Because of the problems associated with antibody-based therapies that have to be given by injection, there is a search for small molecule inhibitors of TNF. TNF-α-converting enzyme (TACE) is a matrix metalloproteinase-related enzyme critical for the release of TNF from the cell surface. Small molecule TACE inhibitors are in development as oral TNF inhibitors (Barlaam et al., 1999).

2.1.12. IFN-γ

Interferon-γ inhibits Th2 cells and should therefore reduce atopic inflammation. In sensitized animals nebulized IFN-γ inhibits eosinophilic inflammation induced by allergen exposure (Lack et al., 1996) and adenovirus-mediated gene transfer of IFN-γ inhibits allergic inflammation in mice (Behera et al., 2002). However, administration of IFN-γ by nebulization to asthmatic patients did not significantly reduce eosinophilic inflammation, possibly due to the difficulty in obtaining a high enough concentration locally in the airways (Boguniewicz et al., 1995). Interestingly, allergen immunotherapy increases IFN-γ production by circulating T cells in patients with clinical benefit (Benjaponpitak et al., 1999) and increases the numbers of IFN-γ expressing cells in nasal biopsies of patients with allergic rhinitis (Durham et al., 1996). A preliminary report suggested that IFN-α may be useful in the treatment of patients with severe asthma who have reduced responsiveness to corticosteroids (Gratzl et al., 2000).

2.2. Inhibition of chemokines

Many chemokines are involved in the recruitment of inflammatory cells in asthma and COPD (Lukacs, 2001). Over 50 different chemokines are now recognized and they activate up to 20 different surface receptors (Rossi and Zlotnik, 2000). Chemokine receptors belong to the 7 transmembrane receptor superfamily of G-protein-coupled receptors and this makes it possible to find small molecule inhibitors, which has not yet been possible for classical cytokine receptors (Proudfoot, 2002). Some chemokine receptors appear to be selective for single chemokines, whereas others are promiscuous and mediate the effects of several related chemokines. Chemokines appear to act in sequence in determining the final inflammatory response and so inhibitors may be more or less effective depending on the kinetics of the response (Gutierrez-Ramos et al., 2000).

2.2.1. Chemokine CC2 receptor

Monocyte chemoattractant protein-1 (MCP-1) activates chemokine CC2 receptor on monocytes and T lymphocytes. Blocking MCP-1 with neutralizing antibodies reduced the recruitment of both T cells and eosinophils in a murine model of ovalbumin-induced airway inflammation, with a marked reduction in airway hyperresponsiveness (Gonzalo et al., 1996). MCP-1 also recruits and activates mast cells, an effect that is mediated via chemokine CC2 receptor (Campbell et al., 1999). MCP-1 instilled into the airways induces marked and prolonged airway hyperresponsiveness in mice, associated with mast cell degranulation. A neutralizing antibody to MCP-1 blocks the development of airway hyperresponsiveness in response to allergen (Campbell et al., 1999). The MCP-1 levels are increased in the bronchoalveolar lavage fluid of patients with asthma (Holgate et al., 1997). This has led to a search for small molecule inhibitors of chemokine CC2 receptor.

2.2.2. Chemokine CC3 receptor

Several chemokines, including eotaxin, eotaxin-2, eotaxin-3, regulated on activation, normal T-cell expressed and secreted (RANTES) and monocyte chemoattractant protein-4 (MCP-4) activate a common receptor on eosinophils designated (Gutierrez-Ramos et al., 1999). Chemokine CC3 receptor has a critical role in allergic inflammation, and therefore, chemokine CC3 receptor inhibitors may be useful targets for asthma treatment.

