

Allergic Bronchial Asthma: Airway Inflammation and Hyperresponsiveness

Minoru SUGITA, Kozo KURIBAYASHI*, Takayuki NAKAGOMI*, Shigeru MIYATA*,
Tomohiro MATSUYAMA** and Osamu KITADA*

Abstract

The international consensus report on diagnosis and treatment of asthma was published in 1992 (Clin Exp Allergy 22: 1-72). According to the report, asthma is a chronic inflammatory disorder of the airways in which many cells play a role, including mast cells and eosinophils. Airway inflammation causes various symptoms of asthma which are usually associated with widespread but variable airflow obstruction and causes an associated increase in airway responsiveness to a variety of stimuli. The definition of asthma, provided in this report, is an epoch-making revision of the conventional recognition of asthma based on respiratory physiology and does not contradict the empirical knowledge that asthma responds well to steroid therapy.

One reason, which led airway inflammation to be understood as a major factor in the pathophysiology of asthma is the technological advance and the widespread use of bronchoscopes. The use of bronchoscopy as a research tool has markedly improved the understanding of the pathology of asthma. It became also possible to link biopsy findings to autopsy findings in patients who died of asthma.

However, it is relatively difficult to repeat a biopsy of the airway mucosal membranes in individual asthmatic patients. Here, animal models of asthma play a significant role. Findings from animal models can provide a clue for the development of new anti-asthmatic drugs.

This paper will deal with the paradigm of allergic asthma and focus on recent topics of interleukin (IL)-4 and IL-5, which seem to play a central role in allergic asthma. The causative relationship between airway inflammation and hyperresponsiveness will be discussed. (Internal Medicine 42: 636-643, 2003)

Key words: allergic bronchial asthma, airway inflammation, airway hyperresponsiveness, interleukin (IL)-4, IL-5

Differences in Data Originate from Differences in Murine Strains and Experimental Protocols

In 1996, Foster et al (1) reported in the Journal of Experimental Medicine that in IL-5 deficient C57BL/6 mice, eosinophilia, airway hyperreactivity and lung damage normally resulting from aeroallergen challenge were abolished. Their experiment was performed as follows. Wild type C57BL/6 mice and IL-5 deficient C57BL/6 mice were sensitized with an intraperitoneal dose of OVA alum (50 µg/dose) on days 1 and 12. Mice were exposed to an aerosol of 1% OVA for 30 minutes three times a day (at 1-h intervals) on days 24, 26, 28, 30 and 32. Three hours after the last aeroallergen challenge, mice were examined. In wild type C57BL/6 mice sensitized with OVA, eosinophils were detected in airway lavage fluid. However, eosinophils were absent in IL-5 deficient mice. OVA-specific IgE was detected at similar levels in sera from both wild type and IL-5 deficient mice after aeroallergen challenge. However, airway hyperreactivity to intravenous administration of β-methacholine was only seen in the wild type mice. Histologically, OVA-treated wild type mice showed increased mucus secretion into the airway lumen and epithelial cell shedding. The mucosal membrane of these animals showed cell infiltration, and marked eosinophil infiltration. On the other hand, IL-5 deficient mice showed only slight morphological changes.

When IL-5 deficient C57BL/6 mice received an injection of VV-HA-IL-5 in the nasal mucosa, using recombinant vaccinia virus as a vector, before and during inhalation of OVA (i.e., on days 24 and 28), to induce re-constitution of IL-5, their peripheral blood eosinophil counts increased with time and airway hyperreactivity appeared. On the basis of these results, Foster et al (1) consider IL-5 as an important cytokine in mouse models of asthma.

