

● Summary

Immune modulators can shape the host response to challenge with innocuous antigens and protect against allergic airway inflammation in asthma and possibly other allergic diseases. Among several potential modulators, Flt3-ligand is a novel agent, which prevented and reversed LAR, AHR, and eosinophilia in a murine asthma model. Flt3-ligand induces the generation and peripheral scattering of dendritic cells with preferential expansion of CD8 α ⁺ and IL-12 producing dendritic

Immune Modulators in Allergic Airway Inflammation

DK Agrawal, MT Hopfenspirger and J Edwan

Center for Allergy, Asthma and Immunology, Creighton University School of Medicine
Omaha, NE 68178, USA

a. IL-4: In two separate studies, recombinant human soluble IL-4 receptor (sIL-4R) has been used to selectively neutralize endogenously

antibodies to IL-5 was observed in mucosal eosinophilia (7). Therefore, more studies are warranted to modulate tissue eosinophilia in asthmatic airways. In addition, since eosinophils are an important source of TGF- β , they might be associated with structural remodeling in chronic asthma.

c. IL-9: CD4⁺ T cells, human eosinophils and mast cells produce IL-9. This cytokine promotes the proliferation and differentiation of mast cells and hematopoietic progenitors, stimulates the proliferation of activated T cell, enhances the production of IgE by B cells, and can increase the expression of the α chain of high affinity IgE receptors. In a recent study in a murine model of allergic airway inflammation, anti-IL-9 antibody given intravenously attenuated pulmonary eosinophilic inflammation, T_H2 cytokine production, T_H2-specific chemokine expression in the airways, and AHR (8). These data suggest that blocking antibodies to IL-9 have a potential therapeutic role in the treatment of human asthma.

d. IL-10: IL-10 is a potent anti-inflammatory cytokine and is produced by regulatory T cells (T_R cells). IL-10 inhibits the production of IL-12 and TNF- α from activated macrophages and regulates both innate and specific cell-mediated immunity. This cytokine inhibits the synthesis of several cytokines including TNF- α , IL-5, iNOS and GM-CSF. In a pre-clinical study in a murine model of allergic airway inflammation intranasally administered recombinant murine IL-10 inhibited antigen-induced recruitment of neutrophils and eosinophils into the airways of ovalbumin-sensitized mice (9). In another study, IL-10 prevented the infiltration of antigen-induced CD4⁺ T lymphocytes and eosinophils as well as IL-5 release (10). Indeed, many currently available therapies including theophylline (11), corticosteroids (12), and immunotherapy (13) have been reported to increase IL-10



Devendra K. Agrawal is Professor of Medicine, Medical Microbiology and Immunology and Biomedical Sciences, Creighton University School of Medicine, Omaha, NE, USA. He received M.Sc. (1973) in Chemistry and Ph.D. (1978) in Biochemistry from Lucknow University, India followed by another Ph.D. (1984) in Medical Sciences from McMaster University, Canada. He is a fellow of the American Academy of Allergy, Asthma and Immunology, and a fellow of the American Heart Association. His current research interest focuses on the immunobiology of pulmonary and vascular diseases with particular emphasis on bronchial asthma, restenosis and plaque rupture.
dkagr@creighton.edu

cells. Additionally, Flt3-ligand may also enhance CD4⁺CD25⁺ regulatory T cells in the lungs. Flt3-ligand may prove to be a novel adjuvant therapy in bronchial asthma.

In view of numerous immune cells and mediators that contribute to the exacerbations and progress of asthma, it is not surprising that there are likewise numerous potential modalities to treat the disease. Since the dynamic of the T_H1/T_H2 phenotype as it relates to allergy and asthma has become more fully appreciated, studies have been focused to modulate this balance with the goal of suppressing type 2 responses (1,2). Largely, these efforts have focused on stimulating antigen-specific T_H1 responses, as the T-helper subsets have been shown to be polarizing and mutually antagonistic in nature (3).

This article focuses primarily on the strategies and approaches that have been attempted to develop novel and effective therapy of asthma by modulating the immune response.

