrosis. This is a powerful regulatory cytokine with activity on different hemopoietic cells which controls the development of harmful immune responses to self or to harmless antigens without affecting immune responses to pathogens.

Chemokines recruit T cells to inflamed tissues. Chemokine receptors are differently expressed on Th1 cells, which express CXCR3 and CCR5, and Th2 cells, which express CCR4 and CCR3. Pulmonary CCR4+CD4+ cells and their ligands TARC and MDC were significantly increased in asthma children BALF [61].

TH CELL SUBSETS IN ASTHMA

In the classical conception of the Th1/Th2 paradigm, these two were considered the unique CD4 T cell subsets for a long time, but in recent years new cell subsets have been described. Different protective as well as pathogenic activities have been ascribed to Th1 and Th2 cells in a polarized and mutually opposed fashion. In normal individuals Th1 is the major phenotype present in the airways. In asthmatic subjects the number of CD4+ T cells in the airways is elevated and Th2 is the main phenotype. These cells produce cytokines that are responsible for many of the typical features of asthma. Signals received by naïve T cells drive differentiation towards the various Th subsets. CD4+ T cells can evolve into Th1, Th2, Th9 or Th17 effector cells, depending on the microenvironment, the presence of different cytokines and the status of cells when they encounter the antigens (Fig. **3**). These Th cell subpopulations can generate diverse inflammatory responses, depending on their secretion of cytokines, their interaction with other cells and their response to chemokines. Th2 cells are generated when IL-4 is present and this cytokine inhibits Th1 differentiation. Several cytokines are secreted by Th2 cells including IL-4, IL-5, IL-9, IL-13 and probably IL-25 [62], IL-31 [63] and IL-33 [64]. They induce eosinophilia, the production of allergen-specific IgE, mucus hypersecretion and permissiveness of endothelium for the recruitment of inflammatory cells [31, 65].

Activities of Th1 cells include induction of epithelial cell apoptosis and it has been proposed that the predominance of Th2 cells in allergic diseases might result from activation-induced cell death of Th1 cells [66]. Some Th2 subsets produce one or two of the typical Th2 cytokines but not others, and their activity depends on the predominant cytokine, resulting in increased eosinophilia for IL-5 or IgE induction for IL-4 [31].

The Th1 subpopulation is generated from naïve T cells in the presence of IL-12 and IFNγ which inhibits Th2 differentiation. Th1 cells produce IFNγ and their role is to protect against intracellular pathogens, while Th2 cells have protective activity against gastrointestinal nematodes. The development of these T cell subsets is associated to different transcription factors. For Th1 cells the factor involved is the T box expressed in T cells (T-bet), which binds the IFNγ promoter, and signal transducer and activator of transcription (STAT)-4 [67]. For Th2 cells, GATA-binding protein (GATA)-3 and STAT-6 are the factors involved [68]. GATA-3 generates epigenetic changes in the Th2 cytokine cluster coding for IL-4, IL-5