Corry et al (2), on the other hand, published a paper in the same issue of J. Exp. Med., reporting that IL-4, and not IL-5 or eosinophils, is required in a murine model of acute airway hyperreactivity. Their experiment was performed as follows. BALB/c mice were sensitized with subcutaneous doses of

From Medical Imaging Center, *Department of Internal Medicine, Respiratory Division, **Division of Neurology, Hyogo College of Medicine, Hyogo
Reprint requests should be addressed to Dr. Minoru Sugita, Medical Imaging Center, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo
663-8501

OVA alum (25 µg/dose) on days 0, 7, 14 and 21. These mice inhaled 5% OVA for 20 minutes on days 26 and 31. On day 32, they received various examinations. This protocol is characterized by the lower frequency of OVA inhalation as compared to the C57BL/6 mice mentioned above. Wild type mice were divided into two groups: a group receiving 4 intraperitoneal doses of anti-IL-4 antibody (1 mg/dose) during sensitization, and a group given two intraperitoneal doses of anti-IL-4 antibody (1 mg/dose) limited to the inhalation period of OVA. Anti-IL-5 antibody was administered intraperitoneally (2 µg/dose, 4 doses) at the time of sensitization. Anti-IL-4 antibody administered during sensitization with OVA abrogated airway hyperreactivity but had little effect on the influx of eosinophils. Administration of anti-IL-4 antibody only during inhalation of OVA did not affect the subsequent response to acetylcholine. Administration of anti-IL-5 at levels that suppressed eosinophils to 1% of recruited cells had no effect on the subsequent airway responses. BALB/c mice had significantly greater airway responses than C57BL/6.

According to Corry et al (2), the mechanism for suppression of airway hyperreactivity by anti-IL-4 antibody given only during sensitization with OVA, may be explained by IL-4 generated during lymphocyte priming with OVA in establishing the cascade of responses required to generate airway hyperreactivity to inhaled antigen. No role for IL-5 or eosinophils could be demonstrated.

The discrepancy in results among different experiments, reported to date, has been attributed to: 1) inter-strain differences, 2) differences in protocol, 3) differences in the route of sensitization, and 4) other factors. The publication of these two original papers, by Foster et al (1) and Corry et al (2), in the same issue of the same journal had a large impact; these two papers have been frequently cited.

Drazen et al (3) published a commentary on these two pa-

pers in the same journal. They were interested in the fact that these two studies yielded totally different results despite the procedure similarities (treatment of mice with OVA to induce allergic asthma and the use of airway hyperresponsiveness as an outcome indicator). To explain this discrepancy, they proposed a hypothesis that BALB/c mice are high IgE responders, while C57BL/6 mice resist induction of airway hyperreaction dependent on mast cells. They stated that the mast cells of C57BL/6 mice have at least two genetic defects.

The first defect pertains to the prevention of the C57BL/6 mouse from producing a mast cell protease. In the naturally hyperresponsive A/J mouse, a locus on mouse chromosome 17, Bhr3, is close to the gene for mouse mast cell protease 7 gene, and this enzyme is a protein that can confer constitutive airway hyperresponsiveness on the naturally hyporesponsive C57BL/6 mouse (4). Point mutation was observed in the analysis of the gene encoding murine mast cell protease 7 which is a tryptase-like molecule (5, 6). A mutation of a gene that encodes a mast cell secretory granule constituent that leads to its loss of expression in C57BL/6 was reported by Hunt et al (7). Administration of extrinsic tryptase induces airway hyperresponsiveness (8), probably because it enzymatically degrades and inactivates VIP (vasoactive intestinal polypeptide which partially serves as an endogenous bronchodilator) (9).

The second defect pertains to phospholipase A2 of mast cells. Phospholipase A2 can be divided into Ca²⁺-independent type (iPLA2) and Ca²⁺-dependent type. The latter can be subdivided into cytoplasmic sol type (cPLA2) and secretory type (sPLA2) (10). The low molecular weight secretory sPLA2 is deficient in C57BL/6 mice (11).

