REVIEW

Current Understanding of the Molecular Mechanisms of Allergy

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Human efforts towards understanding the mysteries of allergy are more than a century old. It was 1902 when Charles Ritchet described the process of Anaphylaxis, which subsequently earned him the Nobel Prize in Medicine in 1913 [1]. The term "Allergy" was coined by Clemens Freiherr von Pirquet in 1906, when he explained the phenomenon of serum sickness [2]. For several years, numerous scientists endeavored to understand the phenomena related to hypersensitivity. Finally, success came to John Freeman and Leonard Noon in 1911, who injected increasing amount of grass pollen extract subcutaneously, until either the intensity of the hypersensitivity reaction decreased or the response was completely abolished [3]. In today's age of molecular biology, our knowledge of the atopic phenomena is much more enhanced, as we have developed greater understanding of the role of cells, inflammatory mediators and the cell-signaling phenomena. These discoveries have provided novel molecular targets for the advent of new therapies for allergic rhinitis, atopic dermatitis and asthma [4]. Since asthma is known to have a family tendency [5, 6] and a very strong heritable component, much research has been directed towards understanding the genetic basis of the disease. In this regard, the genetic mapping studies have revealed that chromosome 5q31-q33 is very important in the pathogenesis of asthma, as it contains the cytokine gene clusters for interleukin (IL) IL-4, IL-5, IL-9 and IL-13 [7, 8] which, along with other factors, are very strongly implicated in the disease processes of allergies and asthma.

The Major Determinants

IgE is the root cause?

Considering the role of different factors associated with hypersensitivity, the most important seems to be Immunoglobulin E (IgE), as it is characteristically elevated in most of the atopic individuals [9]. Moreover, predisposition to allergies and asthma, as well as an increased tendency to produce IgE in greater numbers [7], is known to run in families. Elevated IgE levels can also be linked with the severity of disease and the hyperresponsiveness of the tracheo-bronchial tree [10]. Since its discovery in the 1960s, the role and modus operandi of IgE has gradually started to become clear. It is known to be produced by B lymphocytes under the influence of IL-4 and IL-13. Furthermore, it binds to high-affinity IgE receptors called Fc epsilon RI on mast cells to induce their degranulation [11, 12]. This binding is responsible for eliciting the classic immediate hypersensitivity reaction, which sets the stored vasoactive mediators free, induces transcription of cytokines and stimulates the synthesis of leukotrienes and prostaglandins. All these factors culminate into bronchial constriction due to edema of the bronchial mucosa, increased mucus production and smooth muscle constriction. This manifests itself as acute airway obstruction, with a drop in Forced Expiratory Volume in one second (FEV_1) [13]. IgE also results in chronic airway inflammation and causes secondary delayed phase of airway obstruction (Late-phase response or LPR) by activating low-affinity IgE receptor CD23 [14], which is expressed on B-lymphocytes, T lymphocytes, macrophages and airway smooth muscle cells [15]. Scientific research has demonstrated the fact that the binding of allergen with specific IgE improves its uptake by CD23-bearing cells for antigen processing and presentation [14, 16, 17]. Moreover, cell-bound CD23 has been shown to be elevated in atopic individuals, which rises with the exacerbation of the disease [18]. It is interesting to note that IgE not only binds to CD 23 and Fc epsilon RI, but also positively regulates their expression on the cells, as it has been shown that cells grown in the presence of IgE express greater number of such receptors [19, 20]. The importance of the role played by IgE in the pathogenesis of allergies and asthma can be deciphered more clearly if we

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consider the therapeutic strategies targeting IgE antibodies. The IgE blockers developed for the treatment of allergies and asthma have been quite successful. A humanized mouse monoclonal antibody called E25 directed against IgE has shown a decrease in early and late allergic responses, decreased eosinophil counts in induced sputum and reduced airway reactivity [21-23]. One investigation regarding the effect of omalizumab, the anti-immunoglobulin E monoclonal antibody, on asthma exacerbations in patients with severe persistent asthma revealed a significant reduction in the rate of asthma exacerbations and total emergency visits by 47% [24, 25]. Moreover, a profound reduction in the circulation of IgE with the use of anti-immuno-globulin E monoclonal antibody has also been demonstrated in recent times. Another study reports a significant reduction in steroid requirements among those patients suffering from severe asthma and requiring oral steroids. In this study, a number of patients were able to completely withdraw oral steroids in comparison with a placebo [23, 26].

The Role of Cells

There is a wide range of cells that participate in allergic reactions and contribute to the allergic response in a variety of ways. Antigen presentation is an important step in the induction of allergic response. Cells performing this function include Dendritic cells and Macrophages. Dendritic cells are the macrophage-like cells, present in the airway epithelium [27], and perform an important function in the initiation of the allergic response. They take up allergens, process them to smaller peptide fragments and migrate to local lymph nodes where they present these antigenic peptides to uncommitted T lymphocytes [28]. This action programmes the production of allergen-specific T cells, which are normally Th1 but can also be Th2 [29]. The fine balance between Th1 and Th2 heavily depends upon the inducing phenomena [30]. As there seems to be a disproportion between Th2 and Th1 cells in the atopic diseases, such as allergic rhinitis and asthma, the specific induced type is extremely important in the pathogenesis of the disease. The proportion between Th1 and Th2 helper T cells may be determined by locally released cytokines [31] and other factors, such as Atrial Natriuretic Peptide [32], whose inhibition with the siRNA has shown considerable reduction in the allergen-induced inflammatory lung damage and eosinophilic infiltration as compared to the control groups [33]. Macrophages are yet another type of antigen presenting cells in the airways, which process allergen for presentation to T lymphocytes. They normally have a 'suppressive' effect on lymphocyte function [34], and they are responsible for directing the immunogenic response in the right direction [35]. However, this phenomenon is usually impaired in asthma. Macrophages may play an important anti-inflammatory role by preventing the development of atopic response [36]. These dendritic cells and macrophages, along with the epithelial cells, fibroblasts and smooth muscle cells of the airway release different mediators of allergy, which bring about the inflammatory changes in the airways. Among these mediators, cytokines are of paramount importance. Some cytokines are released by the Th2 cells and include IL-3, IL-4, IL-5, IL-9 and IL-13. The cytokines released by the Th1 cells are IL-10 and interferon (IFN)-γ. Some cytokines are released by both the subsets, such as IL-12 and granulocyte-macrophage colony-stimulating factor [37]. From the large body of research, it has been revealed that the major players orchestrating the immunogenic response are the Th-2 cytokines.

