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Cytokines in atopic diseases: revisiting the Th2 dogma

In the field of allergic diseases, extensive research has demonstrated the implication of a strong T_H2 activation that both initiates and maintains *in situ* inflammation with the help of T_H2 cytokines. However, as anti-T_H2 cytokines therapy is not sufficient to suppress the disease, a more complex immunological mechanism is suspected. In this review, we will revisit the T_H2 dogma in allergic diseases, propose a possible implication of T_H1 inflammation correlated with the severity of atopic disease, to conclude with the therapeutic solutions envisaged.

Key words: asthma, atopic dermatitis, allergy, T_H1/T_H2, cytokines, immunotherapy

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Immunological sensitizations to allergens induce various clinical manifestations such as rhinitis, conjunctivitis, asthma, atopic dermatitis and also food, drug and venom allergy, leading, in some extreme cases, to anaphylactic shock, respiratory failure and death.

The dramatic increase of atopic diseases in recent years, more specifically concerns children from developed countries. In western societies, asthma and eczema affect 10% and 15% of children respectively and asthma is present in 60% of children with severe atopic dermatitis. In adults, asthma is present in about 5% of the Western world population and its prevalence has been increasing since a few decades [1].

Atopic diseases result from complex polygenic inheritance and environmental factor interactions. As genes responsible for susceptibility to these diseases have not changed in a few decades, the allergic disorder increase is rather attributable to changes in life style [2]. Epidemiological hygiene hypothesis suggests that in westernized societies, the decrease of microbial exposure during infancy was responsible for the allergy increase [3]. Indeed microbial exposure is associated with a T_H1 bias in the development of the immune system during childhood, supposed to be protective against T_H2 responses characterizing allergic diseases or reactions against helminthes.

In this review, we will revisit the T_H2 dogma in allergy, and propose the importance of an associated T_H1 inflammation and also of an improved T regulatory cell activation.

Immunological mechanisms (figure 1)

Initial T_H2 deviation: a role for dendritic cells

When a non-pathogen exogenous allergen penetrates into an organism, antigen presenting cells (APC) and notably dendritic cells (DCs) immediately uptake and process the allergen in the endosome. As the allergen is normally not aggressive towards the cells, no inflammatory context occurs, neither expression nor activation of "DANGER" signals, so that DCs produce the non-cytotoxic cytokines IL-4, IL-5 and IL-10. At this step, DCs and T cells from non-

allergic subjects abrogate the development of allergic immune responses. Indeed, an excess of inhibitory cytokines and an appropriate balance of the T_H1/T_H2 profile prevent the T_H2 commitment and initiation of T_H2 inflammation cascade towards allergens [4].

In allergic experimental models, both naturally occurring CD4+CD25^{high} regulatory T cells and inducible populations of antigen-specific interleukin-10-secreting regulatory T cells suppress inappropriate T_H2 responses [5]. In atopic subjects, there is an alteration of the T_H1/T_H2 balance towards a T_H2 bias, because of a missing T_H1 deviation [6]. Immune cells from atopic patients intrinsically display a deficient IFN- γ production compared to healthy volunteers, favoring a T_H2 differentiation of T cells in response to antigens, *i.e.* the preferential production of IL-4, IL-5, IL-9 and IL-13. In this pro-T_H2 context, DCs present allergen peptides loaded on MHC_{II} to naïve T_H0 CD4+ T cells and drive their T_H2 deviation. In this case, T_H2 cytokines secreted from APC, and especially IL-4, initiate the subsequent cascade of T_H2 transcriptional factors in the naïve CD4+ T cells in absence of IFN- γ and IL-12 (figure 2). The transcription factors STAT-6, and then c-maf and GATA-3 allow the T_H2 commitment of T_H0 cells by activating T_H2 cytokine genes promoters [7]. In parallel, GATA-3 inhibits expression of other T_H1 transcription factors [8].

Maintenance of the sustained T_H2 inflammation (table 1)

Once their T_H2 phenotype is acquired, allergen specific CD4+ T cells tightly orchestrate the allergic immune response by cytokines. IL-4 maintains cells activated, amplifies the T_H2 response and recruits other T_H2 cells *in situ*. Indeed, IL-4 acts as a growth and differentiation factor for lymphocytes, but also basophils and mastocytes and deactivates macrophages [9]. In these functions, IL-4 is helped by IL-13, with which it shares a common receptor chain and transduction pathways [10]. These two cytokines allow B cell activation, plasmocyte differentiation and survival, and isotypic commutation towards IgE synthesis [11]. IL-4 and IL-13 also induce chemokine production which is responsible for the recruitment of other T_H2 cells,

maintaining the T_H2 inflammation. More recent experiments suggest that IL-13 and IL-4 are also implied in eosinophil recruitment [12]. However the blockade of IL-4, IL-13 or both genes, impaired, but did not completely abrogate the T_H2 cell development and IgE production [13], demonstrating the complementarity of these two cytokines but not only redundancy. Moreover, when IL-13 transgenic mice were crossed on an IL-4 knock-out background, IgE production was restored, demonstrating that IL-13 can induce IL-4-independent IgE production [14].

IgE-dependent cascade: early phase reaction

Once produced by specific plasmocytes, IgE bind to the high affinity receptor (FcεRI), present on various cell types (DCs, monocytes, eosinophils, and overall mast cells and basophils). Bound on this receptor, IgE half-life reaches several weeks although circulating IgE disappear in a few days. During a new exposure, allergens rapidly bind to IgE at the surface of FcεRI expressing cells, which results in coaggregation of the receptors and as far as mast cells and basophils are concerned, activates the release of granule

content [15]. This content consists of preformed and newly synthesized inflammatory mediators responsible for early phase reactions and initial acute symptoms.