Drazen et al (3) thus assumed two pathways for airway hyperresponsiveness, a pathway involving IgE/mast cells and the other involving IL-5/eosinophils, as shown in Fig. 1. They

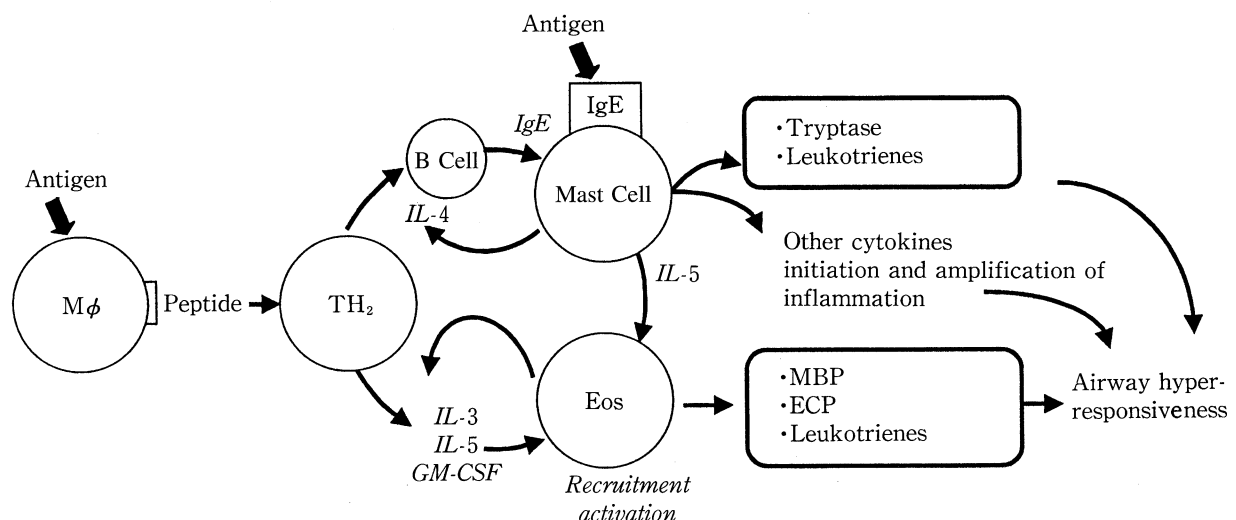


Figure 1. Putative mechanism of mast cell- and eosinophil-dependent airway hyperresponsiveness (3).

thought that the latter pathway is predominant in C57BL/6 mice which have molecular defects in IgE/mast cells.

Zhang et al (12) induced asthma by the same protocol in BALB/c and C57BL/6 mice in order to determine the importance of the route of allergen administration and the genetic background in modulating the physiologic, inflammatory and immunologic features characteristic of allergen-induced asthma. Intraperitoneal immunization with OVA (100 µg/dose) on days 1 and 14 resulted in increased circulating levels of OVA-specific IgE and IgG1 in BALB/c mice on day 24, while mice immunized in this manner did not have increased airway reactivity to methacholine. When mice were sensitized with OVA on days 1 and 14 and nasally inhaled 0.2% OVA on days 14 and 25 (Protocol A), no marked increase in airway responsiveness was noted.

However, when mice were sensitized intraperitoneally with OVA on days 1 and 14 and inhaled OVA on days 14, 25, 26 and 27 (Protocol B), mice had an increased airway reactivity. When these mice inhaled OVA more frequently (on days 14, 23, 24, 25, 26 and 27; Protocol C), their airway hyperresponsiveness did not further intensify. Therefore Zhang et al (12) used Protocol B when comparing BALB/c mice with C57BL/6 mice (Fig. 2).

This comparison in eosinophils (%) of bronchial lavage fluid was revealed to be similar between the two mouse strains. However, OVA-specific IgE and IgG1 levels in blood was about 40% lower in C57BL/6 mice than in BALB/c mice. The dose of methacholine causing a 50% dynamic compliance (ED₅₀) was higher in C57BL/6 mice after OVA treatment. These results suggest that pulmonary hyperresponsiveness in OVA-treated C57BL/6 mice occurred

to a lesser degree than in OVA-treated BALB/c mice. This finding is identical with the fact that the frequency of inhaling OVA was higher in the experiment conducted by Foster et al (1) than in that conducted by Corry et al (2). In other words, this means that in C57BL/6 mice, OVA-treated airway hyperresponsiveness may be demonstrated more easily if the frequency of OVA inhalation is increased. The level of Th2 type cytokine mRNA levels of OVA-treated C57BL/6 mice in bronchial lymph nodes tended to be less than in those of OVA-treated BALB/c mice.