The cytokines

Interleukin-4

IL-4 is a major cytokine that is produced by the Th-2 cells, which helps in switching B lymphocytes to produce IgE [38] differentiating Th2 cells and releasing IL-5 that is essential for the differentiation of eosinophils. It is known to up-regulate the IgE receptors on the cells surfaces, the low-affinity IgE receptor (Fc epsilon RII) on B lymphocytes and mononuclear phagocytes and the high-affinity IgE receptor (Fc epsilon RI) on mast cells and basophils [39]. IL-4 also induces vascular cell adhesion molecule [VCAM]-1 on vascular endothelium, which helps in the migration of T lymphocytes, monocytes, basophils and eosinophils to inflammatory loci [40-42]. It has been identified as an attractive therapeutic target in the treatment of atopic diseases. There exists an endogenous homeostatic mechanism, which decreases the effects of the IL-4 on the body. The soluble form of the IL-4 receptor (Soluble IL-4R) has been found to be capable of interacting and binding to IL-4, thereby leading to the sequestration of the secreted IL-4. The Soluble IL-4R seems to be a perfect model of

endogenous antagonism. Its recombinant form, called soluble recombinant human IL-4 receptor (rhuIL-4R), is being used in recent times and has been demonstrated to be safe and quite effective for the treatment of allergic diseases [43, 44].

Interleukin-5

Another major cytokine released by the Th-2 helper T Cells is IL-5. It is responsible for differentiation of the eosinophil precursors [45], as well as activation [46] and lengthening of the life span and survival of the mature cell in the tissues [47]. It is well-documented that eosinophils possess the ability to induce tissue damage and are abundantly present in the lungs of asthmatic patients. According to numerous authorities, eosinophilia is considered to be the hallmark of asthma and allergic disorders [48]. Another discovery revealed that IL-5 deficient mice were unable to develop the proper inflammatory response, with significant decrease in the number of peribronchial fibrosis and magnitude of smooth muscle thickening, even after exhibiting three months of continuous challenge with the allergens [49]. Due to the significant role played by IL-5, the anti IL-5 antibodies have also received considerable attention. These antibodies have quite effectively decreased the blood and sputum eosinophil count, but were unable to completely abolish them [50-52], thereby indicating that they are not solely responsible for the allergic events.

Interleukin-9

IL-9 is yet another Th-2 cytokine that mainly stimulates the production of mast cell proteases and the high-affinity IgE receptor [Fc epsilon RIα] [53]. It was found to be related to the enhancement of IL-4-mediated production of IgE in human and murine B cells [54]. It also improves eosinophil maturation along with IL-5 [55]. Furthermore, it was identified to be responsible for upregulating the IL-5 response, potentiating the IL-5-mediated maturation of eosinophil precursors [56, 57] and activating the airway epithelial cells by stimulating the production of mucus and several mediators of inflammation, thereby resulting in the inflammatory response in the airways, bronchial hyperresponsiveness, and mast cell hyperplasia [58-60]. It has also been shown that there is a decrease in the reduction in goblet cell and mast cell responses in IL-9-deficient mice [61]. The IL-9 blocking antibody has been tested to yield promising results in the treatment of asthma, as its intratracheal administration successfully reduced airway hyper-resposiveness and serum IgE level. The treatment also resulted in decrease in the pulmonary eosinophilia, lung damage and goblet cell hyperplasia [62, 63].

Interleukin-13

Another interleukin, IL-13 has also been found in increased levels in the airways of asthmatic individuals [64, 65] and is known to have a major role in the pathogenesis of asthma [66]. It has many similarities with the IL-4 [67], in terms of structure [68], receptors and biological activities. The IL-13 receptors are present on most of the inflammatory cells [69, 70] except for the T-Cells. One study has reported that IL-13 prolongs eosinophil survival and induces their chemotaxis [71]. The investigators also claimed that it increases the secretion of mucus, fibrosis of subepithelial tissue and other physiological abnormalities [72]. Further reports in murine models have also depicted that IL-13 is vital, as well as sufficient on its own for the development of Airway Hyper-responsiveness and that in its absence, the airway remodeling does not develop, even in the presence of a large numbers of eosinophils [73]. It has been reported in mice that anti-IL-13 monoclonal antibodies decreased airway hyperresponsiveness [AHR] and prevented the progression of subepithelial fibrosis and inflammation. It also reversed the mucus hyperplasia to normal levels [74]. A peptide base vaccine has also been tested successfully in murine models of asthma. [75]

Interleukin-12

The role of IL-12 was also found to be extremely important, as it is the regulator of Th1 cell development and is responsible for the determination of the balance between Th1 and Th2 cells [76, 77]. Further publications reveal that it directs the balance in favor of Th1 cells, thus decreasing the hyper-responsiveness and inflammatory response provoked by the allergens in the airways [78, 79]. This makes it an ideal potential drug target, as it may halt the disease progression in the earlier stages and thus might offer a method of altering the asthmatic inflammatory response [80]. However, despite all of these successes, the situation is not so encouraging in the case of human studies. In one study, a subcutaneous IL-12 injection did cause a decrease in blood and sputum eosinophil count but was unable to affect

the airway hyper-responsiveness to histamine or inhaled allergen [81].