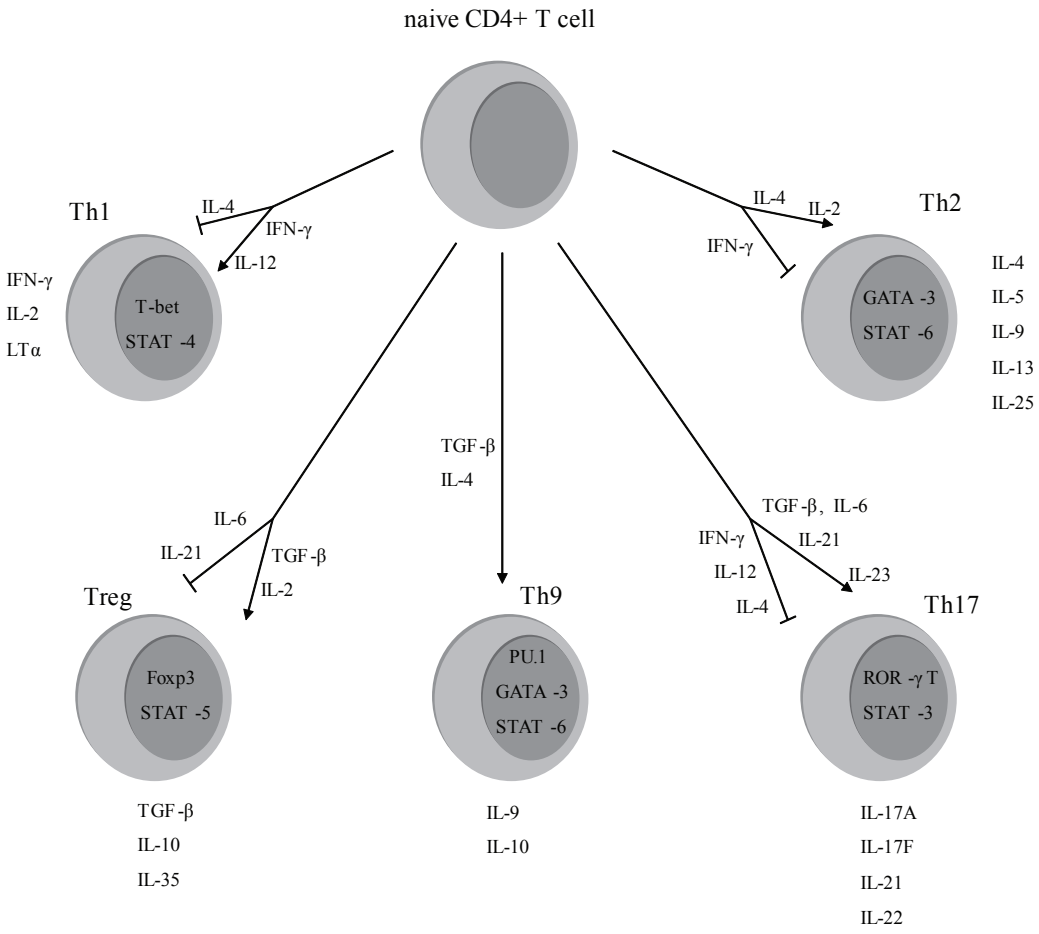

Fig. (3). Th cells subsets in asthma.

Different T cell subsets are derived from naïve T cells, depending on the presence of distinct combinations of cytokines, associated to the activation of different transcription factors. Each phenotype secretes a characteristic set of cytokines. Th1 cells are differentiated in the presence of IL-12 and IFNγ through the expression of T-bet and STAT-4. They secrete, among other CKs, IFNγ which inhibits Th2 differentiation.

Th2 cells differentiate in the presence of IL-4 and IL-2, through the expression of GATA-3 and STAT-6. These cells produce IL-4, IL-5, IL-9, IL-13 and IL-25; in the presence of IL-4 Th1 differentiation is inhibited.

Stimulation of naïve T cells in the presence of TGFβ and either IL-6, IL-21 or IL-23, results in the differentiation into Th17 cells, through the expression of RORγT via STAT-3. Th17 differentiation can be inhibited by the presence of Th1 and Th2 cytokines. If naïve T cells are activated in the presence of TGFβ and IL-2, iTreg cells are generated, by induction of Foxp3, via STAT-5. Treg cell differentiation can be inhibited by IL-6 and IL-21.

When the activation takes place in the presence of TGFβ and IL-4, Th9 cells are generated by expression of the transcription factor PU.1.

and IL-13 [69]. Expression of T-bet is reduced in asthmatic patients' airways T cells. T-bet regulates GATA-3 function and IL-27, a member of the IL-12 family, downregulates GATA-3 expression and upregulates T-bet expression. On the other hand, GATA-3 inhibits STAT-4, factor activated by the T-bet inducing IL-12, therefore inhibiting the production of Th1 cytokines [27]. The transcription factor PU.1 has been identified as the factor that promotes the Th9 phenotype in a recent paper [70].

Asthma and allergies have been described as Th2 mediated conditions when the Th1/Th2 paradigm was first defined [71], but Th1 cells also play a role in allergic asthma pathogenesis and probably contribute to chronicity. They produce IFNγ and increase activation induced cell death, while Th2 cells survive. In the lung Th1 cells secrete IFNγ, TNFα and Fas ligand and induce epithelial apoptosis [72] generating shedding of these cells in asthma patients' tissue [73].

Histamine, one of the autacoids produced in allergic asthmatic patients, induces in DCs production of IL-10 [74, 75]. Human Th1 cells express mainly the histamine receptor (HR) 1, while Th2 cells express HR2. Activation of HR2 suppresses production of IL-4 and IL-13 and T cell proliferation, and potentiates TGFβ suppressive activity [76]. On this basis, some authors have proposed the targeting of T cells for specific immunotherapy of allergy, bypassing IgE [77]. In naturally anergized bee keepers, HR2 is upregulated on Th2 cells and increased numbers of IL-10 producing CD4+CD25+ T cells have been observed.