● 1. Modulation of Receptor-response Coupling to Cytokines

Several studies have been reported using antibodies to a cytokine, soluble receptor, or cytokine itself to prevent and/or treat allergic airway inflammation.

produced IL-4 and thus inhibiting its effects. In double blind placebo-controlled studies, sIL-4R prevented the fall in lung function (maintained FEV1 and forced expiratory flow and stabilized asthma symptom scores) induced by withdrawal of corticosteroids in patients with moderate asthma (4). However, the long-term effect of such therapy with sIL-4R requires further investigation. Furthermore, it will be important to demonstrate anti-inflammatory effects of sIL-4R in allergic airway inflammation and airway remodeling in chronic asthma.

b. IL-5: Although preclinical studies in experimental models showed very promising effect of anti-IL-5 antibodies (5), clinical studies with a single intravenous injection of humanized monoclonal antibody (SB 240563) to IL-5 in moderate to severe asthmatics have been disappointing (6). In the later study, antibodies to IL-5 significantly attenuated sputum and blood eosinophilia. However, there was no significant effect of monoclonal antibody to IL-5 on the late asthmatic response or on AHR to histamine. Interestingly, the authors did not examine the effect of anti-IL-5 antibodies on tissue eosinophilia in the bronchial mucosa. Recently no significant effect of the monoclonal

concentrations. These data suggest a potential therapeutic role of IL-10 in the treatment of allergic airway inflammation and associated changes in pulmonary functions. However, clinical studies with recombinant human IL-10 are clearly warranted to prove its therapeutic efficacy in human allergic asthma.

e. IL-12: Antigen-presenting cells such as macrophages and dendritic cells primarily produce IL-12. This cytokine modulates the differentiation of T_H0 cells towards IFN- γ producing T_H1 cells and determines the balance between T_H1 and T_H2 cells. In allergic airway inflammation model in mice, administration of IL-12 effectively inhibited AHR, BAL and tissue eosinophilia, and serum IgE levels (14). In patients with mild asthma, weekly administration of rhIL-12 in escalating doses over 4 weeks had no significant effect on early or late allergic response to antigen challenge and had no attenuation in the AHR (15). In addition, although rhIL-12 reduced eosinophils suggesting its effect on immune deviation, it elicited several toxic effects including flu like syndrome; transient increases in serum hepatic transaminases and cardiac arrhythmia. This suggests that IL-12 therapy might not be suitable for the treatment in asthma. However, it is possible that IL-12 might be beneficial as an adjuvant in conjunction with allergen immunotherapy.

f. IL-13: Although IL-13 receptors consist of the IL-13R $\alpha1$ and $\alpha2$ chains, this cytokine induces its cellular effect via a shared surface receptor, IL-4R α chain. Receptor-response coupling to both IL-4 and IL-13 cytokines is largely dependent on signal transducers and activator of transcription-6 (STAT6). In a murine model, soluble IL-13R $\alpha2$ has been shown to be effective in attenuating AHR, pulmonary eosinophilia and IgE production (16).

g. IL-18: Antigen presenting cells in response to microbial products produce IL-18, which stimulates the production of IFN- γ from T cells and NK cells. IL-18, an IFN- γ -releasing cytokine, is a structural homolog of IL-1 and involves toll-like receptors to elicit response. In conjunction with IL-12, IL-18 induces cell-mediated immunity. Therefore, it is reasonable to speculate its role in regulating T_H1/T_H2 balance in the airways. Indeed, coadministration of

IL-12 and IL-18 inhibited antigen-induced AHR, eosinophilia, and serum IgE levels in an animal model (17). However, its potential effect in human asthma requires further investigation.

h. Interferons: Both IL-12 and IL-18 are potent inducers of Interferon- γ (IFN- γ) production from T_H1 cells. IFN- γ primarily activates macrophages and is involved in both innate and adaptive immunity. IFN- γ suppresses the activity of T_H2 cells and inhibits the IL-4-induced IgE and IgG4 synthesis by B cells in mice. Therefore, it is reasonable to speculate that the administration of IFN- γ might be useful in the treatment of allergic airway inflammation and associated change in pulmonary functions. In a murine model, IFN- γ was effective in inhibiting the secondary development of secondary allergic responses (18). However, nebulized IFN- γ did not reduce eosinophilic inflammation in asthmatic subjects (19).

● 2. Mycobacterial Antigens

Increased childhood infections may, paradoxically, protect against asthma, a concept known as the 'hygiene hypothesis'. Many studies in human or experimental animals support the notion that the delivery of immune modulators such as the BCG vaccine in early life will facilitate and reestablish conditions for the rapid development of type 1 T cell function during early postnatal life and balance type 1/type 2 T cell response.