Interleukin-18

Another cytokine called IL-18 or IFN-gamma-inducing factor activates NK cells and along with IL-12, polarizes T cells towards Th1 [82]. It also suppresses Th2 cells [83-85] and works in combination with IL-12 for the production of IFN-gamma [86]. IL-18's receptors are mainly expressed on Th-1 type of helper T cells [87]. It is known that the levels of IL-18 are far less in the asthmatic patients as compared to the normal subjects [88]. The synergistic action of the combination of IL-12 and IL-18 has already shown effective activity in inhibiting allergic inflammatory response in the mouse model [89]. However, administration of IL-18 alone can exacerbate the immune response and result in elevated serum IgE, Th2 cytokines and airway eosinophilia in a ragweed mouse model of allergic asthma [84], thereby emphasizing the complex role of IL-12 in the development of the immune response in susceptible individuals.

Interleukin-10, the good cytokine

IL-10 is a powerful cytokine synthesized by macrophages, which exerts its anti-inflammatory action by inhibiting the formation of a number of pro-inflammatory agents that are over-expressed in asthma. It also inhibits antigen presentation and decreases the entrance of inflammatory cells into the airways [90]. Researchers suggest that IL-10 may have a role in the development of tolerance against the allergens [91], especially through the suppression of antigen presentation by dendritic cells in the airways [92]. There are some indications that the IL-10 regulation may be defective in asthmatic individuals. Published reports reveal that IL-10 is effective in suppressing the inflammatory response to allergens in sensitized animals [93, 94]. The IL-10 therapy has also shown promising results in the treatment of asthma [95-97]. Another interesting fact to be brought under consideration is that certain probiotic bacteria possess the ability to induce IL-10 producing regulatory T cells [98], which sheds further light on the possibility of their role in alleviating the condition of allergic patients [99, 100].

Interferon-Gamma: Another good cytokine

*Inter*ieron-gamma is another cytokine which is produced by Th-1 cells and is of paramount importance in developing tolerance against the threatening antigens [101, 102]. It is quite fascinating to note that the defective production of interferon gamma by the allergen triggered neonatal cord blood cells has been found to be associated with increased incidence of atopy in the later age. Therefore, an estimation of interferon gamma production may act as a predictor of probability of an individual developing an atopic response and may help in introducing preventive measures before the development of the disease [103-105].

The role of structural lung cells can never be overlooked, which includes the cells of airway epithelium, smooth muscle and fibroblasts. These cells are an important source of inflammatory mediators, because they are the most abundant cells in the airways and may act as the translators of the environmental stimuli into the inflammatory response through the entrance of leukocytes into the airways, thus orchestrating the inflammation [106]. The airway response to injury is characterized by airway remodeling, which manifests in the form of increased airway smooth muscle mass [107], subepithlial collagen deposition [108] and increased matrix proteins [109]. Increased airway smooth muscle mass correlates with asthma severity and is thought to be the result of hypertrophy with or without hyperplasia [110-112]. These smooth muscles express the high affinity IgE receptors and respond to the release of the IgE [113, 114] by themselves proliferating and releasing the mediators [115]. It has been demonstrated that smooth muscles are the targets for corticosteroid therapy, which effectively inhibits their proliferation and hyperplasia [116-118].

The Chemokines

Various studies have also demonstrated a major role of chemokines in the development of various aspects of the asthmatic response. Chemokines are 8-15kD proteins, which act as important cell signaling molecules for drawing the leukocytes to the site of injury and causing the release of histamine through the activation of different cells [119]. They act on various immune-mediator cells, play a major role in host defense mechanisms and are fundamental for the development, control and operation of the immune system. Chemokines are induced by various cytokines and work in co-ordination with them to produce the inflammatory response in the airways [120, 121]. They have been divided into 4 groups: CXC, CC, C, and CX3C. Among these four, CC

chemokines target a variety of cell types, including macrophages, eosinophils, basophils and dendritic cells. The most important chemokines in the pathogenesis of allergic asthma are the eotaxin subfamily of the CC chemokines, as well as their receptors CC chemokine receptor 3[CCR4]. They have been found to be central regulators of the asthmatic response [122-125], because they act as chemoattractants for the eosinophils, which then in turn leads to airway inflammation through a large array of mechanisms [126-129]. Furthermore, research has demonstrated that the development of Th1 or Th2 type of immune response is also dependent on the presence of specific chemokines, which underlines the importance of the specific immune response in an individual [130]. It has been shown that the chemokine receptors CCR3, CCR4 and CCR8 are present on the Th-2 type of helper T cells [131-133], while CXCR3 and CCR5 are preferentially expressed in human Th1 type of helper T cells [134, 135]. It is worth mentioning here that the CC chemokine receptor 3 antagonists have successfully inhibited the eosinophil migration in murine models, which resulted in the reduction of pulmonary eosinophilic inflammation [136]. Hence, it can be predicted that the development of gene-silencing techniques against various chemokines would open new horizons for the treatment of allergies and asthma in the future [137].