On the other hand, up to date evidence suggests that allergic asthma is a multifaceted condition actively controlled by effector as well as T reg cells. Th2 cells and the more recently described Th17 cells have been shown to increase airway inflammation, while Tregs have anti-inflammatory activity.

As already mentioned, the immune response in allergy and asthma, as well as in other inflammatory diseases, could not be completely explained on the basis of the Th1/Th2 paradigm. The description of the Th17 subset has helped to complete the setting. More recently, another two subsets of effector CD4+ T cells, named Th9 and Th22 cells, have been described, although their pathophysiological meaning is still unclear [78].

TGFβ is involved in the differentiation of several Th cell subpopulations. In the presence of IL-4 it generates the Th9 subset,

secretor of IL-9 and IL-10. These are effector cells that promote tissue inflammation and have no regulatory activity [79, 80]. Up to date reports have demonstrated that TGFβ together with IL-6 is required for the generation of Th17 cells from naïve T cells [81].

The recently described CD4+ T cell subpopulation Th17 secretes IL-17 and several other cytokines. Th17 cells are characterized by the production of IL-17A and IL-17E, also named IL-25. These cytokines induce the release of proinflammatory and neutrophil recruiting cytokines. In an experimental model of asthma, neutralization of IL-17 and Th17 –related functions reduces neutrophil infiltration and increases eosinophil infiltration [82, 83].

Th17 cells also secrete IL-21 and IL-22. IL-21 is involved in the differentiation of Th17 cells and inhibits forkhead box protein 3 transcription factor (Foxp3) expression and Treg cells development [27, 84]. IL-9 is also produced by Th17 cells and can contribute to inflammatory disease [85]. This cytokine, which has largely been regarded as a Th2 cytokine that makes multifocal contributions to allergic disease, is highly expressed by CD45RO+ CCR8+ memory T cells when stimulated with anti-CD3/CD28. IL-9 producing cells are also heterogeneous, including Th2 cells, natural Treg cells as well as induced Treg cells.

Th22 cells are a population of Th cells defined by the production of IL-22 and TNFα, but not IFNγ, IL-4 or IL-17. Nevertheless, some Th22 cells have been observed to produce IFNγ or IL-17 [58]. This may reflect once more the plasticity of CD4 T cell subsets. When naïve T cells are stimulated in the presence of TGFβ and inflammatory cytokines IL-6, IL-21 or IL-23, they differentiate into Th17 cells, which secrete IL-17 and IL-22. Those inflammatory cytokines act by inducing the RAR-related orphan receptor (ROR)γt and RORα, *via* STAT3 [86]. If naïve T cells are activated in the presence of TGFβ and IL-2, induced Treg cells are generated, by induction of Foxp3 [87] *via* STAT5. When the activation takes place in the presence of TGFβ and IL-4, Th9 cells are generated. These cells can proliferate and enhance T cell proliferation, hence are neither anergic nor suppressive [79].

It has been recently demonstrated that Treg and Th17 cells are not stable populations and can dedifferentiate [41, 88]. T cell plasticity would be the norm, allowing a strong response from the host against a variety of microbiological challenges along the host's life, by keeping these cells to be dynamic and responsive rather than terminally differentiated [89]. Several reports support the role of TGFβ in the regulation of airway remodeling, favoring skewing towards a given CD4+ cell subset or reprogramming Th2 cells into Th9 cells [41].

REGULATORY T CELLS

A few years ago, another Th cell subset termed regulatory T cells was described, reviving the concept of T suppressor cells abandoned in the eighties by lack of specific surface markers [90].T reg cells have immunosupressive function, they inhibit the development of allergic Th2 cells responses as well as Th1 effector cell responses.

Several populations of regulatory T cells have been described which control homeostasis and regulate immune responses [31], among them a cell population defined by the expression of CD4 and CD25 ^{high}. These cells were later characterized by the transcription factor Foxp3. Foxp3 T reg cells are required for self-tolerance and immune homeostasis.

Two CD4 T subsets of Treg cells have been described: naturally occurring, thymus-derived CD4+CD25+ Foxp3+ nT reg cells and inducible type 1 Treg cells Tr1. Other cell types may have a suppressive or regulatory activity: γδ Tcells, subsets of CD8+ T cells, double negative CD4-CD8- T cells, IL-10 producing B cells, IL-10 producing NK cells, IL-10 producing DCs and macrophage subsets with suppressive activity [31]. Besides, many interactions among cells: T effector, Treg cells, with neutrophils, with B lymphocytes, with NK cells, with NKT cells, may contribute to suppressive functions [31].