Eosinophil-dependent cascade: late and chronic phase reaction

In parallel, another inflammation cascade is initiated with IL-5 synthesis. IL-5 is an important mediator of eosinophil differentiation and proliferation in bone marrow and also a chemotactic factor for their homing from bone marrow to inflamed tissues [16]. In activating eosinophils *in situ*, IL-5 induces the release of other T_H2 chemotactic agents such as eotaxin and basic proteins responsible for tissue damage [17]. To a lesser extent, IL-5 also activates basophils to release toxic mediators such as histamine and leukotrienes. In IL-5 knockout mice, lung eosinophilia could not be induced in sensitized mice after specific challenge, and lung damage was markedly suppressed as well as airway hyper-responsiveness (AHR) [18]. The lack of effect on other cell types or on antibody production confirmed the

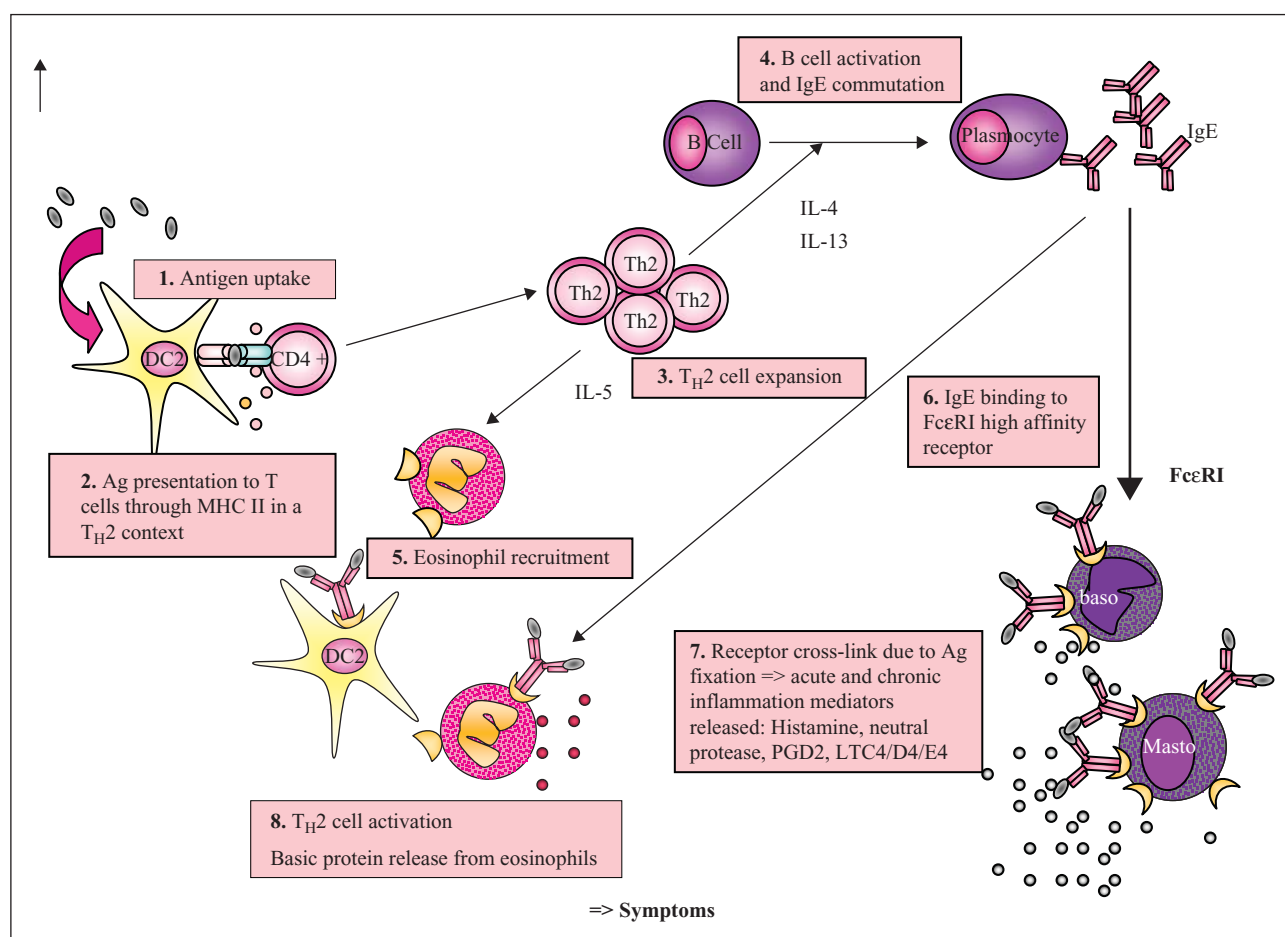


Figure 1. The Allergic T_H2 dogma.

Once an allergen penetrates into the organism, the allergen is uptaken by sentinel dendritic cells. DC migrate to the nearest lymphoid organ, process and present the allergen coupled with MHC_{II} molecule to T lymphocytes. Once a naive T cell recognize the allergen, T cells expand clonally, and migrate at the site of inflammation. *In situ*, activated T_H2 cells produce T_H2 cytokines: IL-5 allows eosinophil recruitment and activation, leading to tissue damage; IL-4 and IL-13 mainly activate B cells and allow IgE commutation and synthesis. IgE binds FcεR1 on non-specific immune cells. During new contact with the allergen, already bound IgE immediately capture the allergen, which causes receptor cross-linking and rapid mast cell and basophil activation. These cells are responsible for the release of the most inflammatory and toxic mediators that cause immediate allergic symptoms.

quasi-unique specificity of IL-5 for the eosinophil lineage and its responsibility in tissue damage [19].

Other Th2 cytokines

Other T_H2 cytokines are implied in allergic diseases. Among them IL-9 increases mast cell and eosinophil differentiation, proliferation, survival and homing [20]. The IL-9 gene was pointed out as an important candidate in genetic studies. In a mouse asthma model, anti-IL-9 inhibited airway inflammation and AHR [21]. However, in IL-9 knockout mice allergic reactions were not abolished, indicating that IL-9 plays a secondary or redundant role in allergy [22].

IL-16 is localized in bronchial epithelial cells from allergic patients, even in the absence of allergen, whereas no immunoreactivity was found in non allergics [23]. IL-16 is chemotactic, induces activation and proliferation of $CD4^+T_H2$ cells, eosinophils and monocytes [24]. However, several recent studies have demonstrated that both *in vitro* and *in vivo*, IL-16 down regulates rather than exacerbates

antigen-driven T cell activation, $Th2$ cytokine production and allergic airway inflammation.

IL-25 is secreted by mast cells after $Fc\epsilon RI$ -mediated activation, and induces the production of IL-4, IL-13 and IL-5, thus helping mast cells to enhance and sustain the $Th2$ activation [25].