A study of the childhood antecedents of allergic sensitization in young British adults, with asthma or asthmatic-like complaints and controls, concluded that factors related to small families and relative affluence in childhood promote atopic sensitization (20). These observations are consistent with the suggestion that early infection may protect against subsequent allergic disease. Thus, children with older siblings and a larger number of siblings and an increased number of infections in early life have reduced allergic symptoms and allergen sensitization. Respiratory syncytial virus infection in early childhood or infancy may increase the risk for subsequent allergen sensitization and asthma. Measles infections, however, are associated with a reduced risk of asthma and allergen sensitization. In Japan, Shirakawa and colleagues (21)

observed predominantly a type 1 T cell response in PPD positive children whereas type 2 profiles was seen in children with negative tuberculin skin test. BCG vaccination at birth induced a memory T_H1 -type response, suggesting that human newborns can be immunized against pathogens controlled by a type 1 immune response. Alternatively, it is also possible that in the findings of Shirakawa and colleagues (21) atopic state itself may result in a suppression of delayed hypersensitivity to the PPD skin test. However this could be refuted by the observations of Marchant and colleagues (22), who observed that BCG vaccination at birth induced a memory Th1-type response, suggesting that human newborns can be immunized against pathogens controlled by a T_H1 immune response. In another study BCG vaccination during the first week of life was associated with a decreased risk of atopy in children in Guinea-Bissau (23). In a recent study while BCG vaccination and serum IgE did not correlate, BCG-vaccinated children did show lower rates of atopic sensitization and clinical manifestations before the 2nd year of life (24). In contrast, other investigators observed no correlation between tuberculin response and atopy (25,26). The exact reason for discrepancy in these studies is not clear. However, this could be related to the differences in the atopic status of the children from high-risk vs. low-risk allergy families, timing of BCG vaccination, exposure of children to environmental mycobacterium, and variations in the strain of BCG vaccination.

Erb and Holloway (27) demonstrated that BCG infection of the lung strongly inhibits the development of airway eosinophilia. They showed that the BCG infection-induced inhibition of airway eosinophilia was strongly inhibited in IFN- γ receptor deficient mice. Sano and colleagues (28) also reported the inhibitory role of IFN- γ in *M. tuberculosis*-induced regulation of eosinophilia. These investigators also observed that exposure to an allergen along with *M. tuberculosis* switched development of allergen-specific T cells towards a T_H1 phenotype, which in turn, down-regulated allergic manifestations in an antigen-specific manner.

All these studies in human or experimental animals support

the notion that BCG vaccination in early life will facilitate and reestablish conditions for the rapid development of T_H1 functions during early postnatal life. This is a necessary step for the adaptation of the fetus to outside world, and for the establishment of a balanced T_H1/T_H2 response, in which both components of the response system are present and reciprocally control the over-expression of each other. These studies suggest that *specific* childhood infections may modulate the immune system to either increase or decrease the risk of allergen sensitization and asthma. In 1975, McGeady and Buckley (29) demonstrated that in a group of 16 children with atopic eczema, who also had asthma, there was decreased cellular immune response to antigen stimulations such as tuberculin skin test, *Candida*, and other delayed type cellular antigens. This observation needs to be studied prospectively. BCG vaccine stimulates a T_H1 response with increased IFN- γ and IL-12, which in turn inhibits cytokines from T_H2 like cells and also inhibits proliferation of T_H2 like cells. Holt et al (30) showed that production of IFN- γ by mitogen stimulated $CD4^+$ T-cell clones was low in babies and young children, and was significantly lower in babies born to families with a history of allergic disease. Thus, a low level of T_H1 cytokine, IFN- γ may predispose to a risk for an allergic disease. By stimulating the Th1 response with BCG vaccine and thus increasing IFN- γ and IL-12, it may be possible to decrease the T_H2 profile seen in allergic persons.

Very recently Umetsu and colleagues (31) reported that not only mycobacterial antigens, other infections such as with hepatitis A virus, gastrointestinal pathogens including *Helicobacter pylori* and *Toxoplasma gondii* are strongly associated with protection against the development asthma. Human *Tim-1*, a newly identified asthma susceptibility gene, is selectively expressed on T_H2 cells. Hepatitis A virus may elicit significant effect on T_H2 differentiation and survival of T_H2 cells. It is therefore possible that *Tim-1* by directly interacting with hepatitis A virus may alter the T_H balance of the infected individual to protect against atopy and asthma (31).