An important role has been ascribed to CD4+CD25+ T cells in asthma. Both n Tregs and Tr1 inhibit the development of allergy *via* several mechanisms, including suppression of other effector Th1, Th2, Th17 cells; suppression of eosinophils, mast cells and basophils, antibody isotype change from IgE to IgG4; suppression of inflammatory DC and suppression of inflammatory cell migration to tissues [91].

Treg cells represent one of the mechanisms responsible for control of inflammation and several reports support their role in the control of allergies. Adaptive Foxp3 Treg cells are generated during induction of tolerance in mucosa and after immunization. In a mouse model, they have been described as regulating the response to allergens and chronic inflammation, while the cells which secrete IFNγ are useful to control eosinophil mediated inflammation [92]. Constitutive CD4+CD25+ T reg cells represent 5-10% of CD4+ peripheral blood T cells. They inhibit the activation of effector T cells. like the Th2 response to allergens in non-atopic individuals, as well as the specific proliferation of peripheral T cells [93].

Naïve CD4+CD25- T cells are converted into CD4+CD25+ by TGFβ induction of Foxp3 [87], generating Foxp3-expressing induced Treg cells, and TGFβ is a requirement for the *in vivo* expansion and immunosuppressive activity of CD4+CD25+ T cells [94].

Inducible T reg cells are characterized by the production of IL-10 and TGFβ. Suppression by Tr1 cells is exerted through IL-10 and TGFβ, but also through cytotoxic T lymphocyte antigen (CTLA)-4 and programmed death (PD)-1 molecules [95]. Migration to the allergic site may reduce the number of allergen specific T reg cells in peripheral blood [96]. Treg cells inhibit the development of allergic Th2 responses in animal models and allergen specific immunotherapy has been shown to induce both allergen-specific IL-10 and TGFβ secreting Tr1 cells as well as CD4+CD25+ reg T cells [97]. Use of T reg cells activated and expanded *in vitro* has been proposed as allergy therapy [98].

Recent reports suggest that Treg cells are required in the regulatory axis operating within the respiratory mucosa and that the number of Treg recruited to the airways may be crucial for the inhibition of AHR associated with exacerbations of asthma [99]. T reg cells exert a strong suppressive activity on Th2 cells. It has been reported that CD4+CD25+ expressing Foxp3 are diminished in subjects with hay fever. This could explain the increase in Th2 cells in allergic diseases [93]. However, higher levels of these T reg cells have been observed in the peripheral blood of asthmatic patients when compared to mild asthma subjects [100].

CD4 CD25 ^{high} T cells represent a small proportion of circulating cells and no exclusive marker has been described, making difficult the isolation of this population. Foxp3 is critical for the development of CD4+CD25 ^{high} T reg cells, but it can be induced in activated T cells that also express CD25. In healthy subjects the majority of circulating Treg cells express several skin-homing receptors: cutaneous lymphocyte- associated antigen (CLA), CCR4, CCR6 [101].

In allergic children, T reg cells increase during the pollen season. It is not known whether these cells contribute directly to inflammation or whether they avoid worsening of inflammation [31]. Some researchers observed similar levels of circulating allergen-specific CD4 CD25 ^{high} Foxp3+ cells in nonatopic and in atopic individuals [102]. Another group demonstrated a negative correlation of Foxp3 expression and IgE, eosinophilia and IFNγ levels. Also the Foxp3+/CD4+ ratio is significantly low in asthma patients and in those with atopic dermatitis [103].

We evaluated CD4+ CD25^{high} T cells in the peripheral blood mononuclear cells of pediatric atopic asthma patients, also both in

steady state and crisis or exacerbation. As it can be seen in Fig. (**4**), we observed no differences in the percentage of CD4+ $CD25^{high}$ T cells of patients in steady state compared to normal controls, in accordance to some reports [102]. A slightly higher percentage, but non significant, was observed in PBMC of patients in crisis. Contradictory results have been published about the number of Tregs in the peripheral blood of asthmatic patients compared to control individuals, all the possible situations, the same number, a reduced number or a higher number, have been reported [104].