When the distribution of eosinophils in the airway was studied in mice with bronchial asthma, eosinophils were primarily detected around the bronchi in BALB/c mice, while they were primarily detected in the lung parenchyma or peripheral lung tissue (bronchioles and tissue around alveoli) in C57BL/6 mice.

Nakajima et al (13) reported that OVA-induced eosinophils and CD4⁺ T cells in the submucosal tissue of the trachea 24 hours after inhalation of OVA in BALB/c mice were more than 20–10 folds as abundant as those in C57BL/6 mice, respectively.

Takeda et al (14) reported that after sensitization and airway challenge with ovalbumin, the antibody responses in BALB/c mice far exceeded those in C57BL/6 mice. In contrast, although the responsiveness to methacholine was much higher in BALB/c mice, the number of eosinophils in the bronchoalveolar lavage fluid was higher in C57BL/6 mice. In BALB/c mice, the eosinophils were primarily observed in the peribronchial regions, whereas in C57BL/6 mice, the eosinophils were primarily detected in the parenchyma or peripheral lung tissue around the smallest airways or alveoli.

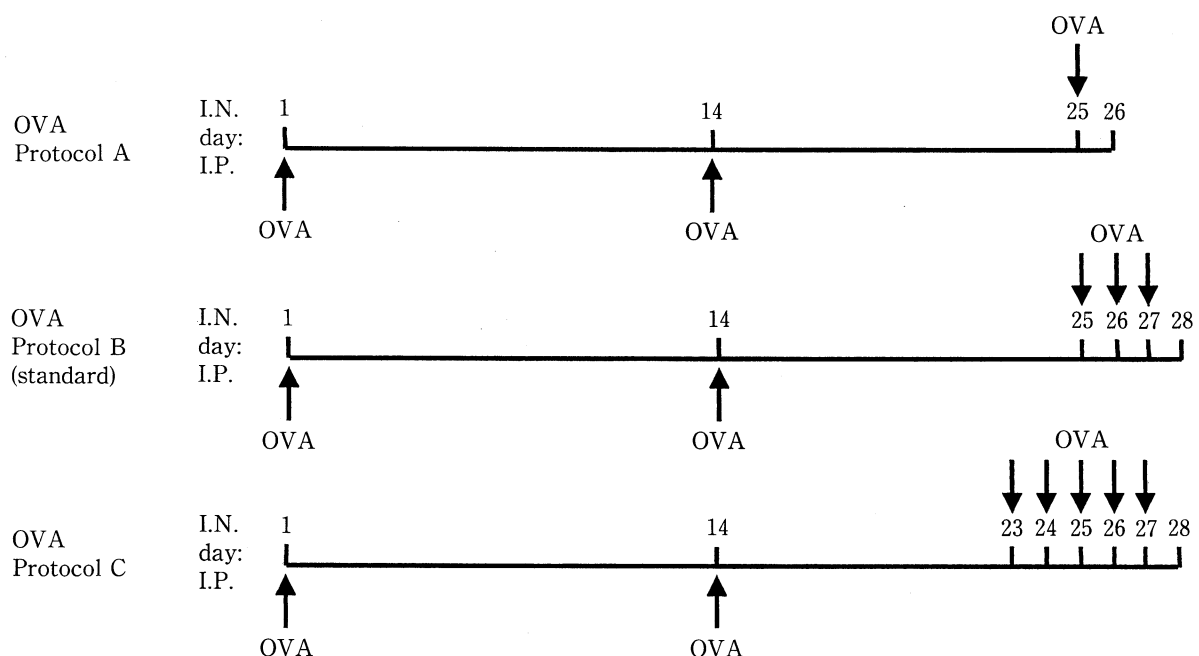


Figure 2. Experimental protocols (12).

Table 1 summarizes the differences observed between C57BL/6 and BALB/c mice.