We have recently reported that in a mouse model of allergic airway inflammation both BCG and *M. vaccae*

significantly attenuated late response, AHR to methacholine, and airway eosinophilia (32). We have also reported that a single-dose of mycobacterial treatment can suppress the LAR, AHR to methacholine and BAL IL-5 and eosinophilia in pre-sensitized mice without affecting serum IgE levels (33). Recently, Zuany-Amorim and colleagues (34) reported that killed *Mycobacterium vaccae*-suspension suppressed airway eosinophilia and this effect was mediated through IL-10 and TGF- β released from the activity of $CD4^+CD45RB^{lo}$ regulatory T cells. These studies suggest that *specific* childhood infections may modulate the immune system to either increase or decrease the risk of allergen sensitization and asthma. Drs. Steven McGeady and Rebecca Buckley at Duke University demonstrated that in a group of 16 children with atopic eczema, who also had asthma, there was decreased cellular immune response to antigen stimulations such as tuberculin skin test, *Candida*, and other delayed type cellular antigens (29). This observation needs to be studied prospectively. BCG vaccine stimulates a T_H1 response with increased IFN- γ and IL-12, which in turn inhibits cytokines from T_H2 like cells and also inhibits proliferation of T_H2 like cells. Holt et al (30) showed that production of IFN- γ by mitogen stimulated $CD4^+$ T-cell clones was low in babies and young children, and was significantly lower in babies born to families with a history of allergic disease. Thus, a low level of T_H1 cytokine, IFN- γ may predispose to a risk for an allergic disease. By stimulating the Th1 response with BCG vaccine and thus increasing IFN- γ and IL-12, it may be possible to decrease the T_H2 profile seen in allergic persons. While more investigation is needed to define the durability of this effect, these results support the hypothesis that mycobacterial antigens may be effective immunotherapeutic agents in bronchial asthma.

● 3. CpG DNA motifs

Bacterial DNA possesses repeated unmethylated CpG dinucleotide sequences that can potently stimulate innate and acquired immune responses in a host (35). Decreases in IL-4, IL-5, IgE and eosinophilia have been observed in mouse models using both antigen-conjugated and unconjugated approaches

(36). However, the conjugated CpG oligos with allergen effectively reverse established atopic eosinophilic airway disease, at least partially through redirecting a T_H2 to a T_H1 response (36). There is some concern about the potential for host development of autoimmunity. Furthermore, as CpG motifs are potent immune stimulators, there has also been concern for the potential of cytotoxic shock development, although a recent report showed that this might not be an issue (37).

● 4. Suplatast tosilate (IPD)

Suplatast tosilate is an anti-allergic drug, which is currently used in Japan to treat allergic asthmatic patients. IPD, a dimethylsulphonium drug, inhibits IL-4, IL-5 and reduces IgE (38). Oda and colleagues (39) recently reported that IPD inhibited thymus- and activation-regulated chemokine production by antigen-specific human T_H2 cells. IPD has been shown to attenuate allergen-induced late allergic response, AHR, infiltration of $CD4^+$ T cells, eosinophilia, and goblet cell metaplasia. Addition of 100 mg IPD three times a day induced steroid reduction by 50% in steroid-dependent asthmatics (40). Furthermore, Suplatast tosilate attenuated serum ECP and IgE levels and improved FEV₁, peak expiratory flow, symptoms and β_2 -agonist use at both 4 and 8 weeks after treatment in these patients. Suplatast tosilate also inhibits eosinophil chemotaxis and eosinophil adhesion to vascular endothelial cells (41). However, the exact mechanism of action of this drug is still unclear.

● 5. Probiotics

Probiotics are cultures of potentially beneficial bacteria of healthy gut microflora. It has been proposed that gastrointestinal microflora promote T_H1 type immunity, IgA production and generation of TGF- β to suppress T_H2 -induced allergic inflammation and induce oral tolerance (42). These data suggest potential anti-allergic and antiasthmatic properties of probiotics, such as *Lactobacillus rhamnosus*, in atopic diseases. Indeed administration of *Lactobacillus GG* prenatally to mothers and postnatally for 6 months to their infants at high risk of atopic diseases was effective in preventing chronic recurring atopic eczema in children at high risk of atopic diseases

(43). Treatment with probiotics was efficacious and safe in patients with food allergy (44). These data suggest that gut microflora have endogenous immunomodulatory property to combat against the increasing frequency of atopic diseases.

