

Immunopharmacological Approach to Elucidating the Mechanism of Allergic Inflammation

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ABSTRACT

Allergy has now become amongst the commonest disease in the world and the patients are increasing. There have been significant improvements in our understanding of allergic disease and its management. However there are still many unclear points in the precise mechanism of allergic diseases and therapy. Recent wide and extensive investigations suggest that the underlying mechanism of allergic disease is closely related to the allergic inflammation. The aim of this review is to offer a state-of-the-art description in our research concerning the allergic inflammation from the immunopharmacological standpoint.

KEY WORDS

allergic inflammation, allergy, anti-allergic drug, eosinophil, mast cell

INTRODUCTION

Research in the past decade has provided extensive evidence regarding the manner of onset and therapy for immuno-inflammatory diseases including allergic asthma, allergic rhinitis, atopic dermatitis¹⁻⁵ and some autoimmune diseases. Bronchial asthma, a typical allergic disease, has been described as involving sub-chronic eosinophilic epithelial desquamative inflammation of the airway. Allergic rhinitis and atopic dermatitis are also inflammatory diseases, as their names indicate. In other words, allergic inflammation is the basic pathophysiology of allergic diseases and is closely associated with their progression and exacerbation. Recent advances in immunological and molecular-biological research have improved our understanding the mechanism of allergic inflammation (Fig. 1). The focus has shifted from the overall body to tissues, from tissues to cells, and from cells to functional molecules and genes. However, the precise mechanism of allergic inflammation is still unclear. In this paper, an immunopharmacological approach to elucidating the mechanism of allergic inflammation will be described. Since our final purpose is to develop a new remedy for allergic diseases, we tried to study the role of inflammatory cells and molecules in allergic reactions as a target for new therapeutic agents.

While much effort has been paid to developing new anti-allergic drugs, complete understanding of the allergic disease process has yet to be conquered. Notably, many problems remain to be solved, including airway hyperresponsiveness and airway wall remodeling in bronchial asthma, nasal obstruction in allergic rhinitis, and itching and eczema-like symptoms in atopic dermatitis. All of these symptoms are intractable conditions. The development of new therapeutic agents is necessary. This paper focuses on our research into the mechanism of allergic inflammation, which is the basis of these intractable conditions, and development of new therapeutic agents.

As a first step, we created a mouse model that reflected some clinical allergic conditions. To study the role of inflammatory cells in allergic diseases, mice lacking a certain kinds of cells were used. Then, in order to identify the functional molecules of allergic reactions, a comprehensive gene analysis and certain gene-deficient mice were used. After the identification of functional molecules, we studied the efficacy of inhibitors of these functional molecules in experimental allergic disease models. This paper does not touch upon nasal obstruction in allergic rhinitis and itching in atopic dermatitis, because, for the former, antithromboxane agents and steroids are currently

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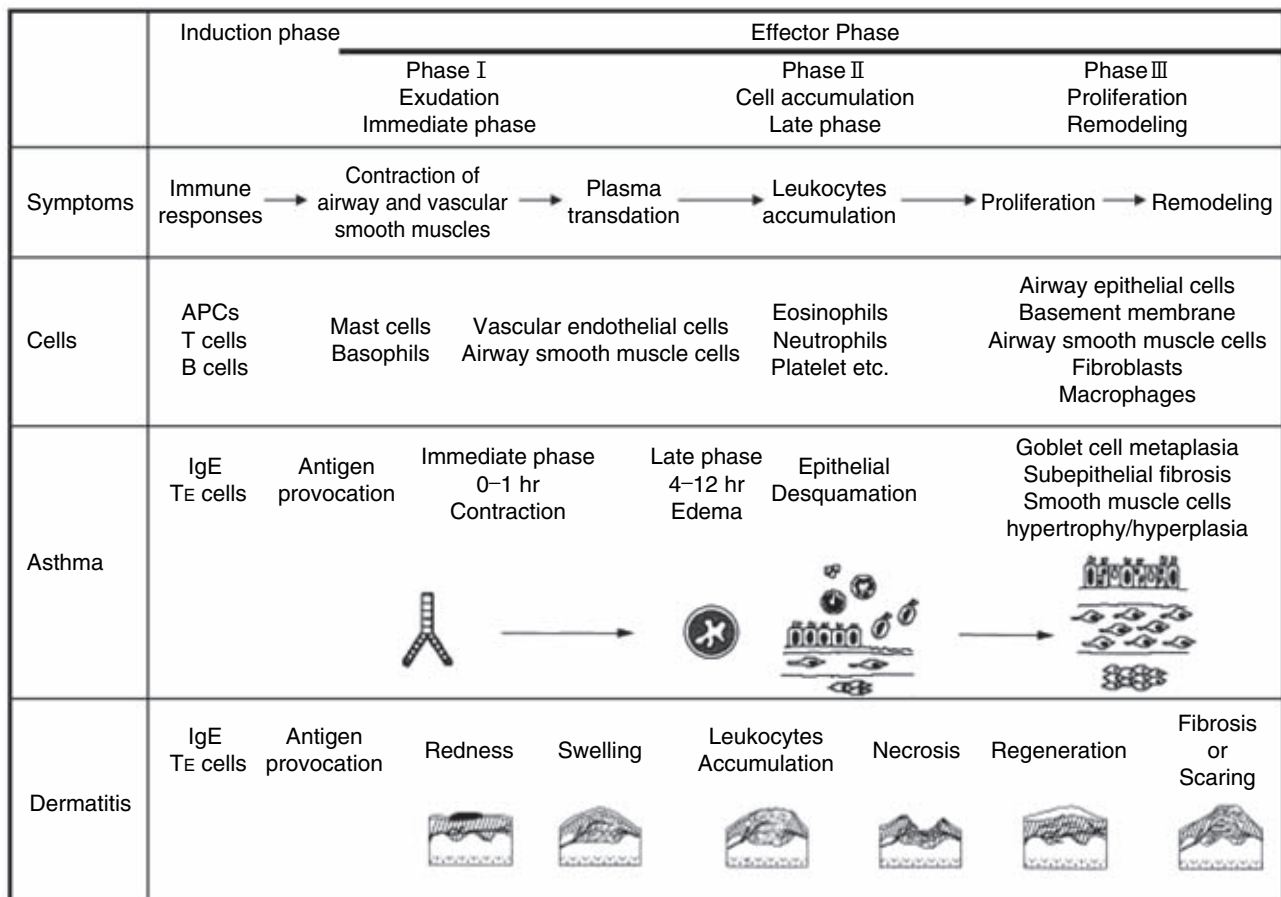