However, even if T_H2 cytokines remain the corner stone of allergic reaction, several works tend to demonstrate that T_H1 cytokines, notably $IFN-\gamma$, act concurrently with T_H2 cytokines during the chronic inflammation. This was first described in atopic dermatitis and now appears in other allergic diseases, especially in uncontrolled asthmatic reactions. The best approach to get the right picture of the respective role of T_H2/T_H1 activation in these diseases is to describe current knowledge of their immunopathology.

Cytokines in human atopic diseases

Atopic dermatitis (figure 3)

Atopic dermatitis (AD) results from a combined IgE-mediated hypersensitivity and delayed type hypersensitivity-

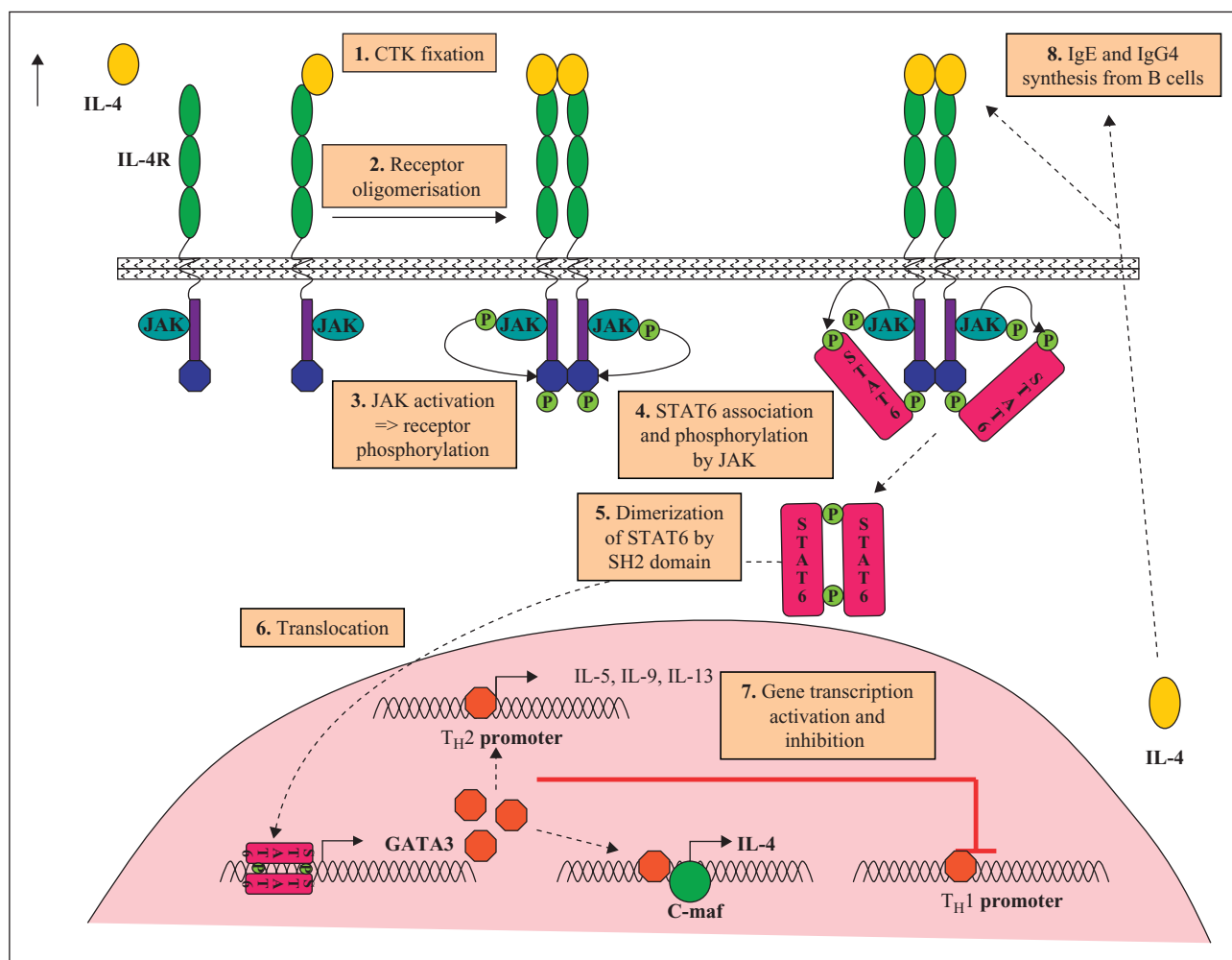


Figure 2. From protein to gene regulation.

Once bound onto their receptors, cytokines induce receptor oligomerisation. JAK phosphorylates the receptor, which recruits STAT to the receptor intracellular chain, and is in turn phosphorylated by JAK. Phosphorylated by JAK, STAT dimerizes and is imported to the nucleus and specifically recognizes the promoter site to induce GATA-3 transcription. GATA-3, the T_H2 transcription factor, simultaneously induces transcription of IL-5, IL-9 and IL-13 cytokines and inhibits T_H1 promoters. Associated to C-maf, GATA-3 increases IL-4 production, inducing irreversible T_H2 phenotypes.

Table 1. Chromosome location, cellular source, cellular receptor and main functions of cytokines and chemokines implicated in allergic reactions