● **6. Monoclonal Anti-IgE**

For allergic asthmatics, the targeting of IgE antibodies is a desirable strategy, as these antibodies initiate the cascade of events leading to narrowed airways and inflammatory cell recruitment. A humanized monoclonal anti-IgE antibody (rhuMAB-E25; Omalizumab) has been developed as a potential therapeutic option for allergic diseases (45,46). These antibodies, when injected, bind to the Cε3

domain of circulating free monomeric IgE antibodies, thus remove free IgE from circulation and decrease the expression of FcεRI on basophils and mast cells (45). Studies using injected rhuMAB-E25 have shown efficacy in reducing AHR, IgE levels, and steroid dependency (45,46). In addition, omalizumab prevented the release of histamine and other inflammatory mediators in response to IgE. Several other studies have also reported a reduction in symptoms and exacerbation rate in allergic rhinitis and allergic asthma with no adverse effects (47). Omalizumab has also been reported to reduce sensitivity to food antigens in causing anaphylaxis. Recently, Finn and colleagues (48) reported that in patients requiring moderate-to-high

doses of inhaled corticosteroids for severe allergic asthma, add-on therapy with omalizumab clinically improved in all aspects of asthma-related quality of life. Additionally, omalizumab reduced asthma-related emergency room visits and hospitalizations in patients with allergic asthma (49). Thus, monoclonal anti-IgE antibody is a promising therapy for the treatment of allergic disorders.

● **7. Corticosteroids**

More moderate cases of asthma require the addition of an anti-inflammatory drug for further disease control. Indeed, inhaled corticosteroids are considered the cornerstone of asthma management (50). These drugs bind the intracellular glucocorticoid receptor, which then