Fig. 1 The process of allergic inflammation

Table 1 Comparison of allergic and non-allergic inflammation

Item	Allergic Inflammation	Non-allergic inflammation
Cause	Antigen	Primary; Infection Secondary; Physicochemical stimuli Immunological stimulation
Cells	APC, T cell, B cell, Mast cell, Eosinophil	Tissue cells, Neutrophil
Pathology	Flare, Swelling Tissue Dysfunction Remodeling	Flare, Fever, Swelling, Pain Tissue Dysfunction, Remodeling
Pharmacology	Anti-inflammatory steroid Anti-histamine, Anti-LT Anti-TxA ₂ , Mast cell stabilizer Desensitization, etc	Anti-inflammatory steroid Non-steroidal anti-inflammatory drug Gold, etc

APC: Antigen presenting cells LT: Leukotriene TxA₂: Thromboxane A₂

highly effective, and, for the latter, no sufficiently good outcomes have been obtained.

ALLERGIC INFLAMMATION AND NON-ALLERGIC INFLAMMATION

Table 1 shows a comparison between allergic inflammation and non-allergic inflammation. There are roughly two stages in allergic inflammation, the induction phase due to an immunological mechanism and the effector phase due to an inflammatory mechanism (refer to Fig. 1). In the induction phase, the im-

munoglobulin or the effector T cell produced as the result of activation of the immunological mechanism works as a trigger of disease. In this stage, antigen-specific immunoglobulin or effector T cells are detected in the blood. In the immunological mechanism, immunocompetent cells including antigen-presenting cells, T cells and B cells play a role. When antigen exposure occurs repeatedly, inflammatory cells, such as mast cells and eosinophils, participate in the activation of the immunological mechanism, blurring the distinction between the induction stage

Table 2 Summary of pathological changes in antigen-induced airway hyperresponsiveness (AHR) in mice

Defective Cell	Mice	IgE	Th2 cytok	Eo	AHR
	Wild	++	++	++	++
T cell	Nu/nu ^{-/-}	—	—	—	—
Mc	W/W ^V	++	++	++	—
	W/W ^V + BMMC	++	++	++	++
Eo	sIL-5R α	++	++	+	++
	a-IL-5	++	++	+	++

++: Moderate increase +: Slight increase —: no change Mc: mast cell
Cyt: Cytokine Eo: Eosinophil

Table 3 Summary of pathological changes in antigen-induced airway hyperresponsiveness (AHR) in mice

Defective	Mice	Molecule	IgE	Th2 Cyt	Eo	AHR
		Wild	++	++	++	++
Th2 Cyt		IL-4	—	—	+	—
		IL-13	+	+	—	—
Co-s		NGF-p75	—	—	—	—
LO		5-LO	++	++	+	—
PG		EP ₁	++	++	++	++
		EP ₃	+++	+++	+++	++
		EP ₄	++	++	++	++
		IP	+++	+++	+++	++
		DP	++	+	+	—
		TP	++	++	++	—

+++ : Marked increase ++: Moderate increase +: Slight increase —: no change
NGF: Nerve growth factor LO: 5-Lipoxygenase Cyt: Cytokine Eo: Eosinophil

and the effector stage. In order for a response to be formed, many mediators, including cytokines and adhesion molecules, must play roles in the respective phases.