The view proposed by Drazen et al (3) that the discrepancy in results between C57BL/6 and BALB/c mice is related to mast cells, has been argued by Hogan. Hogan, a co-author of the paper by Foster et al (1), proposed IL-5 as an important cytokine using C57BL/6 mice. Hogan carefully considered the results in BALB/c mice reported by Corry et al (2). Hogan et al (15) used IL-4 and IL-5 deficient BALB/c mice in combination with inhibitory antibodies to these cytokines. As a result, the inhibition of the actions of IL-4 and / or IL-5 did not abolish airway hyperreactivity, and in the case of IL-4 deficient mice pretreated with anti-IL-5 antibody, airway hyperreactivity persisted in the absence of pronounced airway inflammation. In contrast, treatment of IL-4 deficient mice with anti-IL-5 and anti-CD4 antibody (or anti-CD4 antibody alone) significantly reduced the lymphocyte number in the BALF and abolished airway hyperreactivity. Hogan et al (15) proposed the existence in BALB/c mice of a new CD4⁺ T cell pathway for modulating airway hyperreactivity.

It was not Hogan et al (15) who first administered anti-CD4 antibody to a mouse model of bronchial asthma. Gavett et al (16) had injected intraperitoneally anti-CD4 mAbGK1.5 to sheep red blood cell-sensitized A/J mice before antigen intratracheal challenge and found complete suppression of airway hyperreactivity and the infiltration of eosinophils. The paper published by Hogan et al (15) is unique in that it proposed a new pathway, involving T cells, in addition to the hypothesis proposed by Drazen et al (3) on two pathways of the onset of airways hyperreactivity in BALB/c mice (a pathway involving IgE/mast cells and the other involving IL-5/eosinophils). Briefly, Hogan et al (15) believe that the mechanism for the onset of airway hyperreactivity is not simple but involves multiple pathways, and that the pathway involving T cells markedly regulates airway reactivity and does not involve marked morphological changes of the airway.

Lee et al (17) generated transgenic mice that constitutively express murine IL-5 in the Clara cells of the lung epithe-

lium, by using fetuses yielded from a cross of F1 (CBA/CaJ × C57BL/6J) female with male C57BL/6 mice. In the lung of these mice, marked morphological changes (remarkable accumulation of peribronchial eosinophils and striking pathologic changes including the expression of bronchus-associated lymph tissue, goblet cell hyperplasia, epithelial hypertrophy, focal collagen deposition, etc.) were also accompanied by eosinophil infiltration of the airway lumen. The mice showed airway hyperresponsiveness to methacholine in the absence of aerosolized antigen challenge. These findings demonstrate that lung-specific IL-5 expression can induce pathologic changes characteristic of asthma.

Hamelmann et al (18) showed that treatment of OVA-challenged BALB/c mice with anti-IL-5 antibody during the sensitization protocol completely abolished the infiltration of eosinophils into the lung tissue and prevented the development of airway hyperresponsiveness. In their study, BALB/c mice were sensitized with inhalation of 1% OVA for 20 minutes a day for 10 consecutive days. On days 4, 6 and 8, mice received intravenous doses of anti-IL-5 antibody (100 µg/day; 300 µg in total). On day 12, i.e., 2 days after the last nebulization, the trachea was removed to examine its reactivity by the electrical contraction test *in vitro*.

Unlike the study conducted by Corry et al (2) in which anti-IL-5 antibody was intraperitoneally administered at a cumulative dose level of 8 µg, Hamelmann et al (18) administered larger amounts of anti-IL-5 antibody (300 µg in total) via the intravenous route. Soluble IL-4R (sIL-4R) is regarded as particularly promising, and sIL-4R is physiologically formed *in vivo*.

Renz et al (19) sensitized BALB/c mice through the airways to OVA by ultrasonic nebulization once a week for 4 weeks, which developed increased serum anti-OVA IgE and IgG1 antibody titers and were associated with the development of increased airway responsiveness. Sensitized mice treated by intraperitoneal injection of sIL-4R (systemic) as well as administration of sIL-4R via the airways (local) developed significant suppression of anti OVA IgE and IgG1 antibody production and airway responsiveness. Importantly

Table 1. Inter-strain Differences in Characteristics of Allergic Bronchial Asthma in Mice

	C57BL/6 mouse	BALB/c mouse
Th2 cytokine levels (12)	40% lower as compared with BALB/c	
Mast cell molecular deficiency (3)	Protease 7, Secretory phospholipase A2	
Eosinophil distribution in the airway following sensitization and inhalation of antigen (14)	Primarily in lung parenchyma or peripheral lung tissue (bronchiole and peri-alveolar tissue),	Primarily in peri-bronchial tissue
Airway contraction to non-specific substances (e.g. β-methacholine etc.) (12)	Less reactive	More reactive

local administration of sIL-4R was more potent in normalizing airway responsiveness.