A neutralizing antibody against eotaxin reduces eosinophil recruitment in the lung after allergen challenge and the associated airway hyperresponsiveness in mice (Gonzalo et al., 1996), and blocking eotaxin reduces the trafficking of Th2 cells and eosinophils (Lloyd et al., 2000). There is increased expression of eotaxin, eotaxin-2, monocyte chemoattractant protein-3 (MCP-3), MCP-4 and chemokine CC3 receptor in the airways of asthmatic patients and this is correlated with increased airway hyperresponsiveness (Ying et al., 1997, 1999). Several small molecule inhibitors of chemokine CC3 receptor, including UCB35625, SB-297006 and SB-328437, are effective in inhibiting eosinophil recruitment in allergen models of asthma (Sabroe et al., 2000; White et al., 2000), and drugs in this class are currently undergoing clinical trials for asthma. Although it was thought that chemokine CC3 receptors were restricted to eosinophils, there is some evidence for their expression on Th2 cells and mast cells, so that these inhibitors may have a more widespread effect than on eosinophils alone, making them potentially more valuable in asthma treatment.

RANTES, which shows increased expression in asthmatic airways (Berkman et al., 1996), also activates chemokine CC3 receptor, but has effects on chemokine CC1 receptor and chemokine CC5 receptor, which may play a role in T cell recruitment. Modification of the N-terminal of RANTES, met-RANTES, has a blocking effect on RANTES by inhibiting these receptors (Elsner et al., 1997). This Met-RANTES can prevent the recruitment of eosinophil in allergen-sensitized and -challenged mice (Elsner et al., 1999).

2.2.3. Chemokine CC4 receptor and chemokine CC8 receptor

Chemokine CC4 receptor and chemokine CC8 receptor are selectively expressed on Th2 cells. Chemokine CC4 receptor is activated by the monocyte-derived chemokine (MDC) and thymus and activation dependent chemokine (TARC) (Lloyd et al., 2000), and chemokine CC8 receptor is activated by I-309 (Roos et al., 1997; Tiffany et al., 1997). Neutralized antibody to MDC prevented airway hyperresponsiveness in a murine asthma model (Gonzalo et al., 1999). Blocking TARC also attenuates the airway eosinophilia and airway hyperresponsiveness induced by allergen challenge (Kawasaki et al., 2001). Blocking I-309 reduces airway eosinophilia, but not airway hyperresponsiveness and Th2 cytokine production (Bishop and Lloyd, 2003). Inhibitors of chemokine CC4 receptor and

chemokine CC8 receptor may therefore inhibit the recruitment of Th2 cells and thus the persistent eosinophilic inflammation in the airways. However, blockade of chemokine CC4 receptor has no effect on the recruitment of cells or the production of chemokines in guinea pig (Conroy et al., 2003), and chemokine CC8 receptor gene deletion does not have any effects on allergic inflammation in mice (Chung et al., 2003), suggesting that these receptors may not be an effective target. Chemokine CC7 receptor plays a role in the migration of dendritic cells to regional lymph nodes and therefore blocking this receptor might suppress antigen presentation (Sallusto and Lanzavecchia, 2000).

2.2.4. Chemokine CXC4 receptor

Chemokine CXC4 receptor is also selectively expressed on Th2 cells and is activated by stromal cell-derived factor 1 (SDF-1). Neutralized antibody to chemokine CXC4 receptor reduced airway eosinophilia and airway hyperresponsiveness in a murine model of allergic airway disease. In addition, blocking SDF-1 also reduced both airway inflammation and airway hyperresponsiveness (Gonzalo et al., 2000). A small molecule inhibitor, AMD3100, inhibited allergen-induced inflammation in a murine model of asthma (Lukacs et al., 2002).

2.3. Other approaches to cytokine inhibition

Although there have been several attempts to block specific cytokines, this may not be adequate to block chronic inflammation in asthma, as so many cytokines are involved and there is considerable redundancy in their effects. This suggests that the development of drugs that have a more general effect on cytokine synthesis may be more promising. However, these drugs also affect other inflammatory processes, so their beneficial effects cannot necessarily be ascribed to the inhibition of cytokine synthesis alone.

Corticosteroids are the most effective treatment for asthma (Ichinose et al., 2000b) and part of their efficacy is due to the inhibition of inflammatory cytokine production. This is mediated through an effect on glucocorticoid receptors to reverse the acetylation of core histones that is linked to the increased expression of inflammatory genes, such as those encoding cytokines and chemokines (Ito et al., 2000). New steroids have recently been developed, including prodrug (Reynolds and Scott, 2004) or dissociated corticosteroid (Belvisi et al., 2001).