From a pathological standpoint, allergic inflammation does not always involve all of the five major characteristics of non-allergic inflammation, flare, fever, swelling, pain and tissue dysfunction. Fever and pain appear only in special cases of allergic inflammation. The inflammatory response in allergic inflammation progresses in stages in the same order as in non-allergic inflammation, namely, exudation, cellular infiltration and tissue repair. However, in the cellular infiltration stage, while neutrophils are dominant in non-allergic inflammation, eosinophils are dominant in allergic inflammation. In the tissue repair stage, while granulation and rheumatic progressive changes occur relatively frequently in non-allergic inflammation, this stage in allergic inflammation is characterized by tissue remodeling following exacerbation and chronicity. In the tissue repair stage of either type of inflammation, macrophage series cells play a leading role by producing growth factors. In allergic inflammation, in addition to macrophages, the mast cells, eosinophils and epithelial cells work as modulators.

Pharmacologically, while the usefulness of steroidal anti-inflammatory drugs is common to both types,

so-called non-steroidal anti-inflammatory drugs are effective for non-allergic inflammation but often ineffective for allergic inflammation.

As described above, allergic inflammation and non-allergic inflammation resemble each other in some points but differ in many others. However, in accordance with the final objective of our research which is the development of therapeutic agents for intractable allergic symptoms, from cellular and molecular standpoints we planned to elucidate the characteristics of the mechanisms of inflammation, which from the basis of these intractable conditions.

THE MECHANISM UNDERLYING ALLERGIC AIRWAY HYPERRESPONSIVENESS, AND DEVELOPMENT OF THERAPEUTIC AGENTS

The mechanism of airway hyperresponsiveness, an important symptom of bronchial asthma, remains unclear, even though many clinical and basic studies have been conducted on the subject.⁶⁻²⁰ First, we tried to produce a mouse model of airway hyperresponsiveness due to an allergic mechanism.^{21,22} Mice were immunized systemically, then made to inhale antigen 3 times. Figure 2 shows the results of the airway hyperresponsiveness model. In this model, the dose response curve for the contraction by acetylcholine in allergic mice shifted to the left and the area