Application of IL-4 and -5 Analogues to New Treatments for Asthma

On the basis of a general assessment of these studies from various animal models, Leckie et al (20) did a double-blind randomized placebo-control trial, in which a single intravenous infusion of humanized monoclonal antibody to IL-5 or placebo given to atopic patients with mild allergic asthma who were maintained on short-acting inhaled beta-agonist treatment as required.

As a result, administration of anti-IL-5 antibody caused pronounced long-term suppression of circulating eosinophils and greatly lowered the degree of sputum eosinophilia after allergen challenge. However, despite these clear effects, treatment did not protect against the allergen-induced late asthmatic response and did not have effects on postallergen airway hyperresponsiveness. This finding by Leckie et al (20) suggests that eosinophils might not be a prerequisite for the late asthmatic response and airway hyperresponsiveness in relation to inhaled allergen challenge, and it has relevance to the pathogenesis and treatment of asthma.

To date, however, clinical application of anti-IL-5 antibody to humans has not yet been done on a full scale. Attempts of administering human anti-IL-5 antibody to crab-eating monkeys repeatedly or at large dose levels have been made (21). Clinical trials in humans have also been continued. Emphasis has been laid on evaluating the efficacy of anti-IL-5 antibody in humans.

Borish et al (22, 23) reported the results of treating asthmatic patients by soluble recombinant human IL-4 receptor. The subjects of their study were patients with moderate allergic bronchial asthma, accompanied by atopy who had received steroid inhalation therapy for 6 months or more. In their study, inhalational steroids were stopped the day before IL-4R inhalation. In the short-term nebulized IL-4R protocol, the patients were followed for 2 weeks (22). In the long-term protocol, the patients visited the clinic and inhaled IL-4R once a week and were followed for 84 days (23). During the IL-4R inhalation period, concomitant therapy, other than nonantihistamine decongestants and beta-stimulants, was not allowed. The randomized, double-blind long-term inhalation protocol involved 4 groups, i.e., the placebo group (n=16), the 0.75 mg dose group (n=15), the 1.5 mg dose group (n=16) and the 3.0 mg dose group (n=15). The percentage of study discontinuation by the 84th day was not significantly different between 56% in the placebo group and 47% in the 3.0 mg dose group. When the efficacy was evaluated 3 months later, there was no significant difference between the placebo group and the 3.0 mg dose group in terms of improvement in symptom scores or the decrease in the frequency of needing β_2 -stimulant inhalation. However, efficacy was demonstrated by a decline in FEV₁, observed in the placebo group (−0.4 l and −13%, predicted) which did not occur in the group receiving 3.0 mg of IL-4R (−0.1 l and −2%, predicted). Thus, despite the suspended use of inhalational steroids, IL-4R favorably affected the lung function of these patients. It seems possible that IL-4R may be used clinically as a medicine in the future.