Cyclosporin A, tacrolimus and rapamycin inhibit the transcription of nuclear factor of activated T-cells which regulates the secretion of IL-2, IL-4, IL-5, IL-13 and GM-CSF by T-lymphocytes (Rao et al., 1997). Although some beneficial steroid-sparing effects in asthma have been reported (Lock et al., 1996), the toxicity of cyclosporin A limits its usefulness, at least when given orally. More selective Th2 selective drugs may be safer for the treatment of asthma in the future. An inhibitor of Th2 cytokines, suplatast tosilate (Oda et al., 1999), has been reported to provide clinical benefits in asthma (Tamaoki et al., 2000).

Phosphodiesterase 4 (PDE4) inhibitors inhibit the release of cytokines and chemokines from inflammatory cells via an increase in intracellular cyclic AMP (Torphy, 1998). Their clinical use is limited in asthma by side effects such as nausea, which seems to be mainly due to the inhibition of PDE4D subtype (Lamontagne et al., 2001), while PDE4B is thought to be more important to reduce airway inflammation (Jin and Conti, 2002). Thus, a PDE4B selective inhibitor may be a more useful tool for asthma.

NF-κB that is a pro-inflammatory signaling molecule that regulates the expression of many cytokines and chemokines involved in asthma (Barnes and Karin, 1997). There are several possible approaches to the inhibition of NF-κB, including gene transfer of an inhibitor of NF-κB (IκB), inhibitors of IκB kinase-2 (IKK2), NF-κB-inducing kinase and IκB ubiquitin ligase, which regulate the activity of NF-κB, and the development of drugs that inhibit the degradation of IκB (Delhase et al., 2000). One concern about this approach is that effective inhibitors of NF-κB may result in immune suppression and impair host defenses, since knockout mice which lack NF-κB proteins succumb to septicemia. However, there are alternative pathways of NF-κB activation that might be more important in inflammatory disease (Nasuhara et al., 1999). Several small molecule inhibitors of IKK2 are now in development (Adcock and Caramori, 2004b; Castro et al., 2003).

Mitogen-activated protein (MAP) kinases play a key role in chronic inflammation, and several complex enzyme cascades have now been defined. p38 MAP kinase pathway is one of these kinases, which is involved in expression of several inflammatory cytokines and chemokines (Kumar et al., 2003; Meja et al., 2000; Underwood et al., 2000). Small molecule inhibitors of p38 MAP kinase, such as SB 203580, SB 239063 and RWJ 67657, also known as cytokine-suppressive anti-inflammatory drugs (CSAIDS), have been developed and these drugs have a broad range of anti-inflammatory effects (Lee et al., 2000). In addition, p38 MAP kinase inhibitors reduce eosinophil survival through the enhancement of apoptosis (Kankaanranta et al., 1999). It has been also shown that p38 MAP kinase is associated with steroid resistant asthma, and that p38 MAP kinase inhibitors may improve the response to steroid in asthma (Irusen et al., 2002). However, there may be issues of safety, as p38 MAP kinases are involved in host defense. It is possible that using the inhaled route of delivery may reduce the risk of side effects.

3. Cytokine directed therapies for COPD

3.1. Inhibition of cytokines and chemokines

Unlike asthma, Th2 cytokines do not play a critical role in the pathogenesis of COPD. There is no evidence that the levels of Th2 cytokine are elevated in COPD airways (Barnes, 2001a). Pro-inflammatory cytokines such as IL-1β and TNF-α may be involved in the inflammatory response not only in asthma but also in COPD. Although an IL-1 receptor antagonist is now in a clinical trial for some inflammatory diseases (Cohen, 2004),

there have been no published studies on the usefulness of an IL-1 receptor antagonist in COPD.

It has been reported that the levels of TNF-α and soluble TNF receptor are increased in the sputum of COPD (Keatings et al., 1996; Vernooy et al., 2002). TNF-α enhances airway inflammation through the induction of IL-8 and other chemokines via the activation of NF-κB. Therefore, TNF-α or its soluble receptor may be a target for reducing COPD inflammation. Trials of anti-TNF therapy in patients with the systemic features of COPD are now underway (Barnes and Stockley, 2005). TACE is required for soluble TNF-α release. Thus, small molecule of TACE inhibitors may be an attractive therapeutic target not only for asthma but also for COPD.