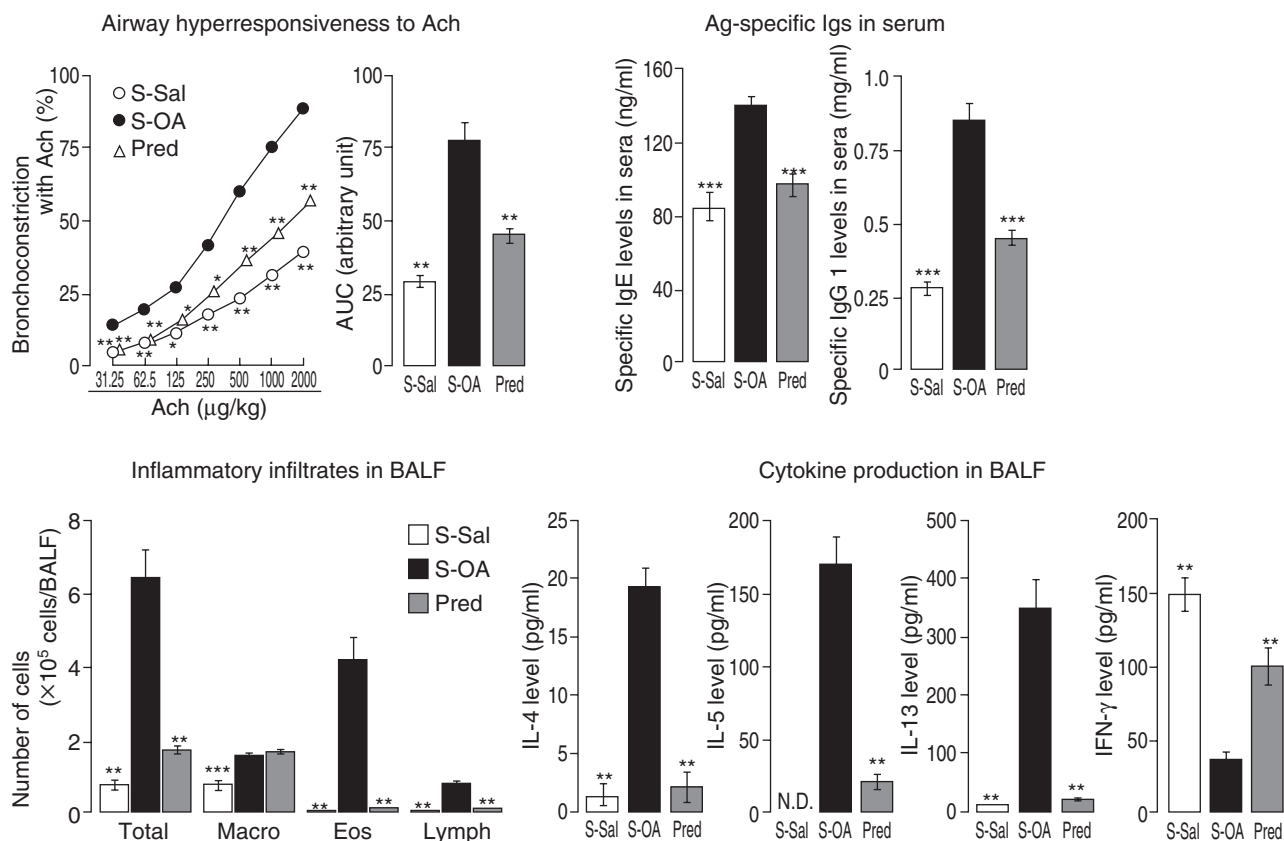


Fig. 2 Changes in airway hyperresponsiveness with acetylcholine, immunoglobulin levels in serum, the number of infiltrated cells in airway and cytokine levels in bronchial alveolar lavage fluid after repeated allergen provocation and treatment with prednisolone. Each value represents the mean $\pm$ SEM of 8–12 mice. S-Sal; Injection and inhalation of saline instead of allergen, S-OA; Immunization and inhalation of ovalbumin (allergen), Pred; Administration of 5 mg/kg prednisolone after immunization and inhalation of ovalbumin, **: $P < 0.05$, ***: $P < 0.01$.

under the curve (AUC) increased when compared to normal saline-treated mice. A high level of IgE production and eosinophilic infiltration were observed in allergic mice. The levels of Th2 cytokines such as interleukin 4 (IL-4), IL-5 and IL-13, were elevated, while the level of interferon gamma (IFN- γ), a Th1 cytokine, was lowered in antigen exposed mice. The above symptoms improved following administration of prednisolone, suggesting that the model was useful as a model for immuno-pharmacological studies. In the next experiment, cells and molecules associated with the onset of hyperresponsiveness were investigated using the model with defective cells or defective genes that coded molecules. Table 2 summarizes findings in mice that were defective in specific cells.^{21–24} Results suggested that T cells and mast cells played important roles in the onset of airway hyperresponsiveness, while eosinophil were not a major player. In particular, the role of the mast cell was confirmed, because the airway hyperresponsiveness of mast cell-defective mice recovered following the transfer of cultured bone marrow-derived mast cells.

In molecular studies, changes in genes were analyzed comprehensively before and after the onset of airway hyperresponsiveness. Although not all genes have been fully analyzed, those encoding for cytokines, chemokines, their receptors, ion-channels, enzymes and others were altered significantly. Table 3 shows the results of the investigation employing cytokine and arachidonate receptor gene-deficient mice. The results suggest that the Th2 cytokines and among lipid mediators including Leukotriene (LT), prostaglandin D₂(PGD₂) and thromboxane A₂(TxA₂), played important roles in inducing airway hyperresponsiveness in the model.^{25–29} The Th1 cytokines, prostaglandin E₂(PGE₂) and I₂(PGI₂) worked as inhibitory factors against the onset of allergic inflammation. In particular, PGE₂ seemed to express its inhibitory action principally due to the interference of inflammatory cell activation through the EP₃ receptor. Results also demonstrated that prostaglandin I₂ inhibited the inclination from Th₀ toward Th₂ in T cells. Allergic capillary permeability was markedly increased in IP (PGI₂ receptor) gene-defective mice compared to wild mice. In T cells obtained from immunized IP-

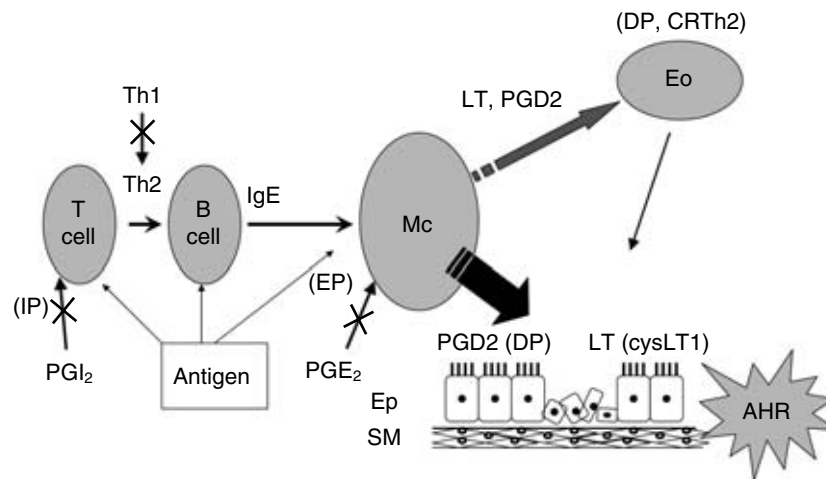


Fig. 3 Possible mechanism of allergic inflammation underlying airway hyperresponsiveness. Mc; Mast cells, Eo; Eosinophils, Ep; Epithelial cells, SM; Smooth muscle, IP; Prostaglandin I₂ receptor, EP; Prostaglandin E₂ receptor, DP; Prostaglandin D₂ receptor, CRTh2; Chemoattract receptor homologous molecule expressed on Th2 cells, cysLT1; cytenyl leukotriene receptor 1, AHR; Airway hyperresponsiveness

Table 4 Summary of drug efficacy against antigen-induced airway hyperresponsiveness (AHR) in mice