Table 2. Relation between Airway Eosinophilia and Hyperresponsiveness

1) Eo influx ↓ and AHR ↓		Eo, effector cell		
Hartley guinea pig (32)	OVA/OVA	anti-IL-5 ip	30 mg/kg*	Mauser (1993)
C57BL/6 mice (1)	OVA/OVA	IL-5 (KO)		Foster (1996)
BALB/c mice (18)	OVA	anti-IL-5 iv	300 μ g*	Hamelmann (1997)
Monkey (33)	Ascaris	anti-IL-5 iv	0.3 mg/kg	Mauser (1995)
2) Eo influx ↑ and AHR without antigen ↑		Eo, effector cell		
C57BL/6 mice (17)		IL-5 (TG) Clara cell limited		Lee (1997)
3) Eo influx ↓		Eo, questionable		
Hartley guinea pig (32)	OVA/OVA	anti-IL-5 ip	10 mg/kg*	Mauser (1993)
BALB/c mice (2)	OVA/OVA	anti-IL-5 ip	8 μ g*	Corry (1996)
BALB/c mice (15)	OVA/OVA	IL-4 (KO)		Hogan (1998)
Allergic asthma with atopy (20)		anti-IL-5 iv	10 mg/kg	Leckie (2000)
4) AHR ↓				
BALB/c mice (2)	OVA/OVA	anti-IL-4 ip	4 mg	Corry (1996)
5) Eo influx ↑				
C57BL/6 F1 mice (34)		IL-4 (TG) Clara cell limited		Rankin (1996)

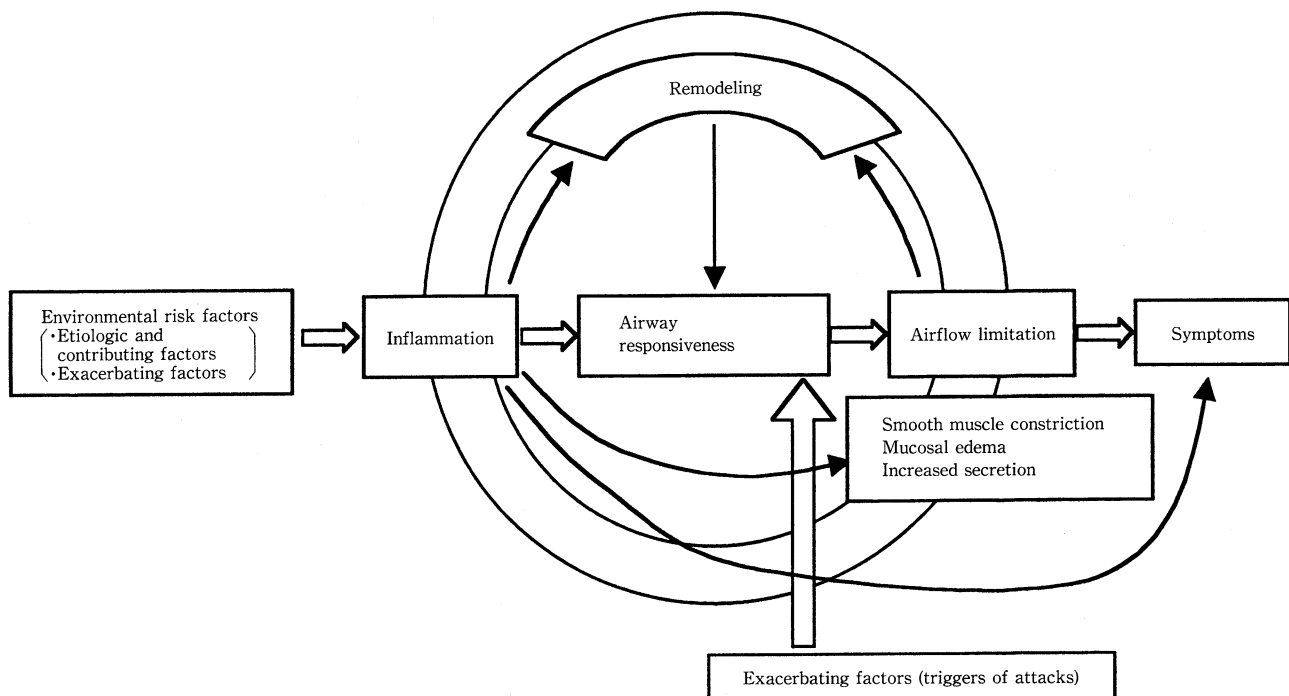
*dose dependent. KO: knock out, TG: transgenic, AHR: airway hyperresponsiveness.