Various chemokines also play an important role in the recruitment of inflammatory cells in COPD airways and have been shown to be elevated in COPD. These include IL-8 and growth-related oncogene-α (GRO-α) (Keatings et al., 1996; Traves et al., 2002). The effects of these chemokines are mediated by chemokine CXC receptors, which are G-protein-coupled receptors. Neutrophils, the major contributors to the airway inflammation of COPD, express both chemokine CXC1 receptor (IL-8 specific low affinity receptor) and 2 (high affinity receptor for several chemokines). Thus, inhibitors of these chemokines or antagonists of chemokine CXC receptors may be a therapeutic target for COPD.

Blocking IL-8 reduces the neutrophil chemotactic activity of sputum from COPD patients (Beeh et al., 2003). A monoclonal antibody to IL-8 has been developed and tested in COPD. Although this antibody had a small effect in improving dyspnea, no significant differences were observed in the lung function and health status (Mahler et al., 2004). Antagonism of chemokine CXC2 receptor may be a more effective strategy. Several small molecule inhibitors of chemokine CXC2 receptor are now in clinical development for the treatment of COPD (Hay and Sarau, 2001; White et al., 1998). In chemokine CXC2 receptor knockout mice, there is a marked reduction in mucus secretion in response to viral infection, suggesting that this receptor may be also involved in mucus hypersecretion (Miller et al., 2003).

Growth related oncogene-α (GRO-α) is one of the CXC chemokines that is produced by several cells such as monocytes, endothelial cells and fibroblasts. GRO-α is also secreted in alveolar macrophages and airway epithelial cells by the stimulation with lipopolysaccharide, TNF-α and IL-17 (Becker et al., 1994; Schulz et al., 2004; Jones and Chan, 2002; Prause et al., 2003). GRO-α is a powerful activator and chemoattractant of neutrophils and exerts its effect through the activation of chemokine CXC2 receptor (Geiser et al., 1993). The levels of GRO-α are significantly increased in COPD sputum (Traves et al., 2002). In addition, the expression of chemokine CXC2 receptor is increased during exacerbations of COPD and there is a correlation between the airway neutrophilia and chemokine CXC2 receptor expression (Qiu et al., 2003). These data suggest that GRO-α and chemokine CXC2 receptor play a critical role in the recruitment of inflammatory cells in COPD. SB 225002, a small molecule antagonist of chemokine CXC2 receptor, which is now in clinical trials (Widdowson et al., 2004), potently inhibits the chemotaxis of neutrophils induced by IL-8 and GRO-α (White et al., 1998). This selective antagonist may be a useful tool for COPD treatment.

3.2. Inhibition of signal transduction

Like asthma, several signal transduction pathways are involved in the pathophysiology of COPD and some inhibitors of these pathways are now in clinical development (Barnes and Stockley, 2005; Cohen, 2002). Unlike asthma, corticosteroids are not effective in preventing the decline of the lung function and airway inflammation (Culpitt et al., 1999; Hattotuwa et al., 2002; Keatings et al., 1997).

PDE4 inhibitors may be useful for COPD treatment. Indeed, several PDE4 inhibitors have been developed and are in clinical trials. It has been demonstrated that PDE4 inhibitors reduce the production of TNF-α by LPS stimulation in a mononuclear cell line and in whole blood cells from COPD patients (Draheim et al., 2004; Ouagued et al., 2005).