Drug	(No. of examined drugs)	IgE	Th2 Cyk	Eo	AHR
Wild		++	++	++	++
T cell immunosuppressor	(2)	ND	—	—	—
anti-IL-4 antibody	(1)	—	+	+	++
anti-IL-5 antibody	(1)	++	++	+	++
IFN- γ (β)	(2)	+	+	+	++
Cytokine inhibitors	(3)	++	++	++	++
Mast cell stabilizer	(5)	++	++	++	++
Anti-histamines	(5)	++	++	++	++
PGI ₂ agonist	(2)	++	++	++	++
EP3 agonist	(1)	++	++	++	++
Anti-PGD2	(1)	++	+	+	+
Anti-LT	(5)	++	+---	+---	+---
Anti-TxA ₂	(2)	++	++	++	+---
Anti PAF	(2)	++	++	+	+---
PDE inhibitor	(3)	++	++	+	++
Eosinophil inhibitor	(3)	+--+	+--+	+	++

++: Marked increase +: Slight increase —: no change
Cyk: Cytokine Eo: Eosinophil

defective mice, while the production of the Th2 cytokine IL-4 increased following addition of antigen, the production of IFN- γ , a Th1 cytokine, decreased when compared to each production by T cells from wild type mice. Consequently, it was thought that the responsiveness in IP-defective mice inclined toward a Th2 response. Figure 3 shows a schema of the allergic inflammation underlying the onset of airway hyperresponsiveness, that is, the Th2 cells activated upon antigen invasion stimulate B cells to produce the IgE antibody under control of the Th1 cytokines.

The IgE antibody is fixed to mast cells through its Fc ϵ . When the antigen invades again, mast cells are activated and produce many mediators including PGD₂, LT and chemokines. Chemokines gather inflammatory cells such as eosinophils at the responsive locale, amplifying allergic inflammation. However, it was thought that mast cells are the leading player in airway hyperresponsiveness, while eosinophils are a minor player. The present results indicate that among prostanoids possibly produced by mast cells, PGD₂, TxA₂ and LT facilitate the onset of airway hyperre-

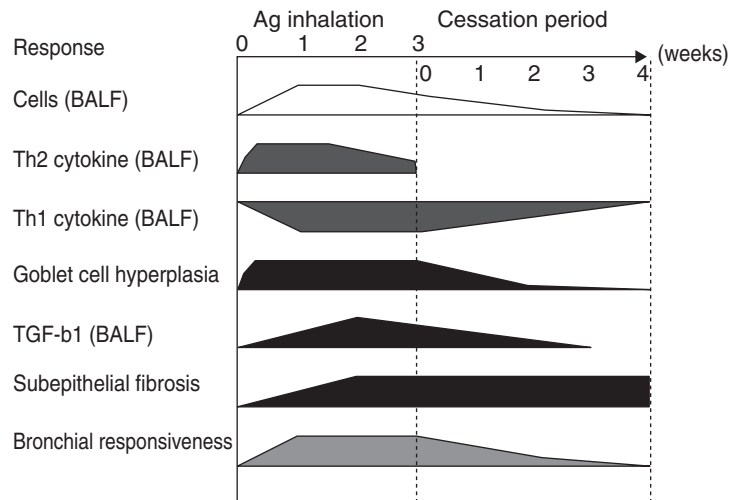


Fig. 4 Time course studies on the changes of various parameters in airway wall remodeling in mice. BALF; Bronchial alveolar lavage fluid, TGF; Transforming growth factor

sponsiveness. PGI₂ and PGE₂ control the progress of an allergic inflammatory response through the IP receptor in T cells and the EP₃ receptor in tissue inflammatory cells, respectively.

Next, efforts were made to find a new remedy for airway hyperresponsiveness based on the above mechanism of inflammation. The pharmacological effects on airway hyperresponsiveness were studied in T cell immuno-suppressors, Th2 cytokine depressants, Th1 agonists, mast cell stabilizers, antihistamines and inhibitors for lipid mediators.³⁰⁻³⁷ Results are summarized in Table 4. The drugs useful for treating airway hyperresponsiveness were T cell immunosuppressors and agents affecting lipid mediators. Among the immunosuppressors, cyclosporine A and tacrolimus hydrate, which act on T-cells, demonstrated strong inhibitory actions against allergic airway inflammation and hyperresponsiveness. Cyclophosphamide did not affect airway hyperresponsiveness, although the agent suppressed airway inflammation. Th2 cytokine depressants and Th1 agonists did not affect airway hyperresponsiveness, while they were useful for suppression of airway inflammation. Mast cell stabilizers, antihistamines and phosphodiesterase inhibitors had no effect on airway hyperresponsiveness, although they partially suppressed airway inflammation. Cysteinyl LT, TxA₂ and platelet activating factor (PAF) inhibitors showed apparent inhibitory actions against airway hyperresponsiveness.

The above results demonstrated that T cell immunosuppressors and lipid mediator inhibitors were effective in suppressing airway hyperresponsiveness. These results suggest that airway hyperresponsiveness induced by ongoing inflammation could be controlled only by the potent suppression of inflamma-

tion at either the initial stage or the final stage.