Cytokine	Chromosome location	Cellular source	Cellular receptor and cellular expression	Main activities in allergic reaction
IL-2	4q26-27	T cells, eosinophils	IL-2R/CD25 T cells	T cell proliferation
IL-4	5q23-31	Activated T _H 2 lymphocytes, mast cells, basophils, NK1.1+ T cells	IL-4R/CD124 Lymphocytes, macrophages, mast cells, fibroblasts, epithelial and endothelial cells	Regulation of T _H 2 cell differentiation, of IgE and IgG1 production by B cells. Growth and survival factor for mast cells. Induction of eotaxin production by lung epithelial cells
IL-5	5q23-31	Activated T _H 2 cells, B cells, mast cells and eosinophils	IL-5R/CD125 Eosinophils	Eosinophil differentiation, activation, homing and survival
IL-7	8q12-13	DC, APC...	IL-7R/CD127 Thymocytes, Lymphocytes	Growth factor for lymphocytes and B cells
IL-8	4q13-21	Monocytes, lymphocytes, granulocytes, fibroblasts, endothelial cells, bronchial epithelial cells, keratinocytes	CXCR1/CXCR2 Neutrophils	Chemoattractant of polynuclear neutrophils
IL-9	5q31-q35	Activated T cells,	IL-9R/CD129 Mast cells, B cells, eosinophils	Differentiation and proliferation of mast cells, T _H 2 and B cells growth factors, increased IgE production from B cells
IL-10	1q	Naive and memory T cells T _H 1, T _H 2 and Tr1 subsets, B cells, NK cells, monocytes, macrophages	IL-10Rα & β monocytes, macrophages, and Dendritic Cells	Inhibition of macrophages and lymphocyte activation
IL-12	5q31-q33 3p12-p13.2	APC, dendritic cells, eosinophils	IL-12R1 & 2 /CD212	Regulation of T _H 1 cell differentiation, optimal IFN- γ production, T _H 2 differentiation and IgE synthesis inhibition
IL-13	5q31	Activated CD4+T _H 2 cells, mast cells, NK cells	IL-13R/CD213	IgE commutation, induction of T _H 2 cell reaction, enhanced mucus and eotaxin secretion, suppression of inflammatory responses due to negative regulation of macrophage function
IL-15	4q31	Macrophages, bone marrow stromal cells, endothelial cells	IL-15R	T cell growth factor
IL-16	1q25-35	T cells, epithelial cells and fibroblasts	IL-16R CD4+ T cells, monocytes, eosinophils, dendritic cells	Chemotactic factor and growth factor with specificity for CD4+ T cells, monocytes, eosinophils, dendritic cells
IL-17	2q31	T cells, eosinophils	IL-17R neutrophils	Macrophage activation, neutrophil chemotaxis
IL-18	11q22.2-22.3	Activated macrophages, mononuclear cells, keratinocytes, dendritic cells	IL-18R TH1 Cells NK cells	IFN-inducing factor, enhances production of IL-13 in T and NK cells, augments NK cell activity
IL-25	14q11.2	TH2, mast cells, APC	IL-17BR T cells, APC2 eosinophils, epithelial cells...	Promotes T _H 2 responses by inducing cytokines such as IL-4, IL-5 and IL-13 => epithelial cell hyperplasia, increases mucus secretion, and airway hyperreactivity
IL-27	16	Macrophages and DCs	IL-27R CD4+	IL-27 plays 2 distinct roles: • In early efficient induction of Th1 differentiation • In limiting the intensity and duration of adaptive immune responses

Cytokine	Chromosome location	Cellular source	Cellular receptor and cellular expression	Main activities in allergic reaction
IFN- γ	12q24.1	CD4+ TH1, CD8+ and NK cells	IFN-γR Receptors exist on virtually all cell types of the body	Main activator of macrophages. Activates endothelial cells. Directly antagonizes T _H 2 response and IgE production
GM-CSF	5q23-31	Epithelium, T cells, eosinophils, fibroblast	GMCSFR Eosinophils, myeloid precursors	Eosinophil differentiation, survival and proliferation, myeloid precursor for cell differentiation
TGF- β 1	19q13 2.5	Epithelium, macrophages, eosinophils, fibroblasts	TGF-βRI, II, III T cells, eosinophils, epithelial cells...	Activation of collagen from fibroblast synthesis, inhibition of T cell proliferation and eosinophil activation
SCF	12q22-24	Epithelial cells, macrophages	SCFR/ CD117	Proliferation and differentiation factor for mastocytes
PDGF	7 and 22	Epithelium, eosinophils	PDGFR / CD140a & b	Fibroblasts, smooth muscle cells and epithelial cell proliferation
CTACK CCL-27	9p13	Keratinocytes	CCR10 Lymphocytes	Cutaneous T-cell attracting chemokines
TARC	16q13	Dendritic cells	CCR4 CD4+CD45RO+ T cells T _H 2 polarized	Chemotactic factor for lymphocytes
MCP-1/3/4	17q11.2		High affinity for CCR2 fibroblasts epithelium, alveolar macrophages	T lymphocyte trafficking chemoattractants for monocytes, activated memory (CD45RO+) T lymphocytes, eosinophils and NK cells
Eotaxin	17 q21.1-21.2	Airway epithelium and endothelium, macrophages smooth muscle, eosinophils, dermal fibroblasts, mast cells	CCR3 eosinophils, basophils, T _H 2 cells, mast cells	Eosinophil recruitment
RANTES	17q11.2-q12	Release from platelets and eosinophil granules	CCR1, CCR3, and CCR5 eosinophils, T cells, monocytes, macrophages, dendritic cells, basophils	Chemotaxis of eosinophils, monocytes, T cells, and basophils. Eosinophil cationic protein release and rapid histamine release from human basophils

ity due to T lymphocytes. AD evolves in two successive phases: an early phase that is associated with a predominance of T_H2 cytokine and chemokine synthesis, followed by a delayed and chronic cutaneous inflammation associated to increased production of IL-5, eosinophil infiltration and IFN- γ synthesis, leading to a T_H0 profile.

Indeed, in atopic dermatitis, the allergen is rapidly up-taken by Langerhans cells in the epidermis and DCs in the dermis [26]. These APCs secrete classical T_H2 cytokines and also IL-16, MDC and TARC. TARC induces CTACK (literally a cutaneous T cell-attracting chemokine for T_H2 cells) production from keratinocytes [27]. Then CTACK, and the other chemokines (IL-16, RANTES, MDC), allow CD8+T_H2 cells to infiltrate the epidermis. Once activated, recruited cells, through the secretion of MCP-4, RANTES and eotaxin, activate eosinophils and maintain the T_H2 phenotype [28]. The toxic mediator release due to T_H2 cell activation leads to the early lesions, which give rise to pruritus and subsequent scratching of the epidermis. In a mouse model of atopic dermatitis, a recent report demonstrated that T_H2 cells also produce IL-31, which could be responsible for itch sensation, promoting scratching behavior and severe skin lesions [29]. Thereafter, tissue damage

induces an inflammatory mediator synthesis, such as IL-6, TNF- α and IL-1 β , or IFN- γ , which recruits and stimulates new T_H2 cells and allows T_H1 inflammatory response development in the dermis [30]. TNF- α induces keratinocytes to produce more RANTES and CTACK, maintaining the T_H2 phenotype [31]. However in parallel, a very recent study has demonstrated that antigen binding on Fc ϵ RI at the surface of IDEC (Inflammatory Dendritic Epidermal Cell)-DCs induces the production by these cells of IL12, IFN- γ and GM-CSF [32]. IDEC cells are then programmed to prime naïve T cells toward a T_H1 phenotype. Rapidly, *i.e.* 48 hours after a novel contact with allergen, T_H1 CD4+ and CD8+ cells infiltrate the epidermis and produce IFN- γ , leading to chronic T_H0 skin lesions [33, 34].