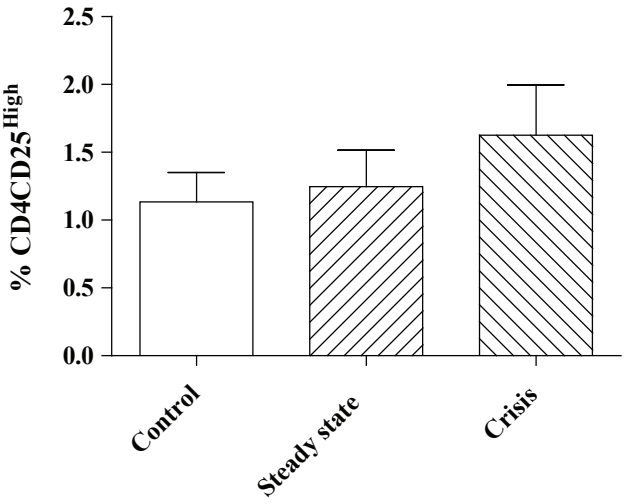

Fig. (4). Frequencies of CD4CD25 ^{high} T cells.

The frequencies of CD4CD25 ^{high} T cells in CD4 T cells were determined in recently isolated peripheral mononuclear cells of pediatric atopic asthma patients (aged 7 to 16), both in steady state and in crisis, and normal controls by flow cytometry.

No significant differences between asthmatic patients and normal controls were observed. Data are presented as mean $\pm$ standard error of the mean (SEM), and significance was analyzed by unpaired t test.

It was demonstrated that healthy and allergic individuals all have allergen-specific T cells of the Th1, Th2 and Tr1 phenotype, but differ in their proportions. In those healthy subjects with detectable allergen-specific IgG, Tr1 are the dominant subset. By contrast, in allergic subjects a high proportion of IL-4 secreting allergen-specific T cells was observed. These allergen–specific Tr1 cells mediate suppression by using IL-10, TGFβ, CTLA-4 and PD-1 [73]. The presence of pathogen derived molecules, and exogenous signals such as histamine, adenosine, retinoic acid or vitamin D3 metabolites can induce new populations of Treg cells [31].

A summary of the main cells involved in asthma and their activities is presented in the Table below (Table **1**).

ASTHMA THERAPY

Currently available asthma treatment based on β2-agonists and glucocorticoids is effective in 90-95% of patients [105]. The remaining group of patients cause high health care costs because they require more sophisticated interventions or hospitalization. New treatments are required to provide the effectiveness of glucocorticoids with fewer side effects and to treat the patients that do not respond to conventional therapies. These new approaches intend to improve the existing ones or develop new drugs that target specific mediators or pathways, and would be employed alone or in combination with compounds already in use [66]. Differences in response to available treatments have led to the recognition of several asthma phenotypes [66]. Many of the treatments, both currently applied and new, involve directly or indirectly cytokines.

Most asthmatic patients respond to glucocorticoid treatment. These drugs suppress inflammation through the nuclear enzyme histone deacetylase 2 (HDAC2), which acts on multiple activated inflammatory genes deacetylating them and in such a way suppressing inflammation [106]. Subjects with severe asthma have a reduced response to corticosteroids probably due to impaired HDAC2 function [106]. Corticosteroids (fluticasone) used in combination with beta-2 agonists (salmeterol) increased IL-10 and reduced IL-5 and IL-13 cytokine synthesis in allergen stimulated human CD4+ T cells, compared to glucocorticoids alone [107].

The usual therapy for allergic diseases is based on medications classified either according to their structure: corticosteroids, or their effects: anti-histaminics, leukotriene inhibitors, or immunotherapy based on subcutaneous injections of the allergen. Inhaled glucocorticoids reverse several aspects of vascular remodeling such as vasodilatation, angiogenesis and increased vascular permeability. Anti-angiogenic agents used for cancer treatment might be a new therapeutic approach for asthma therapy, decreasing edema and the number of vessels [108].

Allergen-specific immunotherapy (SIT) has been successful in the treatment of hay fever, resulting in reduction in inflammation, nonspecific hyperresponsiveness, prevention of new sensitivities, and progression of allergic rhinitis to asthma [109], but not so efficient for the treatment of asthma where more than one allergen may be involved. Even more, potential complications may arise by trig-

Table 1. Cells Involved in Asthma and their Activity

Eosinophils	Release of active proteins from granules, lipid mediators, cytokines, growth factors, and reactive oxygen species; induction of fibrosis (TGFβ); elicit chronic Th2 inflammation
Basophils	Th2 cell induction, stimulation of IgE synthesis, histamine, leukotriene C4, VEGF and Th2 mediators release
Mast cells	Histamine, VEGF, lipid mediators, cytokines release
Dendritic cells	Allergen presentation, immunotolerance through Treg induction
Airway epithelial cells	Hyperplasia, increased mucus production
Smooth muscle cells	Production of chemokines, cytokines and extracellular matrix proteins. Increased proliferation.
B cells	IgE production
Th1 cells	Inflammation
Th2 cells	IgE class switch, mucus production, mast cell recruitment
Th9 cells	IgE class switch, mucus production, mast cell recruitment
Th17 cells	Cytokine and chemokine production, neutrophilia induction
Treg cells	Suppression of inflammation