THE MECHANISM OF ALLERGIC INFLAMMATION AT THE BASIC LEVEL OF AIRWAY WALL REMODELING AND DEVELOPMENT OF THERAPEUTIC AGENTS

When airway allergic inflammation takes place repeatedly, tissue remodeling, that is, the reconstruction of tissues, occurs as a defense reaction against injurious stimuli. The remodeling results in a chronic narrowing of the airway lumen and decreased reactivity of airway smooth muscle relaxants, which are a partial cause of asthmatic death. We attempted to elucidate the mechanism of allergic inflammation at the basic level of airway wall remodeling and development of therapeutic agents. Many trials were carried out to produce an airway wall remodeling model due to an allergic mechanism.³⁸⁻⁴³ A suitable model was obtained in mice by systemic immunization and following inhalation of allergen once a day for 3 weeks before observation for 4 weeks. Results are shown in Figure 4. During the period of allergen inhalation, the number of inflammatory cells and the production of Th2 cytokines increased. The production of TGF-β₁ peaked 2 weeks after the initial inhalation of antigen. Subepithelial fibrosis, one of the characteristics of airway wall remodeling, continued to be observed over 4 weeks after the discontinuation of allergen inhalation, suggesting that the change was irreversible.

The roles of cells were investigated using the above model. Table 5 shows results obtained from mice that were genetically or artificially defective in certain cells. In mice with defective T cells or deprived of CD4-positive T cells using anti-CD4 monoclonal antibody, epithelial hypertrophy, subepithelial

Table 5 Summary of pathological change in antigen-induced airway remodeling and hyperresponsiveness (AHR) in mice

Defective Cell	Mice	IgE	Th2 Cyk	Th1 Cyk	Eo	Ep hy	TGF- β 1	HP	SEF	AHR
T cell	Wild	++	++	sd	++	++	++	++	++	++
	nu/nu	—	—	—	—	—	—	—	—	—
	CD4	—	—	—	—	—	—	—	—	—
	CD8	++	++	sd	++	++	++	++	++	++
Mc	W/WV	++	++	sd	++	++	++	+	+	++
	Sl/SIV	++	++	sd	++	++	++	+	+	++
	W/WV + BMMC	++	++	sd	++	++	++	++	++	++
	IL-5R α KO	++	++	sd	—	±	±	±	±	±
	a-IL-5	++	++	sd	+	+	+	+	+	++

++: Moderate increase +: Slight increase sd: Slight decrease ±: Trace —: no change

Cyk: Cytokine Eo: Eosinophilia Ep hy: Epithelial cell hyperplasia (Goblet cell)

HP: Hydroxyproline SEF: Sub-epithelial fibrosis

Table 6 Summary of pathological changes in antigen-induced airway remodeling and hyperresponsiveness (AHR) in mice

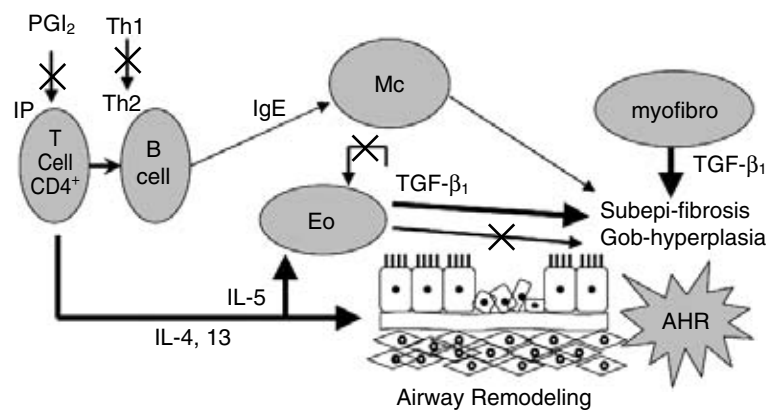
Defective Mice	Molecule	IgE	Th2 Cyk	Th1 Cyk	Eo	EP hy	TGF- β 1	HP	SEF	AHR
Th2 Cyk	Wild	++	++	md	++	++	++	++	++	++
	IL-4	—	—	—	+	+	+	+	+	+
	IL-13	+	+	sd	—	—	+	—	—	—
5-LO	5-LO	++	++	md	++	++	++	+	++	++
PG	DP	++	++	md	++	++	++	++	++	++
	IP	+++	+++	md	+++	+++	+++	+++	+++	++
	EP3	++	++	md	++	++	++	++	++	++

+++ : Marked increase ++: Moderate increase +: Slight increase

sd: Slight decrease md: Moderate decrease —: no change Cyk: Cytokine

Eo: Eosinophilia EP hy: Epithelial cell hyperplasia (Goblet cell)

HP: Hydroxyproline SEF: Sub-epithelial fibrosis

**Fig. 5** Potential mechanism of allergic inflammation underlying airway wall remodeling. Mc; Mast cells, Eo; Eosinophils, Myofibro; Myofibroblast, Subepi; Subepithelial cells, Gob; Goblet cells, TGF; Transforming growth factor, AHR; Airway hyperresponsiveness