After more than hundred years of research in allergy, this vast discipline still seems to be quite complex, and at times vague. However, it is evident that the cascade of airway responses is the consequence of a constellation of events orchestrated by lymphocytes, macrophages, denritic cells, smooth muscle cells, endothelial cells and eosinophils. These cells unleash a number of mediators that bring about the changes in the airways, ultimately resulting in the condition we call asthma. Although, the various pieces of the allergy and asthma puzzle are yet to be united to form a complete and clear picture, our current understanding and discoveries illustrate ample proof of the fact each and every one of these molecular mediators has unlimited potential to become a drug target in the coming years. It is hoped that in the near future, a thorough understanding and a clearer picture of the role of each of these components in the allergic reaction would lead to the development of tailor-made drug therapies that would enable patients to be treated individually according to their specific allergy and underlying pathological phenomena.

References

1. G. Richet and P. Estingoy. *Hist. Sci. Med.*, **37**, 501 (2003).
2. M. A. Silverstein. *Nature Immunology*, **1**, 453 (2000).
3. M. Jackson. *Stud. Hist. Philos. Biol. Biomed. Sci.*, **34**, 473 (2003).
4. P. J. Barnes. *J. Allergy Clin. Immunol.*, **106**, 5 (2000).
5. S. T. Holgate, M. K. Church, P. H. Howarth, E. N. Morton, A. J. Frew and R. Djukanovic. *Int. Arch. Allergy Immunol.*, **107**, 29 (1995).
6. P. Parronchi, F. Brugnolo, S. Sampognaro and E. Maggi. *Int. Arch. Allergy Immunol.*, **121**, 2 (2000).
7. D. S. Postma, E. R. Bleecker, P. J. Amelung, K. J. Holroyd, J. Xu, C. I. Panhuysen, D. A. Meyers and R. C. Levitt. *N. Engl. J. Med.*, **333**, 894 (1995).
8. D. G. Marsh, J. D. Neely, D. R. Breazeale, B. Ghosh, L. R. Freidhoff, E. Ehrlich-Kautzky, C. Schou, G. Krishnaswamy and T. H. Beaty. *Science*, **264**, 1152 (1994).
9. T. Matsumoto, T. Miike, K. Yamaguchi, M. Murakami, T. Kawabe, and J. Yodoi. *Clin. Exp. Immunol.*, **85**, 288 (1991).
10. H. C. Oettgen and R. S. Geha. *J. Allergy Clin. Immunol.*, **107**, 429 (2001).
11. D. MacGlashan Jr, L.M., J. McKenzie-White, K. Chichester, A. J. Henry, B. J. Sutton and H. J. Gould. *J. Allergy Clin. Immunol.*, **104**, 492 (1999).
12. G. Fattakhova, M. Masilamani, F. Borrego, A. M. Gilfillan, D. D. Metcalfe and J. E. Coligan. *Traffic*, **6**, 673 (2006).
13. P. H. Howarth, S. R. Durham, A. B. Kay and S. T. Holgate. *J. Allergy Clin. Immunol.*, **80**, 703 (1987).
14. M. R. Kehry and L. C. Yamashita. *Proc. Natl. Acad. Sci. U.S. A.*, **86**, 7556 (1989).
15. A. Tsicopoulos and M. Joseph. *Clin. Exp. Allergy.*, **30**, 602 (2000).
16. F. L. van der Heijden, R. J. van Neerven, M. van Katwijk, J. D. Bos and M.L. Kapsenberget. *J. Immunol.*, **150**, 3643 (1993).
17. U. Pirron, T. Schlunck, J. C. Prinz and E.P. Rieber. *Eur. J. Immunol.*, **20**, 1547 (1990).
18. K. M. Muller, M. Rocken, D. Joel, J. Y. Bonnefoy, J. H. Saurat and C. Hauser. *J. Dermatol Sci.*, **2**, 125 (1991).
19. W. T. Lee, M. Rao and D. H. Conrad. *J. Immunol.*, **139**, 1191 (1987).