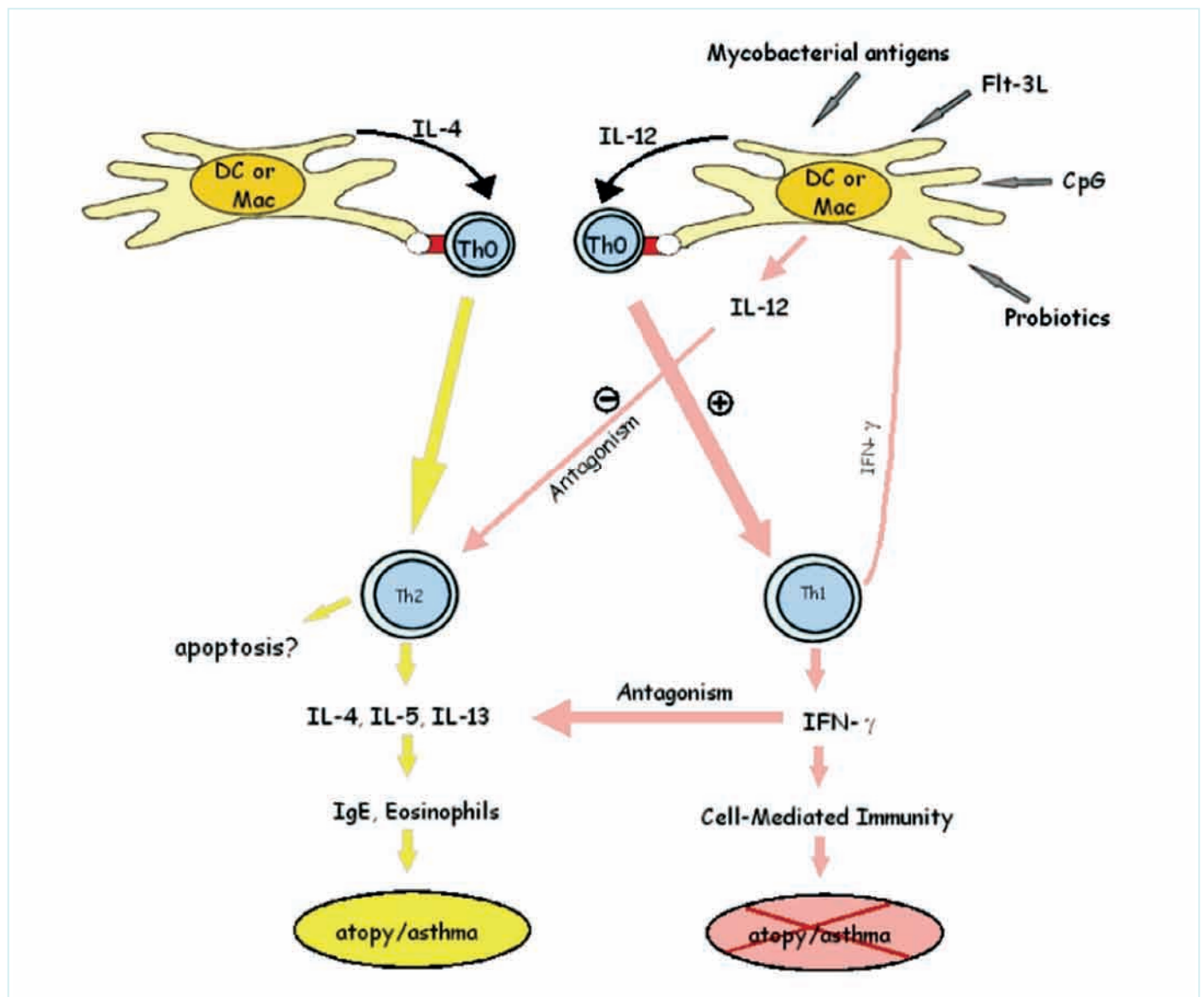


Figure 1: T_H1 cells can antagonize T_H2 cells. Undifferentiated T_H0 cells can mature into T_H1 or T_H2 cells, depending upon the costimulatory signals presented to them, along with antigen, by antigen presenting cells (dendritic cells or macrophages). Type 1 immune modulators such as BCG, CpG and Flt-3 Ligand may preferentially differentiate DC1 (CD8α⁺) cells, which in turn induce the development of T_H1 cells that secrete IFN-γ, a cytokine that suppresses IL-4- and IL-5-secreting T_H2 cells. This could result in the prevention and/or reversal of atopy and/or asthma symptoms.

translocates to the nucleus, mediating the transcription of anti-inflammatory proteins and inhibiting the transcription of inflammatory proteins. These drugs have very wide-ranging effects, decreasing inflammatory cytokines (e.g., IL-4, IL-5), suppressing eosinophil, mast cell, macrophage and dendritic cell numbers, decreasing mucus secretion and edema formation and increasing the density of β_2 receptors in the airways (50). Currently, several pharmaceutical industries are developing newer "safe" steroids either alone or in combination with long-acting β_2 agonists, which hopefully would be available in the market in next 2-3 years (51).

● 8. *Flt-3 Ligand*

Flt-3 ligand (FL) is a recently described growth factor affecting primitive hematopoietic progenitor cells (CD34⁺) both in humans and mice (34). FL selectively induces several cell populations including dendritic cells, natural killer cells, and B cell progenitors (52). One subset (CD8 α^+ ; also termed 'lymphoid') secretes relatively higher concentrations of type 1 cytokines such as IL-2 and IL-12, and another subset (CD8 α^- ; also termed 'myeloid') secretes relatively higher concentrations of type 2 cytokines such as IL-4 and IL-5 (53), with FL-treatment inducing a preferential increase of lymphoid over myeloid dendritic cells (52). A potential

pathogenic role for myeloid-derived dendritic cells in eosinophilic airway inflammation has also been reported.

Since FL has been shown to preferentially induce the proliferation of dendritic cells secreting IL-12 and type 2 T cells predominate in asthma and IL-12 prevents the differentiation of naïve T lymphocytes to a type 2 phenotype, we examined if FL could prevent the development of asthma-like conditions in a mouse model. AHR to methacholine was completely blocked with FL treatment (54). Analysis of bronchoalveolar lavage (BAL) fluid performed 24 hours after challenge demonstrated a significant reduction of eosinophils. Interestingly, BAL supernatant analysis showed decreases in IL-5 and increases in IFN- γ ($p < 0.05$) with no change in IL-4. Serum IgE level was statistically unchanged in FL-treated mice (55). In a follow-up study, FL treatment attenuated LAR and abolished AHR in an established model of asthma (55). These data suggest that FL-treatment prevents and reverses allergic airway inflammation and associated changes in pulmonary function in a mouse model of asthma (Fig. 1). Effect of FL in human asthma requires further studies.