Asthma (figure 4)

The immunopathology of asthma was first considered as a disease distinct from atopic dermatitis, and as pure T_H2 inflammation, as suggested by animal and genetic studies. Allergen exposure leads to IL-4, IL-13 and IL-5 production. IL-4 and IL-13 are essential in inducing AHR [35]. Moreover, IL-4 and IL-13, as IL-9, induce goblet cell hyperplasia and mucus secretion. Several reports also dem-

onstrated that IL-4 and IL-13 induce T_H2 cell activation and recruitment in the respiratory tract, by increasing the expression of VCAM-1, ICAM-1, E-Selectin and other adhesion molecules on pulmonary endothelial cells [36]. IL-4 and IL-13 thus facilitate the diapedesis of inflammatory cells. These cytokines are also responsible for *in situ* inflammatory and toxic mediator release (histamine, protease, basic proteins, leukotrienes...), leading to subsequent bronchial epithelium damage and smooth muscle contraction. Finally, IL-4 and IL-13 were recently implied in *in situ* eosinophil activation through the induction of eotaxin. In atopic and non-atopic asthmatic subjects, IL-4 positively correlated to bronchial hyper-responsiveness (BHR) and after allergenic challenge, the IL-13 increase was closely related to BHR [37].

IL-5 regulates eosinophil accumulation and activation in the respiratory tract of allergic asthmatics, as elegantly shown in occupational asthma [38]. It is noteworthy that in other allergic reactions, such as allergic rhinitis, chronic sinusitis, allergic eye diseases, a tight positive correlation between IL-5 and eosinophil activation has also been reported compared to control subjects, after specific allergen

challenge. Subsequent activation of eosinophils by IL-5 provokes local release of major basic protein, eosinophil cationic protein and eosinophil-derived neurotoxin, leading to the destruction of the asthmatic bronchial epithelium [39].

Currently, IL-5 is considered as an important biological predictive marker for asthma exacerbation. Indeed, it has been demonstrated that more the disease was uncontrolled, the more IL-5 and ECP were produced. In asthmatic patients, induced sputum eosinophils, directly or indirectly recruited by IL-5, are considered as predictive markers of exacerbations [40].

Recent evidence suggests that whereas this exclusive T_H2 face of allergic asthmatic inflammation could be relevant to the initial phase of the disease, a T_H1 face could arise during uncontrolled episodes and chronic phases. Indeed, in humans, we and others have shown that uncontrolled chronic asthma was associated to IFN- γ increase [41], both in blood [42] and in induced sputum [43]. More recently, in induced sputum from asthmatics, Cho *et al.* also demonstrated an increase in both CD4+ and CD8+ type 1 and type 2 cytokines, absent in healthy controls [44]. Moreover, this pro-

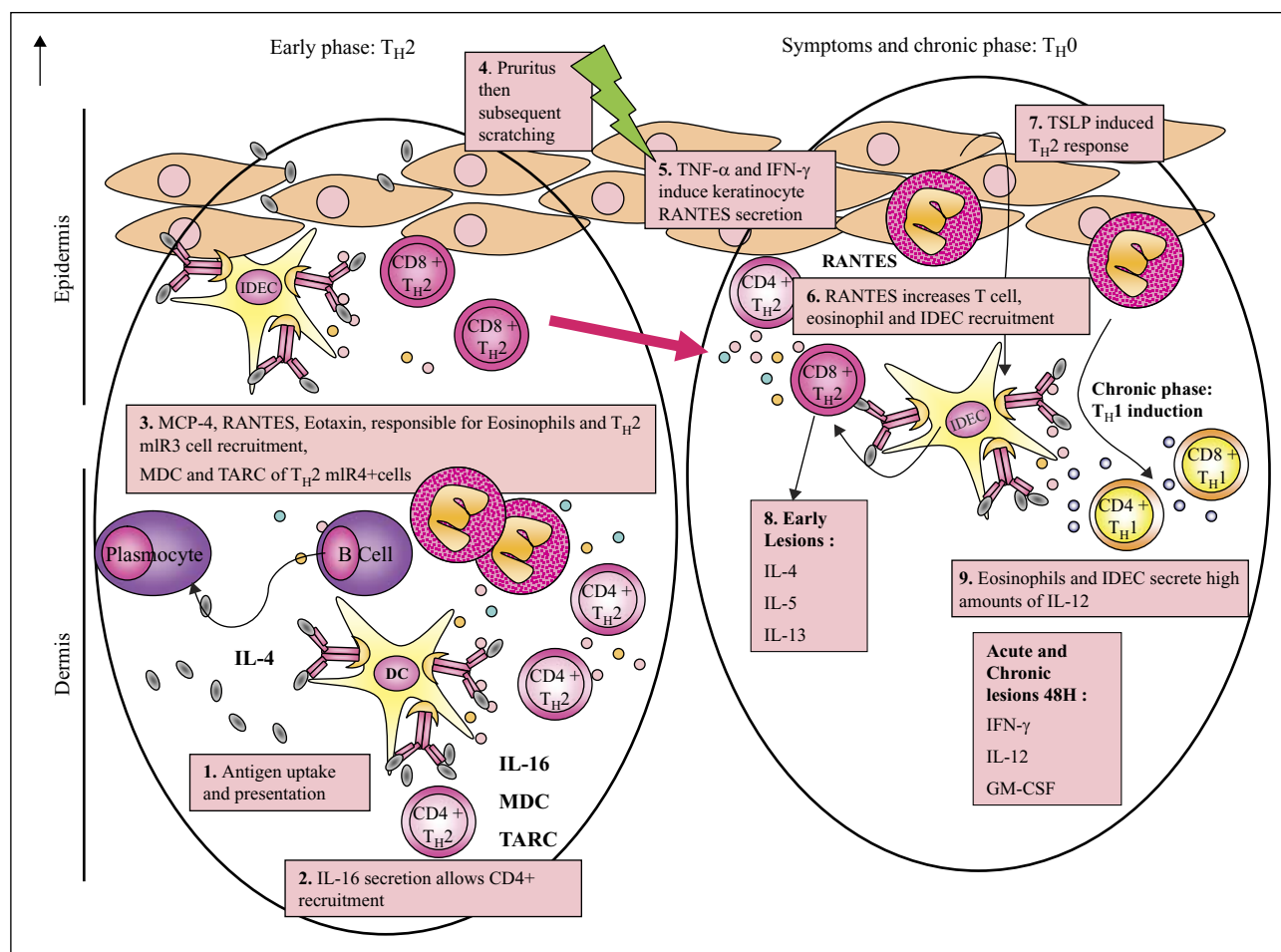


Figure 3. Immunopathology of atopic dermatitis.