20. M. Yamaguchi , C. S. Lantz , H. C. Oettgen, I. M. Katona, T. Fleming, I. Miyajima, J. P. Kinet and S. J. Galli. *J. Exp. Med.*, **85**, 663 (1997).
21. J. V. Fahy, H. E. Fleming, H. H. Wong, J. T. Liu, J. Q. Su, Frick RB Jr. and H. A. Boushey. *Am. J. Respir. Crit. Care Med.*, **155**, 1828 (1997).
22. P. M. Jardieu and R. B. Fick Jr. *Int. Arch. Allergy Immunol.*, **118**, 112 (1999).
23. H. Milgrom, R. B. Fick Jr, J. Q. Su, J. Reimann, R. K. Bush, M. L. Watrous and W. J. Metzger. *New. Engl. J. Med.*, **341**, 1966 (1999).
24. J. Bousquet, P. Cabrera, N. Berkman, R. Buhl, S. Holgate, S. Wenzel, H. Fox, S. Hedgecock, M. Blogg and G. Della Cioppa. *Allergy*, **60**, 302 (2005).
25. P. P. Belliveau and M. R. Lahoz. *Disease Management & Health Outcomes* **15**, 65 (2007).
26. E. H. Walters, J. A. Walters and R. Wood-Baker. *Expert Opinion on Pharmacotherpy*, **8**, 585 (2007).
27. P. G. Holt and P. A. Stumbles. *J. Allergy. Clin. Immunol.*, **105**, 421 (2000).
28. N. Bhardwaj. *Springer. Semin. Immun.*, **26**, 215 (2005).
29. P. A. Stumbles, J. A. Thomas, C. L. Pimm, P. T. Lee, T. J. Venaille, S. Proksch, and P. G. Holt. *J. Exp. Med.*, **188**, 2019 (1998).
30. W. W. Busse and R. F. Lemanske Jr., Asthma. *N. Engl. J. Med.*, **344**, 350 (2001).
31. T. R. Mosmann and R. L. Coffman. *Ann. Rev. Immuno.*, **7**, 45 (1989).
32. R. Morita, N. Ukyo, M. Furuya, T. Uchiyama and T. Hori. *A. J. Immunol.*, **170**, 5869 (2003).
33. X. Wang, W. Xu, X. Kong, S. Shirley, R. Lockey and S. Mohapatra. *Journal of Allergy and Clinical Immunology* **119**, S131 (2007).
34. M. A. Spiteri, R. A. Knight, J. Y. Jeremy, P. J. Barnes and K. F. Chung. *Eur. Resp. J.*, **7**, 1431 (1994).
35. C. Tang, M. D. Inman, N. van Rooijen, P. Yang, H. Shen, K. Matsumoto and P. M. O'Byrne. *J. Immunol.*, **166**, 1471 (2001).
36. J. L. M. Vissers, B. C. A. M. van Esch, G. A. Hofman and A. J. M. van Oosterhout. *Eur. Respir. J.*, **26**, 1040 (2005).
37. P. M. O'Byrne. *Chest*, **130**, 244 (2006).
38. R. L. Coffman, J. Ohara, M. W. Bond, J. Carty, A. Zlotnik and W. E. Paul. *J. Immunol.*,**136**, (1986).
39. R. Pawankar, M. Okuda, H. Yssel, K. Okumura and C. Ra. *J. Clin. Invest.* **99**, 1492 (1997).
40. R. Moser, J. Fehr and P. L. Bruijnzeel. *J. Immunol.*, **149**, 1432 (1992).
41. J. T. Kaifi, E. Diaconu, and E. Pearlman. *J. Immunol.*, **166**, 6795 (2001).
42. K. Y. Larbi, A. R. Allen, F. W. K. Tam, D. O. Haskard, R. R. Lobb, P. M. R. Silva and S. Nourshargh. *Blood*, **96**, 3601. (2000).
43. L. Borish, H. Nelson, M. Lanz, L. Claussen, J. Whitmore, J. Agosti and L. Garrison. *Am J. Respir Crit. Care. Med.*, **160**, 1816 (1999).
44. L. Borish, H. Nelson, J. Corren, G. Bensch, W. Busse, J. Whitmore and J. Agosti. *J. Allergy. Clin. Immunol.*, **105**, A828 (2000).
45. Y. Yamaguchi, T. Duda, J. Suda, M. Eguchi, Y. Miura, N. Harada, A. Tominaga and K. Takatsu. *J. Exp. Med.*, **167**, 43 (1988).
46. R. Sehmi, A. J. Wardlaw, O. Cromwell, K. Kurihara, P. Waltmann and A. B. Kay. *Blood*, **79**, 2952 (1992).
47. M. E. Rothenberg, J. Petersen, R. L. Stevens, D. S. Silberstein, D. T. McKenzie, K. F. Austen and W. F. Owen. *J. Immunol.*, **143**, 2311 (1989).
48. Timothy J. Williams. *J. Clin. Invest.*, **113**, 507 (2004).
49. J. Y. Cho, M. Miller, K. J. Baek, J. W. Han, J. Nayar, S. Y. Lee, K. McElwain, S. McElwain, S. Friedman, and D. H. Broide. *J. Clin. Invest.*, **113**, 551 (2004).
50. M. J. Leckie, A. ten Brincke, J. Khan, Z. Diamant, B. J. O'Connor, C. M. Walls, A. K. Mathur, H. C. Cowley, K. F. Chung, R. Djukanovic, T. T. Hansel, S. T. Holgate, P. J. Sterk and P. J. Barnes. *Lancet*, **356**, 2144 (2000).
51. J. C. Kips, B. J. O'Connor, S. J. Langley, A. Woodcock, H. A. M. Kerstjens, D. S. Postma, M. Danzig, F. Cuss and R. A. Pauwels. *Am. J. Respir. Crit. Care. Med.* **167**, 1655 (2003).
52. J. M. Oldhoff, U. Darsow, T. Werfel, K. Katzer, A. Wulf, J. Laifaoui, D. J. Hijnen, S. Plötz, E. F. Knol, A. Kapp, C. A. F. M. Bruijnzeel-Koomen, J. Ring and M. S. de Bruin-Weller. *Allergy* **60**, 693 (2005).
53. J. Louahed, A. Kermouni, J. Van Snick and J. C. Renauld. *J. Immunol.*, **154**, 5061 (1995).
54. B. Dugas, J. C. Renauld, J. Pene, J. Y. Bonnefoy, C. Peti-Frere, P. Braquet, J. Bousquet, J.Van Snick and J. M. Mencia-Huerta. *Eur. J. Immunol.*, **23**, 1687 (1993).
55. J. Louahed, Y. Zhou, W. L. Maloy, P. U. Rani, C. Weiss, Y. Tomer, A. Vink, J. C. Renauld, J. Van Snick, N. C. Nicolaides, R. C. Levitt and A. Haczku. *Blood*, **97**, 1035 (2001).