Table **1**: Different cells involved in asthma and their activities and/or products are listed.

gering anaphylactic reactions. Vaccination with allergens has the purpose of generating a state of specific tolerance against the allergen [110]. Several studies have demonstrated that this treatment induces a significant reduction in asthma symptoms, the use of medication and improvement in bronchial hyperreactivity [111]. Nevertheless, many patients are excluded for safety reasons from this treatment if their forced expiratory volume (FEV) 1 is not over 70% of predicted. SIT is effective in allergic individuals; however some changes are proposed to improve its safety, convenience, and efficacy. Sublingual instead of subcutaneous immunization with allergen has been successfully tested. Side effects appear to be local, B cells response by production of IgG4 is less evident than with subcutaneous treatment [60]. Other trials searching for safer immunotherapy protocols have been performed or are under investigation using T-cell peptides or DNA vaccines [27, 109]. SIT is thought to promote Th1 immunity in allergic patients leading to the alleviation of symptoms that result from allergen specific Th2 responses. This therapy targets the background immunological state in asthma, attenuates T-cell-mediated airway inflammation by down-modulating Th2 and inducing Th1 differentiation. In addition, SIT induces regulatory T cells, which produce IL-10 [112].

Since Th2 cytokines have been associated with most pathological changes of asthma, anti-cytokine therapies have been proposed, targeting IL-4, IL-5, IL-9 and IL-13, and the combination of IL-4 and IL-13 [113] (Table **2**). IL-4 antagonists bind to the receptor but are not able to induce signal transduction and block the effects of IL-4 *in vitro*. In animal models these IL-4 analogues prevent the development of asthma [105]. An anti-IL-4 humanized monoclonal antibody (pascolizumab) was assayed and abandoned for lack of expected results [114]. Soluble IL-4 receptors, which inhibit the binding of IL-4 to its receptor, are effective in an animal model of asthma [105]. One of them (altrakincept) was assayed in asthma patients, showing some improvement of symptoms in a small trial. However, a larger study could not confirm its efficacy [66], probably because the soluble IL-4R can stimulate IL-13 responses [5], and the development was discontinued. An IL-4 peptide-based vaccine has been assayed in a mice asthma model, trying to block permanently IL-4. Many asthma symptoms such as goblet cell hyperplasia and eosinophil accumulation in BAL were markedly reduced in vaccinated mice [105, 115].

Anti-IL-13 monoclonal antibodies or soluble IL-13 receptors have been assayed in animal models with promising results. Human anti-IL-13 monoclonal antibodies are being used in trials; so far no outcome data on asthma improvement have been reported [5, 116]. Soluble IL-13Rα2 blocked IgE production, lung eosinophilia and airway hyperresponsiveness in animal models [105, 117].

Blocking of the shared α chain of the IL-4 and IL-13 receptor is an attractive therapeutic approach. Pitrakinra is an interleukin-4 mutant that targets allergic Th2 inflammation by potently inhibiting the binding of interleukin 4 and interleukin 13 to interleukin-4R alpha receptor complexes. It is still being studied but improved pulmonary function, decreases in exhaled nitric oxide and improvement related to late phase allergic responses have been observed [118].

The role of IL-5 in asthma was confirmed by IL-5 knockout mice. In these animals eosinophilia and airway hyperresposiveness are markedly suppressed [105]. An anti-IL-5 monoclonal antibody (mepolizumab) human trial showed reduction of eosinophils in blood sputum but no differences in BHR or other asthma features [119]. A later trial suggested a potential regulation in tissue remodeling [5]. Another trial with a different monoclonal antibody (reslizumab) yielded similar results [120]. Eosinophils in the airways, as opposed to those in the blood, do not express the IL-5 receptor, hence being less responsive to these treatments.

In human subjects, depletion of eosinophils that express TGFβ with anti-IL-5, resulted in diminution of airway TGFβ levels, eosinophilic TGFβ expression and associated airway remodeling [31]. Higher levels of TGFβ have been observed in the airways of asthmatic patients, compared to normal subjects [121]. This may be a negative feedback mechanism to control airway inflammation and, on the other hand, may be related to the repair of asthmatic airways since TGFβ is important in healing [122].