Initiation of atopic dermatitis is characterized by a T_H2 immune response that occurs in the dermis, involving T_H2 cells, eosinophil recruitment, B cell activation and IgE production. Later, eosinophils and CD8+ T_H2 cells are recruited to the dermis and are responsible for early skin lesions. The subsequent scratching induces TNF- α and IFN- γ secretions. Together with skin damage due to toxic inflammatory mediator release from eosinophils, the epidermis environment favors IL-12 secretion from IDEC, allowing T_H1 inflammation. The resulting chronic phase is a T_H0 inflammation.

duction was close related to the severity of the disease. Accordingly, in asthmatic children, BHR was associated with eosinophilia and IFN- γ production the more atopic they were [45].

Thus, as in atopic dermatitis, it appears that IFN- γ , absent from the initiation of the allergic reaction, is induced after tissue damage during the chronic inflammatory phase.

IFN- γ could actively maintain and increase the reaction severity. Indeed, IFN- γ induces expression of HLA-II, facilitating antigen presentation and T cell activation by increasing the Tcell-APC contact. IFN- γ enhances expression of Fc ϵ RI both on polynuclear cells and mononuclear phagocytes. IFN- γ also increases adhesion molecule expression on various cell types, resulting in enhanced adhesiveness and diapedesis for leukocytes, maintaining an inflammatory state. Importantly, IFN- γ is related to BHR [41]. In a mouse model of asthma, T_H1 cells induced strong AHR independent of IL-4 and IL-13. Moreover, this AHR was associated with the presence of lymphocytes and macrophages, but not neutrophils [46].

The implication of T_H1 cytokines in uncontrolled asthma could be an explanation for the incomplete success of specific anti-T_H2 treatment compared to anti-inflammatory molecules that inhibit both T_H1 and T_H2 cells.

Therapeutic implications

The large panel of cytokines involved in allergic diseases obviously induced hopes for new therapeutics targeting the most relevant of them. However most of the anti-T_H2 trials were disappointing. Although in mice anti-IL-4 blocking antibodies inhibited AHR, eosinophilia and goblet-cell metaplasia after allergenic challenge, in humans IL-4 blocking antibodies were ineffective. IL-4 soluble receptors in moderate asthmatics improved FEV₁, symptoms and medication scores [47]. In mice and primates, IL-5 antibodies inhibited lung eosinophilia and AHR in response to allergen challenge. Humanized IL-5 antibodies have shown to decrease blood eosinophils, airways and skin eosinophil infiltrates after allergen challenge in mild asthmatics [48]. However, IL5 antibodies were insufficient to inhibit BHR [49]. An alternative approach was to target the IL-5 receptor α -chain. Again, although a single injection of anti-IL-5 receptor antibody reduced peripheral blood eosinophils in mice, in humans IL-5 soluble receptors decreased IL-5 induced activation in some cell types but not in eosinophils. The second approach was to enhance T_H1 activation in order to inhibit the T_H2. In mice, administration of recombinant IL-12 suppressed allergen-induced eosinophilia,