56. A. S. Gounni, B. Gregory, E. Nutku, F. Aris, K. Latifa, E. Minshall, J. North, J. Tavernier, R. Levitt, N. Nicolaides, D. Robinson and Q. Hamid. *Blood*, **96**, 2163 (2000).
57. R. C. Levitt, M. P. McLane, D. MacDonald, V. Ferrante, C. Weiss, T. Zhou, K. J. Holroyd and N. C. Nicolaides. *J. Allergy Clin. Immunol.*, **103**, S485 (1999).
58. Q. Dong, J. Louahed, A. Vink, C. D. Sullivan, C. J. Messler, Y. Zhou, A. Haczku, F. Huaux, M. Arras, K. J. Holroyd, J. C. Renauld, R. C. Levitt and N. C. Nicolaides. *Eur. J. Immunol.*, **29**, 2130 (1999).
59. J. Louahed, M. Toda, J. Jen, Q. Hamid, J. C. Renauld, R. C. Levitt and N. C. Nicolaides. *Am. J. Respir. Cell Mol. Biol.*, **22**, 649 (2000).
60. U. A. Temann, G. P. Geba, J. A. Rankin and R. A. Flavell. *J. Exp. Med.*, **188**, 1307 (1998).
61. J. M. Townsend, G. P. Fallon, J. D. Matthews, P. Smith, E. H. Jolin and N. A. McKenzie. *Immunity*, **13**, 573 (2000).
62. M. P. McLane, J. Trepper, C. Weiss, Y.Tomer, P. Tobin, D. Tumas, Y. Zhou, A. Haczku, N.C. Nicolaides and R.C. Levitt. *Am. J. Respir. Crit. Care Med.*, **161**, A593 (2000).
63. T. T. Kung, B. Lou, Y. Crawley, C. G. Garlisi, K. Devito, M. Minnicozzi, R. W. Eqan, W. Kreutner and R. W. Chapman. *Am. J. Respir. Cell Mol. Biol.*, **25**, 600 (2001).
64. M. Humbert, S. R. Durham, P. Kimmitt, N. Powell, B. Assoufi, R. Pfister, G. Menz, A. B. Kay and C. J. Corrigan, *J. Clin. Immunol.*, **99**, 657 (1997).
65. S. K. Huang, H. Q. Xiao, J. Kleine-Tebbe, G. Paciotti, D. G. Marsh, L. M. Lichtenstein and M. C. Liu. *J. Immunol.*, **155**, 2688 (1995).
66. M. Wills-Karp, J. Luyimbazi, X. Xu, B. Schofield, T. Y. Neben, C.L. Karp and D.D. Donaldson. *Science*, **282**, 2258 (1998).
67. R. E. Callard, D. J. Matthews and L. Hibbert. *Immunol. Today*, **17**, 108 (1996).
68. J. De Vries. J. *Allergy Clin. Immunol.*, **102**, 165 (1998).
69. P. Graber, D. Gretener, S. Herren, J. P. Aubry, G. Elson, J. Poudrier, S. Lecoanet-Henchoz, S. Alouani, C. Losberger, J. Y. Bonnefoy, M. H. Kosco-Vilbois and J. F. Gauchat. *Eur. J. Immunol.*, **28**, 4286 (1998).
70. C. E. Brightling, F. A. Symon, S. T. Holgate, A. J. Wardlaw, I. D. Pavord and P. Bradding. *Clinical and Experimental Allergy*, **33**, 1711 (2003).
71. S. Horie, Y. Okubo, M. Hossain, E. Sato, H. Nomura, S. Koyama, J. Suzuki, M.Isobe and M. Sekiguchi. *Internal Medicine*, **36**, 179 (1997).
72. Z. Zhu, R.J. Homer, Z. Wang, Q. Chen, G.P. Geba, J. Wang, Y. Zhang and J. Elias. *J. Clin. Invest.*, **103**, 779 (1999).
73. D. M. Walter, J. J. McIntire, G. Berry, A. N. J. McKenzie, D. D. Donaldson, R. H. Dekruyff and D. T. Umetsu. *J. Immunol.*, **167**, 4668 (2001).
74. G. Yang L. Li , A. Volk, E. Emmell, T. Petley, J. Giles-Komar, P. Rafferty, M. Lakshminaryanan, D. E. Griswold, P. J. Bugelski and A. M. Das. *J. Pharmacol. Exp. Ther., 313*, 8 (2005).
75. Z. Peng, Y. Ma, Y. Fan, G. Srinivasan, X. Yang, A. Becker, F. Simons and K HayGlass, *J. Allergy Clin. Immunol.*, **117**, 250 (2006).
76. M. K. Gately, L. M. Renzetti, J. Magram, A. S. Stern, L. Adorini, U. Gubler and D. H. Presky. *Annu. Rev. Immunol.*, **16**, 495 (1998).
77. J. Magram, S.E. Connaughton, R. R. Warrier, D. M. Carvajal, C. Wu, J. Ferrante, C. Stewart, U. Sarmiento, D. A. Faherty and M.K. Gately. *Immunity*, **4**, 471 (1996).
78. S. H. Gavett, D. J. O'Hearn, X Li, S. -K Huang, F. D. Finkelman and M. Wills-Karp. *J. Exp. Med.*, **182**, 1527 (1995).
79. J. C. Kips, G. J. Brusselle, G. F. Joos, R. A., Peleman, J. H., Tavernier, R. R., Devos and R. A. Pauwels. *Amer. J. Respir. Crit. Care Med.*, **153**, 535 (1996).
80. M. Wills-Karp. *Allergy*, **53**, 113 (1998).
81. S. A. Bryan, B. J. O'Connor, S. Matti, M. J. Leckie, V. Kanabar, J. Khan, S. J. Warrington, L. Renzetti , A. Rames, J. A. Bock , M. J. Boyce, T. T. Hansel, S. T. Holgate and P. J. Barnes. *Lancet*, **356**, 2149 (2000).
82. H. Okamura, S. Kashiwamura, H. Tsutsui, T. Yoshimoto and K. Nakanishi. *Curr. Opin. Immunol.*, **10**, 259 (1998).
83. K. Kohno and M. Kurimoto. *Immunol. Today*, **17**, 138 (1996).
84. C. L. Hofstra, I. V. Ark, G. Hofman, M. Kool, F. P. Nijkamp and A. J. M. Van Oosterhout. *J. Immunol.*, **161**, 5054 (1998).
85. I. P. Lewkowich, J. D. Rempel and K. T. HayGlass. *Immunol.*, **175**, 4956 (2005).
86. K. Tominaga, T. Yoshimoto, K. Torigoe, M. Kurimoto, K. Matsui, T. Hada, H. Okamura and K. Nakanishi. *Int. Immunol.*, **12**, 151 (2000).
87. D. Xu, W. L. Chan, B. P. Leung, D. Hunter, K. Schulz, R. W. Carter, I. B. McInnes, J. H Robinson and F. Y. Liew. *J. Exp. Med.*, 19