NF-κB is one of the important regulators of the production of several cytokines involved in the pathophysiology of COPD including TNF-α, IL-6 and IL-8 (Tak and Firestein, 2001). The expression of NF-κB is increased in COPD airways and this increased expression is correlated with the disease severity (Di Stefano et al., 2002). Thus, NF-κB may be another therapeutic target for COPD. Small molecule inhibitors of IKK2 may be promising not only for the treatment of asthma but also for COPD (Adcock and Caramori, 2004b; Castro et al., 2003).

p38 MAP kinase pathway is also involved in the pathophysiology of COPD through the regulation of inflammatory cytokines such as TNF-α and IL-8 (Barnes and Stockley, 2005). One of the small molecule inhibitors, SB 239063, reduces neutrophil infiltration and IL-6 production in the lung of LPS stimulated rats (Underwood et al., 2000). SB 239063 also inhibits LPS-induced IL-6 production in alveolar macrophages from guinea pig (Underwood et al., 2000). These results suggest that the inhibition of the p38 MAP kinase pathway may be a useful target for COPD (Adcock and Caramori, 2004b).

More recently, it has been reported that CGH2466, a combined adenosine receptor antagonist, p38 mitogen-activated protein kinase and phosphodiesterase type 4 inhibitor, has potent anti-inflammatory activities (Trifilieff et al., 2005). CGH2466 inhibits the production of cytokines and oxygen radicals in human peripheral blood leucocytes, more potently than each inhibitor or antagonist alone. CGH2466 also inhibits LPS-induced airway inflammation in mice. Therefore, this novel compound may be a beneficial therapeutic tool for COPD.

Phosphoinositide 3-kinases (PI3K) have been shown to play an important role in neutrophil chemotaxis (Thomas et al., 2005; Wymann et al., 2003). Among several PI3K isoforms, PI3K gamma has a pivotal role in chemokine-dependent migration of neutrophils and macrophages (Hirsch et al., 2000), suggesting that the PI3K signaling pathway, especially the gamma isoform, may be a promising target for new therapies to treat COPD (Finan and Thomas, 2004). Small molecule

inhibitors of the PI3K family are now in development (Ward et al., 2003; Ward and Finan, 2003).

4. Conclusions

Several specific cytokine and chemokine inhibitors are now in development for the treatment of asthma and COPD. Inhibition of IL-4 with soluble IL-4 receptors showed promising early results for asthma, however, this was not confirmed in subsequent clinical trials. Antibodies that block IL-5 effectively inhibit peripheral blood and airway eosinophilia, but it does not also seem to be effective in symptomatic asthma. Inhibition of IL-13 appears to be more promising. Anti-inflammatory cytokines may also be useful, however, it would be necessary to develop efficient inhaled delivery systems to prevent systemic adverse effects. Inhibition of TNF-α may be useful in the treatment of severe asthma and COPD. As various chemokines are involved in the recruitment of inflammatory cells in asthma and COPD airways, small molecule inhibitors of chemokine receptors are also promising therapeutic targets. Antagonists against chemokine CC3 receptor or chemokine CXC2 receptor are now in development for the treatment of asthma and COPD. Many cytokines are involved in the complexity of the pathophysiology of asthma and COPD, therefore, agents that inhibit the synthesis of multiple cytokines may be more successful. Several such agents are now in clinical development, including PDE4, p38 MAP kinase, IKK2 and PI3K inhibitors. Using the inhaled delivery route may reduce the risk of adverse effects in these non-specific inhibitors.

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Airway cytokine expression measured by means of protein array in exhaled breath condensate: Correlation with physiologic properties in asthmatic patients

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Background: Simultaneous monitoring of airway inflammation and physiology might be useful for asthma management.
Objective: We examined the upregulated molecules in asthmatic airways. Furthermore, we investigated the relationship between these molecules and the airway physiologic properties of asthma.
Methods: Ten nonsmoking healthy subjects and 16 steroid-naive asthmatic patients were enrolled. Exhaled breath condensate (EBC) sampling, spirometry, and methacholine inhalation challenge were performed on one occasion in this cross-sectional study. Peak expiratory flow was also measured for 4 weeks. Airway cytokine–chemokine–growth factor production was analyzed with a protein array.
Results: The expressions of IL-4, IL-8, IL-17, TNF-α, RANTES, IFN-γ–inducible protein 10, TGF-β, and macrophage inflammatory protein 1α and 1β were significantly upregulated in asthmatic airways compared with those of nonsmoking healthy subjects. Among the upregulated molecules, RANTES expression was significantly correlated with the parameters that represent airway caliber, FEV_1 and respiratory resistance values. In addition, the levels of both TNF-α and TGF-β were significantly correlated with the methacholine threshold and peak expiratory flow variability for the week.
Conclusion: Inflammatory molecule analysis with EBC appeared to be useful for monitoring the asthmatic airway condition.
Clinical implications: Measurements of cytokine levels in EBC might be a promising approach to assess the efficacy of pharmacologic interventions and to investigate the pathophysiology of asthma. (J Allergy Clin Immunol 2006;118:84-90.)