Overexpression of IL-9 provokes asthma symptoms in animal models, but the Th2 lung inflammation in IL-9 deficient mice resembles that of wild mice, in spite of lower lung mast cells and goblet cells numbers. However, in an animal model, after allergen exposure, an anti-IL-9 antibody reduced significantly bone marrow eosinophilia as well as blood neutrophils, without affecting BAL neutrophils [105] . An anti-IL-9 monoclonal antibody (Medi-528) has been used in phase I studies without presenting major adverse events. Trial results are not yet available [5, 123].

Biological agents that target IL-13 and modulators of T reg cells and Th17 are other of the suggested therapies. Some authors have proposed that different cytokine-anti-cytokine antibody complexes can selectively be used to boost or inhibit the immune response [124]. Immunomodulators, cytokines such as IL-12 and IL-10 also regulate allergic airway inflammation [125].

Promotion of Th1 cytokines has also been proposed as treatment for asthma. A clinical trial with recombinant human IL-12 in patients with mild asthma produced lower number of blood eosinophils after allergen challenge but showed no asthma symptoms improvement and had several adverse effects [126]. Some authors have recently proposed TNFα as a novel therapeutic target in asthma [127]. It has been associated with the inflammatory response seen in the asthmatic airway. This cytokine is mainly produced by macrophages in response to binding to TLRs, but many other cell types such as monocytes, dendritic cells, B and T lym-

Table 2. Anti-Cytokine Antibodies Tested for Asthma Therapy

Cytokine	Antibody name	References
IL-4	Pascolizumab Altrakincept (s IL-4 R)	[112, 114] [5,66]
IL-13	-, CAT-354	[5, 116]
IL-4 + IL-13	Pitrakinra (IL-4 mutant)	[114, 118]
IL-5	Mepolizumab Reslizumab	[5, 119] [120]
IL-9	Medi – 528	[5, 123]
TNF	Infliximab Adalizumab Etanercept (soluble TNF – R – Fc fusion protein)	[5] [5] [5]

Table **2**: Anti-cytokine antibodies assayed for asthma therapy.
Several antibodies directed against cytokines or their receptors have been tested in different trials. A list of their names and references is given.

phocytes, neutrophils, mast cells and eosinophils, as well as fibroblasts, epithelial cells and smooth muscle cells can also secrete TNFα. A humanized monoclonal antibody (infliximab), a soluble TNF receptor 2-Fc fusion protein (etanercept) and a human monoclonal antibody (adalizumab) have been assayed with partial benefitial outcomes [5]. Other authors have questioned the efficacy of anti-TNFα therapy in asthma [127].

Therapeutic approaches based on the different processes involved in the pathological changes observed in asthma have been proposed. At the antigen presenting cell level, TLRs are one of the interactive mechanisms with antigens. CpG-rich unmethylated DNA is frequently present in bacteria and it can reorient the immune response towards the Th1 phenotype [128]. After identification of TLRs and their ligands, a number of studies were undertaken using CpG DNA, highly enriched in bacteria, which binds TLR9 expressed by dendritic cells. Upon TLR9 activation, intracellular signaling pathways (MAPKs, nuclear factor (NF) κ B, transcription of IFN-α, IFN-β, IL-10, IL-12) are activated and costimulatory molecules like CD40 and B7 are expressed. All of these can regulate the adaptive immune response to allergens, inhibiting Th2 cytokine responses [60]. Some positive initial partial results from trials using synthetic CpG oligodeoxynucleotides, with improvement of some symptoms were not extended in larger trials. Studies are still ongoing using TLR-9 agonists alone or conjugated to allergen [5].

New therapies such as p38 MAPK inhibitors and anti-oxidants have been proposed to be used alone or in combination with corticosteroids.

Suppression of T cells is another possible therapeutic approach. Cyclosporine A inhibited allergen-induced T cell proliferation, production of IL-2, IL-4 and IL-5 by human CD4+Tcells *in vitro* [105] but this immunosuppressive drug has not yielded beneficial results in asthma therapy. Rapamycin and other drugs used to prevent transplantation rejection have not been tested [27]. Use of T reg cells activated and expanded *in vitro* has also been proposed as allergy therapy [98].

Since chemokines mediate recruitment of inflammatory cells to the lungs in asthma patients, they, as well as their receptors, may also be targeted with therapeutic purposes, mainly CCR3 expressed by eosinophils and CCR4, CCR8 and CXCR4 expressed by Th2 cells. Some inhibitors for these receptors are under development [27].