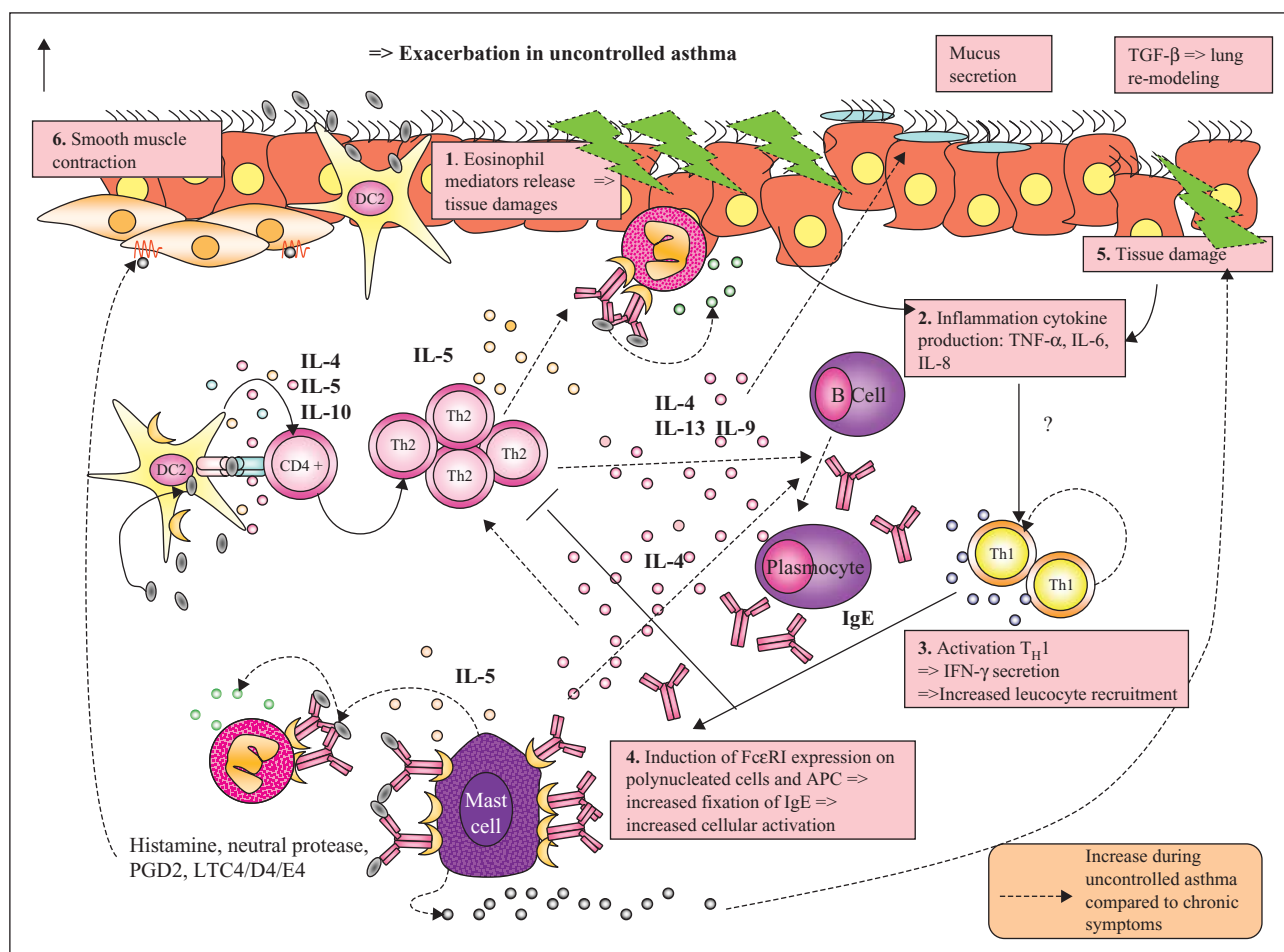


Figure 4. Mechanisms of uncontrolled asthma.

The initial T_H2 activation observed in asthma is responsible for eosinophil recruitment in airways, epithelium damage (IL-5), mucus secretion (IL-13), T_H2 cell expansion, and B cell activation (IL-4, IL-13). Antigen binding of IgE at the surface of mast cells, is responsible for degranulation.

AHR and inhibited allergen sensitization. However, complete inhibition of allergen-specific IgE synthesis was obtained only when IL-12 was administered during sensitization, but not during subsequent allergen challenges [50]. IL-12-induced suppression of IgE synthesis by IL-4-stimulated lymphocytes was dose-dependent. However in humans, IL-12 administration was inefficient. IL-18 gene transfer prevented the development and reversed established allergen-induced AHR in mice [51]. Recently IL-18 injected simultaneously with antigen, induced severe airway inflammation and AHR in a mouse model previously injected with T_H1 cells [52]. IFN- γ nebulized in mice induced a decrease in IgE production and airway inflammation, and normalized airways functions, but parenteral administration had no effect. IFN- γ gene transfer prevented and suppressed allergen-induced AHR [53]. The use of plasmids encoding either IFN- γ or IL-12 genes was also promising in animal models. However administration of

IFN- γ was inefficient in human asthma. In AD, some IFN- γ trials were encouraging. In a double-blind, placebo-controlled trial, recombinant IFN- γ improved clinical parameters. However, failure of IFN- γ therapy in children with severe refractory AD was also reported. Long-term IFN- γ therapy for severe atopic dermatitis is currently considered as a safe procedure [54].

To induce a T_H1 profile, *in vivo* administration of infectious agents was also tested and yielded various results according to the type of agent used. Suppression of allergen-induced airways eosinophilia via IL-12 and IFN- γ production caused by BCG administration has been reported in some experimental models. These findings are consistent with human reports, showing reduced prevalence of allergy in BCG vaccinated children [55].

The more promising treatment to induce a T_H1 profile was provided by the use of bacterial CpG motifs. CpG motifs introduced with allergen, during or after the sensitization

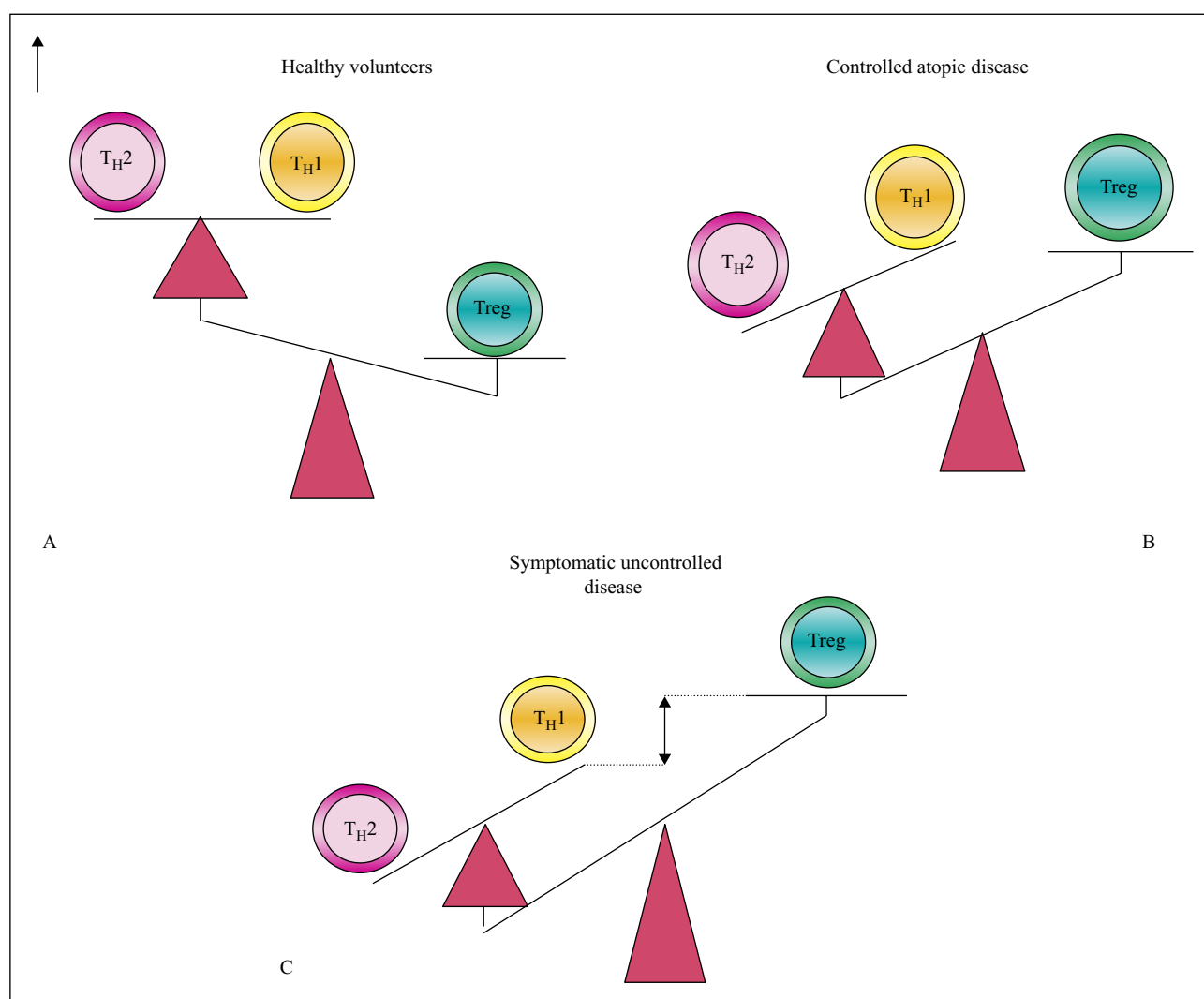


Figure 5. Conclusions.