Key words: Airway hyperresponsiveness, airway lability, airflow limitation, bronchial asthma, exhaled breath condensate, protein array, RANTES, TGF-β, TNF-α

Asthma is a chronic inflammatory disorder of the airways.[1] The inflammation causes airway physiologic changes, such as airway obstruction and airway hyperresponsiveness (AHR). Therefore establishing a simple monitoring system of airway inflammation would be useful for asthma management. In addition, examination of the relationship between the physiologic properties and molecules upregulated during inflammation would also be important.

Exhaled breath condensate (EBC), which is formed by breathing through a cooling system, contains both volatile compounds and nonvolatile compounds.[2-5] Analyses of EBC could provide useful information for possible clinical applications. Because this method is noninvasive, repeated measurements can be made, which could be useful for monitoring the airway inflammation.[2]

Several inflammatory molecules, such as eicosanoids and cytokines, have been identified in the EBC,[3,4] which is likely to reflect the composition of the airway-lining fluid.[5] In the present study the cytokine expression in EBC obtained from asthmatic airways was simultaneously analyzed by using a chemiluminescence-based membrane protein array.[6-9] Furthermore, we examined the relationship between these molecules and the physiologic properties of asthma, such as airway obstruction and AHR.

METHODS

Study subjects

Ten nonsmoking healthy subjects and 16 nonsmoking, steroid-naive asthmatic patients took part in the study after providing informed consent. The study was approved by the local ethics committee. All patients satisfied the American Thoracic Society criteria for asthma.[10] The clinical characteristics of these subjects are shown in Table I. All asthmatic patients were stable and had been without regular asthma treatment, including steroid therapy, before the study, but rescue use of short-acting inhaled β_2-agonists as needed for symptom relief was permitted.

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Abbreviations used
AHR: Airway hyperresponsiveness
EBC: Exhaled breath condensate
IP-10: IFN-γ–inducible protein 10
MIP: Macrophage inflammatory protein
PEF: Peak expiratory flow
Rrs: Respiratory resistance

TABLE I. Baseline characteristics of the study subjects

	Control subjects	Asthmatic subjects
Number	10 (F/M = 7/3)	16 (F/M = 12/4)
Age (y)	34.4 ± 6.6	37.1 ± 12.6
FVC (L)	3.38 ± 0.82	3.19 ± 0.58
FEV_1 (L)	3.10 ± 0.70	2.47 ± 0.47
FEV_1% (%)	92.2 ± 3.1	77.5 ± 5.2
%FEV_1 (%)	103.9 ± 9.0	81.3 ± 8.9

F, Female; *M*, male; *FVC*, forced vital capacity.

Study design

The study was cross-sectional. Subjects attended the outpatient clinic at the Wakayama Medical University hospital on one occasion for clinic examination, spirometry, EBC collection, and methacholine inhalation challenge. Peak expiratory flow (PEF) monitoring had been performed for at least 4 weeks before this attendance.

EBC collection

The EBC was collected by using a condenser, which permitted noninvasive collection of condensed exhaled air and froze it to −20°C (Ecoscreen; Jaeger, Hoechberg, Germany).[11] The subjects breathed through a mouthpiece and a 2-way nonrebreathing valve, which also served as a saliva trap. Subjects were asked to breath at a normal frequency and tidal volume while wearing a nose clip for 15 minutes. The collected EBC was melted and transferred to 1-mL Eppendorf tubes and immediately stored at −70°C. The mean volume collected was 1.6 mL (range, 1.2-2.0 mL).