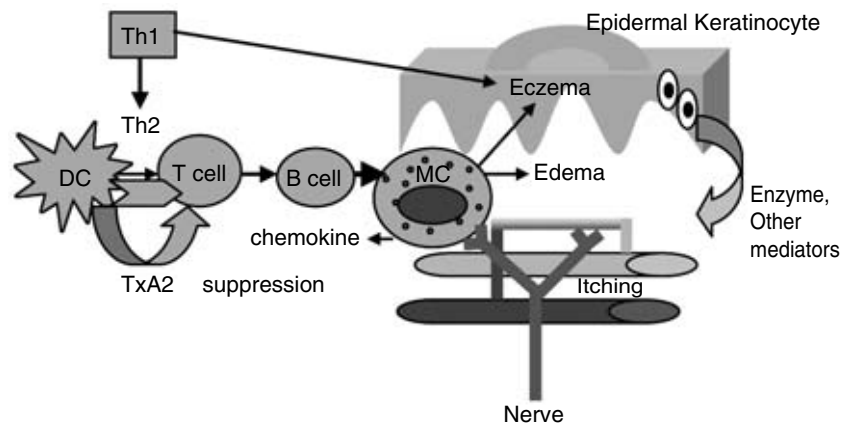
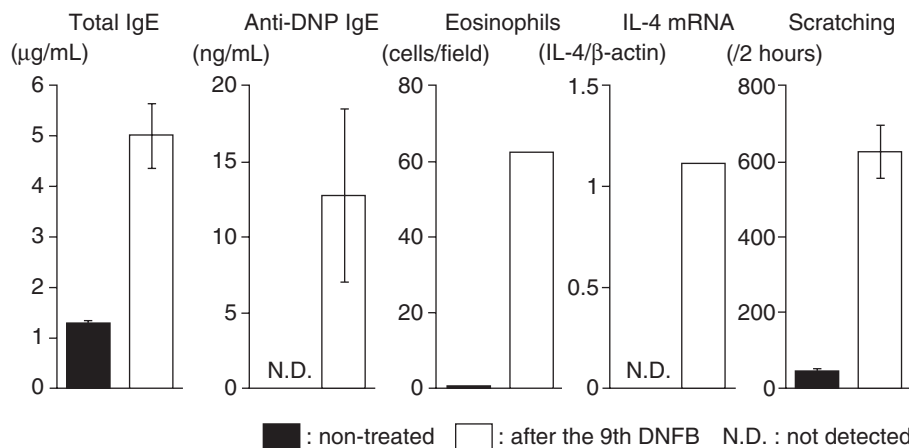
fibrosis and the production of TGF- β 1, all indicators of airway wall remodeling induced by the inhalation of allergen were completely absent. In addition, airway hyperresponsiveness did not occur in these mice. In

mice deficient in CD8-positive T cells, none of the reactions or responses to the allergen were affected. In mice with defective mast cells, that is, W/W^v (c-kit mutant) or Sl/Sld (c-kit ligand mutant) mice, the ele-

Table 7 Summary of drug efficacy against antigen-induced airway remodeling and hyperresponsiveness (AHR) in mice

Drug (No. of drugs)	IgE	Th2 Cyt	Th1 Cyt	Eo	EP Hyp	TGF- β1	HP	SEF	AHR
Wild	++	++	md	++	++	++	++	++	++
anti-IL-4 antibody (1)	±	±	++	+	+	+	++	+	++
anti-IL-5 antibody (1)	++	++	md	+	+	+	+	+	++
PGI ₂ agonist (2)	++	++	md	++	++	++	++	++	++
PGE ₂ (EP3) agonist (1)	++	++	md	+ / ++	ND	++	+	++	++
LT antagonist (2)	++	++	ND	+	ND	++	++	ND	++
PDE inhibitors (2)	++	++	md	++	++	++	++	++	++
MMP inhibitors (2)	++	++	md	++	++	++	++	++	++
CCR3 antagonist (1)	++	+	ND	+	+	+	+	ND	+
Pirprofen	++	++	ND	++	ND	+	++	ND	++
Curcumin	++	++	ND	++	ND	++	++	ND	++

++ : Moderate increase + : Slight increase md : Moderate decrease ± : Trace
 ND : Not done LT : Leukotriene PDE : Phosphodiesterase MMP : Matrix metalloproteinase
 Cyt : Cytokine Eo : Eosinophilia EP Hyp : Epithelial cell hyperplasia
 HP : Hydroxyproline SEF : Sub epithelial fibrosis

**Fig. 6** Potential mechanism of allergic inflammation underlying contact hypersensitivity as a model for atopic dermatitis. DC; Dendritic cells, MC; Mast cells**Fig. 7** Elevation of serum total and DNP-specific IgE levels, and increases in infiltrated eosinophil count, IL-4 mRNA expression and numbers of scratching behavior caused by repeated painting with DNFB. Each column represents the mean ± SEM of 5 to 10 mice.

vation of hydroxyproline content and subepithelial fibrosis were suppressed slightly with no other parameter being affected. In mice having defective eosinophils due to depletion of the IL-5 receptor α -chain gene and treatment with anti-IL-5 monoclonal antibody, immunological responses were not affected but epithelial cell hypertrophy and subepithelial cell fibrosis were dramatically suppressed.