Inflammation and Airway Hyperresponsiveness

As stated above, Leckie et al (20) sensationally raised a question whether eosinophil-induced inflammation is really involved in the antigen-induced delayed asthmatic responses or airway hyperresponsiveness which is a condition underlying allergic bronchial asthma. Eosinophils play three possible roles: 1) innocent bystander, 2) modulating cell, and 3) effector cell. "Innocent bystander" means that eosinophils are accidentally present in inflamed areas and they are nothing more than bystanders. According to this view, it is just a coincidental event that the degree of eosinophil infiltration is widely correlated with the severity of asthma. "Modulating cell" refers to the view that eosinophils play favorable roles by controlling allergic inflammation. This view is based on the findings that eosinophils contain arylsulfatase (24), histaminase (25) and phospholipase D (26). "Effector cell" refers to the view that eosinophils are responsive in many of the inflammatory responses, namely, play unfavorable roles. This view is based on the findings that eosinophils contain eosinophil peroxidase (27), major basic protein (28–30), LTC₄ (28) and PAF (31).

Initially, eosinophils were first believed to play favorable roles. Subsequently, a predominant view is that they play unfavorable roles. However, since the report by Leckie et al was published (20), there has been confusion about regarding to interpret the significance of eosinophil infiltration of the airway.

Table 2 summarizes the relationship between eosinophil infiltration of the airway and hyperresponsiveness of the airway, as far as only IL-4 and IL-5 are concerned. Column 1) of this table shows that suppression of both eosinophils and airway hyperresponsiveness was observed in antigen-treated guinea pigs, mice and a monkey given high doses of anti-IL-5 antibody (18, 32, 33) and in IL-5 knock out mice (2). Column 2) shows that both eosinophil infiltration and airway responsiveness were enhanced in transgenic mice that constitutively express murine IL-5 in the lung epithelium (17). Airway expression of IL-5 resulted in a dramatic accumulation of peribronchial eosinophils. In addition, transgenic mice displayed airway hyperresponsiveness to methacholine in the absence of aerosolized antigen challenge (17). This result, together with the result shown in Column 1), suggests that eosinophils are involved in airway hyperresponsiveness. Column 3) shows that only suppression of eosinophils occurred and airway hyperresponsiveness was not suppressed in antigen-treated guinea pigs and mice given low doses of anti-IL-4 antibody (2, 32), IL-4 deficient mice (15) and atopic patients with allergic bronchial asthma given anti-IL-5 antibody (20). Column 4) shows that airway hyperresponsiveness was suppressed but eosinophil infiltration of the airway was not suppressed in antigen-treated mice given anti-IL-4 antibody (2). The last column 5) shows that IL-4 transgenic mice, confined to Clara cells of the airway epithelium, were characterized by epithelial cell hypertrophy, and the accumulation of macrophages, lymphocytes, eosinophils,



Asthma Prevention and Management Guidelines 1998, Japan (JGL 1998) (35)

Figure 3. Mechanism of asthma onset and exacerbation.

and neutrophils without resulting in an alteration in airway reactivity to inhaled methacholine (34).

The findings shown in Columns 3), 4) and 5) indicate that there are various forms of discrepancy between eosinophil infiltration of the airway and hyperresponsiveness of the airway. Taken together, these findings, implicate that eosinophil infiltration of the airway is not a factor indispensable for the onset of airway hyperresponsiveness.

The Japanese Guideline for the Prevention and Management of Asthma (1998) present the mechanism for the onset and exacerbation of asthma, as shown in Fig. 3 (35). This mechanism suggests that inflammation occurs in the airway as a result of the effects of various factors to which the airway has been exposed, and that this inflammation leads to airway hyperresponsiveness.

There are probably multiple pathways for the onset of airway hyperresponsiveness associated with human asthma. Airway hyperreactivity without airway inflammation and airway inflammation without airway hyperreactivity have been reported. For instance, airway hyperreactivity to histamine following the inhalation of allergen in sensitized guinea pig is expressed within minutes but does not persist for much more than 24 hour, whereas cellular inflammation develops over several hours and is sustained for several days (36). The causative relationship between airway inflammation and hyperresponsiveness may often assume series pathology as shown in Fig. 3 but it may also assume parallel pathological processes (37). It is desirable that the causal relationship between inflammation and hyperresponsiveness of the airway is clarified in the future.

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