Experimental data suggest that the function of several chemokines and chemokine receptors may be redundant in bronchial asthma [105]

Smooth muscle cells in asthma have a deregulated function and present an inadequate expression of C/EBPalpha, a protein required for suppression of inflammation as well as proliferation responses [129]. Evidence is accumulating that the smooth muscle is abnormal in asthma patients, in that it proliferates faster, produces more chemokines and cytokines as well as a different profile of extracellular matrix proteins than its non-asthmatic counterpart [129, 130] This offers a new possible therapeutic target for asthma.

Since a number of transcription factors (NF-κB, AP-1, GATA-3, STAT-1, STAT-6, etc) is involved in the differentiation of Th2 cells, they can also be considered as targets for asthma therapy. Local delivery of GATA-3 antisense oligonucleotides has been proposed as an approach in an experimental model of asthma. This method can suppress the expression of various proinflammatory Th2 cytokines simultaneously rather than that of a single cytokine [105].

CONCLUDING COMMENTS

In asthma patients the respiratory tract is chronically inflamed. This situation results from the expression of a number of inflammatory proteins such as adhesion molecules cytokines, chemokines, inflammatory enzymes and receptors. This inflammation does not affect the lung parenchyma, and it is located in the larger conducting airways. In mild asthma, eosinophilic inflammation, caused by Th2 and DCs, is characteristic, and it is associated to mast cell sensitization by IgE and to the release of mediators with bronchoconstrictory activity [27].

Allergic disease, such as atopic asthma, can result from an inadequate balance between allergen activation of CD4+CD25+ Treg cells and effector Th2 cells. A deficient suppression by Treg cells or strong activating signals that overcome the regulatory effect may be the basis of this imbalance [93]. In human allergies, Foxp3 mRNA expression was significantly increased in asthmatic patients after glucocorticoid treatment [131] and impaired skin infiltration by CD4+CD25+Foxp3+ T reg cells was described in atopic dermatitis lesions [31]. Accumulation of Foxp3+ Treg cells in local draining lymph nodes of the lung correlated with improvement of chronic asthma in a murine model [132].

Some of the currently used therapies like corticosteroids, beta agonists and allergen immunotherapies affect different cytokine production. Other possible treatments may be designed modulating T reg cell activity, but enhancement of their function has to be evaluated considering their beneficial role in controlling infections and tumor development. Taking into account the role of airway epithelium, new approaches may be directed at increasing the resistance of susceptible airways against environmental damage [24]. Since most trials using a single target have not been successful, it has been proposed that focusing on more than one target (cytokines, other biological mediators and specific cell types) may offer new opportunities [23, 133].

Even though many aspects of the role and interactions of these mediators still remain to be determined, several cytokines have been considered as therapeutic targets in those patients.

Although allergic asthma is the most common form of the condition, several distinct clinical forms and phenotypes have been recognized, generated by distinct pathogenetic mechanisms, some dependent and other not depending on Th2 cells and cytokines [134]. This may be the basis for different therapeutical requirements. Severe persistent asthma causes a substantial morbidity and mortality burden and is frequently inadequately controlled despite intensive guideline-based therapy, with lack of response to corticosteroid treatment. New therapeutical approaches are important to improve the outcome of this pathology. The increased knowledge on cytokine interplay and their connections to the cells involved in the immune response in atopic asthma should generate new therapeutical strategies in the near future.

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ABBREVIATIONS

APC	=	antigen presenting cells
BALF	=	broncheoalveolar lavage fluid
BHR	=	bronchial hyperresponsiveness
CLA	=	cutaneous lymphocyte- associated antigen
CTLA	=	cytotoxic T lymphocyte antigen

DCs = dendritic cells
EMTU = mesenchymal trophic unit
FEV = forced expiratory volume
Foxp3 = forkhead box protein 3 transcription factor
GATA = GATA binding protein
HDAC = histone deacetylase
HR = histamine receptor
ICOS = inducible costimulator
IL = interleukin
MAPK = mitogen activated protein kinase
MBP = major basic protein
NF = nuclear factor
NK = natural killer
NOD = nucleotide-binding oligomerization domain
PD = programmed death
PCDH1 = Protocadherin-1
ROR = RAR-related orphan receptor ROR
sIL-4 R = Soluble IL-4 receptor
STAT = signal transducer and activator of transcription
T-bet = T box expressed in T cells
TGF = transforming growth factor
TLRs = Toll-like receptors
Tr1 = inducible type 1 Treg
Treg = regulatory T
TSLP = thymic stromal lymphopoietin
VEGF = endothelial growth factor

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