A. In healthy subjects, immune balance is characterized by high regulatory capacities towards allergens, and tightly equilibrated T_H1/T_H2 balance. **B.** In atopic subjects, studies have clearly demonstrated a T_H2 immune deviation and, more recently, a decrease in the proportion of regulatory T cells compared to healthy subjects. **C.** Few studies have considered atopic diseases during uncontrolled allergic reactions, but all have shown exacerbated T_H2 immune responses, mainly responsible for acute symptoms. Furthermore, a parallel increase of the T_H1 immune responses, was associated to symptoms.

phase, could reduce or eliminate allergic asthma in mice in increasing the IFN- γ /IL-4 ratio, decreasing the IgE allergen specific amount and IgG1, increasing IgG2a and influx of inflammatory cells in the lung [56].

A recent publication showed that IL-27 plays an important role in the down-regulation of airway hyper-reactivity and lung inflammation during the development of allergic asthma through its suppressive effect on cytokine production [57]. This pro-T_H1/ regulatory cytokine is therefore a new potential therapeutic agent for allergy.

The last strategy tested consisted in tolerance induction through allergen specific immunotherapy and/or regulatory populations and related cytokine induction. IL10 favors immunoglobulin isotype switching towards IgG4 production instead of IgE. IL-10 can turn off immune reactions with basophil, mast cell, eosinophil and T cell deactivation. IL-10 can prevent inflammation by inhibiting APC maturation, down regulating MHC_{II} and costimulatory molecules. In a mouse model of asthma, IL-10 was effective in decreasing serum IgE and suppressing eosinophilia and AHR [58]. However IL-10 induces hematological adverse events. The current strategy is thus to induce naturally occurring T cell and spontaneous IL10 producing T cells, notably T regulatory cells (Treg). Several recent reports demonstrated that the absence of deleterious immune responses against allergens in healthy volunteers was due to impaired activation of Treg cells. Regulatory CD4+CD25+^{high} cells have been found to be deficient in allergic subjects [59]. Accordingly, spontaneous recovery from milk allergy in children was related to an increase in CD4+CD25+^{high} Treg cells, which did not occur in non-recovering children [60]. AD patients have normal CD4+CD25+ Treg cells, with normal activity, but when exposed to super antigens, these cells lose their suppressive functions [61]. We have recently demonstrated that in ultra-rush wasp venom immunotherapy, CD4+CD25+ and CD4+IL10+ Treg cell increases were one of the first immune modifications observed during tolerance induction. In addition, both CD4+CD25+^{high} and IL10+ Treg cells increased earlier and in higher proportion in the less severe patients, suggesting a facilitated tolerance induction in these subjects [62]. During grass pollen immunotherapy, Francis *et al.* have shown that CD4+CD25+^{high} Treg cells increase in treated patients and not in controls [5]. Jutel *et al.* also confirmed these results [63] in house dust-mite allergy. In addition specific immunotherapy induced IFN- γ producing T cells correlated to tolerance induction and improvement of symptoms [64].

Conclusions

The combination of epidemiological, genetic and immunological studies has provided a lot of insight into atopic mechanisms (figure 5). Clearly, allergic diseases are characterized by a capacity of T cells to differentiate and produce T_H2 cytokines that maintain the *in situ* chronic airway inflammation. Moreover, these T_H2 cells play a sentinel role and initiate acute inflammation as soon as an allergen penetrates *in situ*. However, when anti-T_H2 cytokines were administered, the pathology was not completely abolished suggesting more complex mechanisms, and notably a concomitant T_H1 activation, correlating with the severity of the disease.

A complete picture of the cytokine network is still lacking in allergic diseases. Such a picture will come from longitudinal studies, evaluating not only patients to controls but also symptomatic and controlled periods in the same patients. ■

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Abbreviations:

AHR:	Airway Hyper Reactivity	LT:	Leukotrienes
BAL:	Bronchoalveolar lavage	MCD:	Mast Cell Degranulating peptide
BCG:	Bacilli Calmette Guerin	MCP:	Monocyte Chemoattractant Protein
BHR:	Bronchial Hyper Reactivity	MHC:	Major Histocompatibility Complex
CCL-5:	CC chemokine ligand 5	MIP-1 α :	Macrophage Inflammatory Protein-1-alpha
CTACK:	Cutaneous T cell-Attracting ChemoKine	mRNA:	messenger RiboNucleic Acid
DC:	Dendritic cells	PBMC:	Peripheral Blood Mononuclear Cells
ELISA:	Enzyme Linked oImmunsorbent Assay	PDGF:	Platelet-Derived Growth Factor
Fc ϵ RI:	Fragment c of the Receptor I	PNN:	Polynuclear neutrophils
FEV ₁ :	Forced Expiratory Volume in one second	RANTES:	Regulated on Activation, Normal T cell Expressed and Secreted
GAPDH:	GlyserAldehyde-3-Phosphate DesHydrogenase	STAT:	Signal Transducers and Activators of Transcription
GM-CSF:	Granulocyte/Macrophage Colony Stimulating-Factor	TARC:	Thymus and Activation Regulated Chemokine
ICAM:	intercellular adhesion molecule	TGF- β :	Transforming Growth Factor beta
IDEC:	Inflammatory Dendritic Epidermal Cell	T _H :	T helper
IFN:	Interferon	TNF:	Tumor Necrosis Factor
Ig:	Immunoglobuline	VCAM:	Vascular Cell Adhesion Molecule
IL:	Interleukine	VIT:	Venom Immunotherapy
IS:	Induced Sputum		