● REFERENCES

1. Erb KJ *Immunol Today* 20, 317, 1999
2. von Hertzen LC et al. *Am J Respir Cell Mol Biol* 22, 139, 2000
3. Garra A et al. *Curr Opin Immunol* 6, 458, 1994
4. Borish LC et al. *J Allergy Clin Immunol* 107, 963, 2001
5. Mauser PJ et al. *Am J Respir Crit Care Med* 152, 467, 1995

6. Leckie MJ et al. *Lancet* 356, 2144, 2000
7. Flood-Page PT et al. *Am J Respir Crit Care Med* 167, 199, 2003
8. Cheng G et al. *Am J Respir Crit Care Med* 166, 409, 2002
9. Zuany-Amorim C et al. *J Clin Invest* 95, 2644, 1995
10. Zuany-Amorim C et al. *J Immunol* 157, 377, 1996
11. Mascali JJ et al. *Ann Allergy Asthma Immunol* 77, 34, 1996
12. John M et al. *Am J Respir Crit Care Med* 157, 256, 1998
13. Akdis CA et al. *J Clin Invest* 102, 98, 1998
14. Gavett SH et al. *J Exp Med* 182, 1527, 1995
15. Bryan SA et al. *Lancet* 356, 2149, 2000
16. Wills-Karp M et al. *Science* 282, 2258, 1998
17. Hofstra CL et al. *J Immunol* 161, 5054, 1998
18. Lack G et al. *J Immunol* 157, 1432, 1996
19. Boguniewicz M et al. *J Allergy Clin Immunol* 95, 133, 1995
20. Strachan DP et al. *J Allergy Clin Immunol* 99, 6, 1997
21. Shirakawa T et al. *Science* 275, 77, 1997
22. Marchant A et al. *J Immunol* 163, 2249, 1999
23. Aaby P et al. *Clin Exp Allergy* 30, 644, 2000
24. Gruber C et al. *Pediatrics* 107, E36, 2001
25. Alm JS et al. *Lancet* 350, 400, 1997
26. Omenaas E et al. *Thorax* 55, 454, 2000
27. Erb KJ et al. *J Exp Med* 187, 561, 1998
28. Sano K et al. *Am J Respir Cell Mol Biol* 20, 1260, 1999
29. McGeady SJ et al. *J Allergy Clin Immunol* 56, 393, 1975
30. Holt PG et al. *Clin Exp Allergy* 22, 1093, 1992
31. Umetsu DT et al. *Nat Immunol* 3, 715, 2002
32. Hopfensperger MT et al. *Int Immunopharmacol* 1, 1743, 2001
33. Hopfensperger MT et al. *J Immunol* 168, 2516, 2002
34. Zuany-Amorim C et al. *Nat Med* 8, 625, 2002
35. Krieg AM *Annu Rev Immunol* 20, 709, 2002
36. Kline JN et al. *Am J Physiol Lung Cell Mol Physiol* 283, L170, 2002
37. Bohle B et al. *J Immunol* 166, 3743, 2001
38. Oda N et al. *Life Sci* 65, 763, 1999
39. Oda N et al. *Clin Exp Allergy* 32, 1782, 2002
40. Tamaoki J et al. *Lancet* 356, 273, 2000
41. Suwaki T et al. *Int Immunopharmacol* 1, 2163, 2001
42. Isolauri E et al. *Clin Exp Allergy* 30, 1604, 2000
43. Kalliomaki M et al. *Lancet* 357, 1076, 2001
44. Majamaa H et al. *J Allergy Clin Immunol* 97, 985, 1996
45. Johansson SG et al. *Ann Allergy Asthma Immunol* 89, 132, 2002
46. Milgrom H et al. *N Engl J Med* 341, 1966, 1999
47. Busse W et al. *J Allergy Clin Immunol* 108, 184, 2001
48. Finn A et al. *J Allergy Clin Immunol* 111, 278, 2003
49. Corren J et al. *J Allergy Clin Immunol* 111, 87, 2003
50. Barnes PJ *J Allergy Clin Immunol* 106, 5, 2000
51. Angus RM *Pulm Pharmacol Ther* 15, 479, 2002
52. Fong L et al. *PNAS USA* 98, 8809, 2001
53. Antonysamy MA et al. *Cytokine* 12, 87, 2000
54. Agrawal DK et al. *Int Immunopharmacol* 1, 2081, 2001
55. Edwan JH et al. *J Allergy Clin Immunol* 109, S24, 2002