The above results suggested that CD4-positive T cells and eosinophils were the leading player in the onset of airway wall remodeling and that mast cells are associated with only part of the changes.

Gene changes in the model at the onset were analyzed comprehensively. Although results have not been fully analyzed, changes occurred in chemokines, enzymes such as matrix metalloprotease and many kinases, and cytokines. Since more than 50 genes changed significantly, further studies concerning the relationship among each gene seemed to be necessary. As in the studies on the airway hyperresponsiveness model, the roles of candidate molecules were investigated using mice defective in genes related to cytokines and prostanoids and the neutralizing antibodies of cytokines. The results are summarized in Table 6. In IL-4 gene-deficient mice, the changes of airway wall remodeling were slight. In the IL-13 gene-deficient mice, no change in airway wall remodeling occurred, suggesting that the role of IL-13 was slightly greater than that of IL-4. Lipid mediators, which played the major roles in airway hyperresponsiveness, seemed to participate little in the onset of airway remodeling. In IP gene-deficient mice, some parameters of airway remodeling including IgE production, Th2 cytokine levels, eosinophilia, epithelial hypertrophy, subepithelial fibrosis and TGF- β production were improved. Studies using the neutralizing antibody of TGF- β 1 demonstrated the possibility of a dual role of the cytokine in the process of airway wall remodeling. TGF- β 1 played a role in epithelial fibrosis as an inducing factor and in the alteration of epithelial cells to goblet cells as an inhibitory factor. The above results can be depicted in a schematic diagram for airway remodeling shown in Figure 5. CD4-positive T cells and eosinophils were the leading players in the remodeling, IL-13 was the facilitator of the onset, and TGF- β 1 worked both in facilitation and suppression. PGI₂ played a role in the suppression of T cell activation. In addition, Th2 cytokines from T cells acted on the airway wall directly to promote the remodeling.

Therapeutic agents useful in airway wall remodeling were investigated using this model. As shown in Table 7, of the neutralizing antibodies of cytokines, anti-IL-5 antibody had a moderate suppressive action in the development of epithelial cell hyperplasia and subepithelial fibrosis. Among the drugs examined, the strongest was a CCR3 inhibitor, which is a migration factor for eosinophils. Pirfenidone and cur-

cumine, which suppress the fibrosis of other organs, did not have apparent effects on airway wall remodeling.

THE MECHANISM OF ALLERGIC INFLAMMATION AT THE BASIC LEVEL OF THE ONSET OF ECZEMA-LIKE SYMPTOMS IN THE ATOPIC DERMATITIS MODEL AND THERAPEUTIC AGENTS

Atopic dermatitis is an intractable disease with severe itching and recurrent eczema-like lesions. By applying allergens repeatedly on the skin, a mouse model for atopic dermatitis was created in which elevations of serum IgE levels, expression of Th1 cytokine mRNA at lesions, expression of Th2 cytokine mRNA at associated lymph nodes and severe scratching behavior were observed (Fig. 6). Using this model, cells and molecules necessary for the onset were investigated.⁴⁴⁻⁵² T cells, specifically Th2 cells, played an important role as an exacerbating factor. From the data for mice deficient in a certain cells, mast cells and eosinophil seemed to play a minor role in both the onset and development of dermatitis. In order to investigate the molecular pathology, gene expression was studied comprehensively at the sites of allergic reaction and at normal sites. Studies employing gene-deficient mice indicated the importance of molecules produced by keratinocytes and Th2 cells. Based on the results from the experiments in mice, the mechanism of allergic inflammation underlying atopic dermatitis is hypothesized in Figure 7. As shown in Figure 7, TxA₂ plays a suppressive role in the cognitive recognition process between dendritic cells and T cells.⁵³ Th1 and Th2 were activated simultaneously. While Th2 worked as an inducer, Th1 worked as an inducer at lesions and an inhibitor of Th2 at other sites. Fucosyltransferase and protease activated receptor 2, which are thought to be appeared in keratinocytes, seemed to play roles in the onset.

Therapeutic agents for atopic dermatitis were investigated using this model. Drugs demonstrated to be effective so far include glucocorticoid and a T cell immuno-suppressant, tacrolimus hydrate. Most of the anti-allergic drugs were found to be ineffective. More studies are planned to investigate the actions of other drugs possessing different mechanisms.

The conclusions described above shed light on the mechanisms of allergic inflammation underlying many disease symptoms and on the ideas for the development of anti-allergic drugs. The mechanism of allergic inflammation was studied principally using comprehensive gene analysis, gene defective mice, and neutralizing antibody with specific neutralizing activity or soluble receptor obtained in genetic engineering. It can be inferred that local gene expression and control will be important methods used in future studies. New anti-allergic drugs will target the inhibition of various molecules such as IL-13 and CCR3. At

the same time, order-made treatments using currently available drugs, that is, treatment suited to individual patients, should be actively sought. Great future advancements in this field can be anticipated.

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