1485 (1998).
88. L. P. Ho, M. Davis, A. Denison, F. T. Wood and A. P. Greening. *Chest*, **121**, 1421 (2002).
89. J. S. Wild, A. Sigounas, N. Sur, M. S. Siddiqui, R. Alam, M. Kurimoto and S. Sur. *J. Immunol.*, **164**, 2701 (2000).
90. C. Zuany-Amorim, S. Haile, D. Leduc, C. Dumarey, M. Huerre, B. B. Vargaftig and M. Pretolani. *J. Clin. Invest.*, **95**, 2644 (1995).
91. A. H. Enk, V. L. Angeloni, M. C. Udey and S. I. Katz. *J. Immunol.*, **151**, 2390 (1993).
92. S. Bhattacharyya, P. Sen, M. Wallet, B. Long, A. S. Baldwin, Jr. and R. Tisch. *Blood*, **104**, 1100 (2004).
93. S. Lim, E. Crawley, P. Woo and P. J. Barnes. *Lancet*, **352**, 113, (1998).
94. L. Borish, A. Aarons, J. Rumbyrt, P. Cvietusa, J. Negri and S. Wenzel. *J. Allergy Clin. Immunol.*, **97**, 1288 (1996).
95. M. Pretolani and M. Goldman. *Immunol. today*, **18**, 2771 (1997).
96. C. A. Akdis, T. Blesken, M. Akdis, B. Wuthrich and K. Blaser. *J. Clin. Invest.*, **102**, 98 (1998).
97. M. Jutel, M. Akdis, F. Budak, C. Aebischer-Casaulta, M. Wrzyszcz, K. Blaser and C. A. Akdis. *European Journal of Immunology*, **33**, 1205 (2003).
. H. H. Smits, A. Engering, D. van der Kleij, E. C. de Jong, K. Schipper, T. M. van Capel, B. A. Zaat, M. Yazdanbakhsh, E. A. Wierenga, Y. van Kooyk and M. L. Kapsenberg. *J. Allergy Clin. Immunol.*, **115**, 1260 (2005).
99. M. F. Wang,Y.Y. Wang, H.C. Lin and C. H. Hsu. *Pediatric Allergy and Immunology*, **15**, 152 (2004).
100. P. V. Kirjavainen, S. *J* Salminen and E. Isolauri. *Journal of Pediatric Gastroenterology & Nutrition*, **36**, 223 (2003).
101. G. Lack, K. L. Bradley, E. Hamelmann, H. Renz, J. Loader, D. Y. Leung, G. Larsen and E. W. Gelfand. *J. Immunol.*, **157**, 1432 (1996).
102. M Boguniewicz, R. J Martin, D Martin, U Gibson and A. Celniker. *J. Allergy Clin. Immunol.*, **95**, 133 (1995).
103. J. A. Warner, E. A. Miles, A. C. Jones, D. J. Quint, B. M. Colwell and J. O. Warner. *Clin. Exp. Allergy*, **24**, 423 (1994).
104. N. Kondo, Y. Kobayashi, S. Shinoda, R. Takenaka, T. Teramoto, H. Kaneko, T. Fukao, E. Matsui, K. Kasahara and Y. Yokoyama. *Clin. Exp. Allergy*. **28**, 1340 (1998).
105. M. L. Tang, A. S. Kemp, J. Thorburn and D. J. Hill. *Lancet*, **344**, 983 (1994).
106. J. L. Devalia and R. J. Davies. *Resp. Med.*, **6**, 405 (1993).
107. A. B. Kay. *Am. J. Respir. Crit. Care Med.*, **154**, S66 (1996).
108. W. R. Roche, R. Beasley, J. H. Williams and S. T. Holgate. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet*, **1**, 520 (1989).
109. P. R. A. Johnson, J. K. Burgess, P. A. Underwood, W. Au, M. H. Poniris, M. Tamm, Q. Ge, M. Roth and J. L. Black. *J. Allergy. Clin. Immunol.*, **113**, 690 (2004).
110. M. Ebina, T. Takahashi, T. Chiba and M. Motomiya. *Am. Rev. Respir. Dis.* **148**, 720 (1993).
111. L. Benayoun, A. Druilhe, M. C. Dombret, M. Aubier and M. Pretolani. *Am. J. Respir. Crit. Care Med.*, **167**, 1360 (2003).
112. P. G. Woodruff, G. M. Dolganov, R. E. Ferrando, S. Donnelly, S. R. Hays, O. D. Solberg, R. Carter, H. H. Wong, P. S. Cadbury and J. V. Fahy. *Am. J. Respir. Crit. Care Med.* **169**, 1001 (2004).
113. A. S. Gounni. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **291**, L312 (2006).
114. A. S. Gounni, V. Wellemans, J. Yang, F. Bellesort, K. Kassiri, S. Gangloff, M. Guenounou, A. J. Halayko, Q. Hamid and B. Lamkhioued. *J. Immunol.*, **175**, 2613 (2005).
115. N. J. Vanacker, E. Palamans, J. C. Kips, and R. A. Pauwels. *Am. J. Respir. Crit. Care Med.*, **163**, 674 (2001).
116. I. S. T. Bos, R. Gosens, A. B. Zuidhof, D. Schaafsma, A. J. Halayko, H. Meurs and J. Zaagsma. *Eur. Respir. J.* **30**, 653 (2007).
117. M. Miller, J. Y. Cho, K. McElwain, S. McElwain, J. Y. Shim, M. Manni, J. S. Baek, and D. H. Broide. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **290**, L162 (2006).
118. R. Tomic, C. C. Lassiter, J. D. Ritzenthaler, H. N. Rivera, and J. Roman. *Chest*, **127**, 257 (2005).
119. A. Zlotnik and O.- Yoshie. *Immunity*, **12**, 121 (2000).
120. N. Zimmermann, G. K. Hershey, P. S. Foster and M. E. Rothenberg. *J. Allergy Clin. Immunol.*, **111**, 227 (2003).
121. C. Stellato, S. Matsukura, A. Fal, J. White, L. A. Beck, D. Proud and R. P. Schleimer. *J. Immunol.*, **163**, 5624 (1999).
122. E. A. Garcia-Zepeda, M. E. Rothenberg, R. T. Ownbey, J. Celestin, P. Leder and A. D. Luster.

Nat. Med., **2**, 449 (1996).

123. E. M. Minshall, L. Cameron, F. Lavigne, D. Y. Leung, D. Hamilos, E. A. Garcia-Zepada, M. Rothenberg, A. D. Luster and Q. Hamid. *Am. J. Respir. Cell Mol. Bio.*, **17**, 683 (1997).
124. A. D. Luster and M. E. Rothenberg. *J. Leukoc. Biol.* **62**, 620 (1997).
125. H. Heath, S. Qin, P. Rao, L. Wu, G. LaRosa, N. Kassam, P. D. Ponathe and C. R. Mackay. *J. Clin. Invest.*, **99**, 178 (1997).
126. N. Zimmermann, B. L. Daugherty, J. M. Stark and M. E. Rothenberg. *J. Immunol* **164**, 1055 (2000).
127. P. C. Fulkerson, C. A. Fischetti, M. L. McBride, L. M. Hassman, S. P. Hogan and M. E. Rothenberg. *Proc. Natl. Acad. Sci. U. S. A.*, **31**, 16418 (2006).
128. M. E. Rothenberg. Eosinophils in the new millennium. *J. Allergy Clin. Immunol.*, **119**, 1321 (2007).
129. H. H. Shen, S. I. Ochkur, M. P. McGarry, J. R. Crosby, E. M. Hines, M. T. Borchers, H. Wang, T. L. Biechelle K. R., O'Neill, T. L. Ansay, D. C. Colbert, S. A. Cormier, J. P. Justice and N. A. Lee. *J. Immunol.*, **170**, 3296 (2003).
130. O'Garra, L. M. McEvoy and A. Zlotnik. *Curr. Biol.*, **8**, R646 (1998).
131. Zingoni, H. Soto, J. A. Hedrick, A. Stoppaciaro, C. T. Storlazzi, F. Sinigaglia, D. D'Ambrosio, A. O'Garra, D. Robinson, M. Rocchi, A. Santoni, A. Zlotnik and M. M. Napolitano. *J. Immunol.* **161**, 547 (1998).
132. B. O. Gerber, M. P. Zanni, M. Uguccioni, M. Loetscher, C. R. Mackay, W. J. Pichler, N. Yawalkar, M. Baggiolini and B. Moser. *Curr. Biol.* 7, 836 (1997).
133. F. Sallusto, C. R. Mackay and A. Lanzavecchia. *Science,* **277**, 2005 (1997).
134. P. Loetscher, M. Uguccioni, L. Bordoli, M. Baggiolini and B. Moser. *Nature,* **391**, 344 (1998).
135. R. Bonnechi, G. Bianchi, P. P. Bordingnon, D. D'Ambrosio, R. Lang, A. Borsatti, S. Sozzani, P. Allavena, P. A. Gray, A. Mantovani and F. Sinigaglia. *J. Exp. Med.*, **187**, 129 (1998).
136. A. M. Das, K. G. Vaddi, K. A. Solomon, C. Krauthauser, X. Jiang, K. W. McIntyre, X. X. Yang, E. Wadman, P. Welch, M. Covinqton, D. Graden, K. Yeleswaram, J. M. Trzaskos, R. C. Newton, S. Mandlekar, S. S. Ko, P. H. Carter and P. Davies. *J. Pharmacol Exp. Ther.* **318**, 411 (2006).
137. K. L. Teixeira, P. F. B. Fonseca, B. A Barboza, A. Bianca and P. B. J. Viola. *Memorias do Instituto Oswaldo Cruz.*, **100**, 1 (2005).