Cytokine measurements were performed within 4 weeks after the collection of the EBC samples.

Cytokine measurements

Human Inflammation Antibody III (Ray Biotech Inc, Norcross, Ga), consisting of 40 different cytokine and chemokine antibodies spotted in duplicate onto a membrane, was used.[6-9] Briefly, the membranes were blocked with 10% BSA in Tris-buffered saline, and then 1.0 mL of EBC obtained from either healthy subjects or asthmatic subjects was added and incubated at room temperature for 2 hours. The membranes were washed, and 1.0 mL of primary biotin-conjugated antibody was added and incubated at room temperature for 2 hours. After a thorough wash, the membranes were incubated with 2.0 mL of horseradish peroxidase–conjugated streptavidin at room temperature for 1 hour. The intensity of signals was detected directly from the membranes by using a chemiluminescence imaging system (Luminocapture AE6955; Atto Co, Tokyo, Japan). Exposure times ranged from 30 seconds to 2 minutes. Chemiluminescence was quantified with Atto imaging and analysis software. Horseradish peroxidase–conjugated antibody served as a positive control at 6 spots and was also used to identify the membrane orientation. For each spot, the net intensity gray level was determined by subtracting the background gray levels from the total raw intensity gray levels. The relative intensity levels in the cytokine amount were normalized with reference to the amount present on the positive control in each membrane on the basis of the average of the cytokine spot intensity levels divided by the average of the positive control spot intensity levels and indicated as a percentage. A list of examined cytokines and their sensitivities is shown in Table II.

Reproducibility for the profiles of cytokine expression was assessed in 5 asthmatic patients in a randomized design in which a second EBC sample was collected while the patient was clinically stable within 7 days of obtaining the first sample.

PEF measurements

PEF was measured twice a day with an Assess peak flowmeter (Respironics HealthScan Co, Cedar Grove, NJ) for at least 4 weeks, according to the standard procedure.[12] The average of the 2 largest values of daily PEF variability from the recent week was determined to represent the PEF variability for the week.[13]

Pulmonary function

FEV_1 and forced vital capacity were measured with a Vitalograph Pneumotrac 6800 (Vitarograph Co, Ennis, Ireland), according to the standard procedure.[14]

Methacholine inhalation challenge

Thus far, the bronchial provocation test for estimating the hyperresponsiveness of the airways has been generally examined by means of spirometric measurement. However, forced expiration itself might introduce bronchoconstriction.[15] To avoid a forced expiratory maneuver during provocation testing, airway responsiveness to inhaled methacholine was measured with a device (Astograph Jupiter21; Chest Co, Tokyo, Japan) that displays respiratory resistance (Rrs) measured by means of the forced oscillation method during tidal breathing with continuous inhalation of the aerosolized drug.[16] Briefly, it consists of an aerosol delivery system, a loudspeaker box system that generates a constant-amplitude sine wave pressure at 3 Hz, and a system for measuring Rrs automatically from the mouth flow and mouth pressure. Aerosols were generated by using a Bird nebulizer (Bird Co, Palm Springs, Calif), each containing 4 mL of solution driven with a constant airflow of 6 L/min by an air compressor to elicit an output of approximately 0.15 mL/min. The output was determined by measuring the change in weight of the nebulizer chamber. Methacholine (Sigma Co, St Louis, Mo) was prepared in 0.9% saline in 2-fold increasing concentrations ranging from 0.049 to 25 mg/mL. After it was confirmed that a 1-minute inhalation of saline did not change the baseline Rrs, each concentration of methacholine solution was inhaled for 1 minute until Rrs reached approximately twice the baseline value or until the maximum concentration was administered. The index of the airway responsiveness was defined as the cumulative provocative dose of methacholine causing a 100% increase in Rrs.

Statistical analysis

Comparisons between 2 groups were performed by using the Kruskal-Wallis test, followed by the pairwise Mann-Whitney *U* test. Pearson correlation coefficients were calculated to determine the correlation between the relative cytokine levels and pulmonary physiologic parameters. All data were expressed as means ± SD, and significance was defined as a P value of less than .05.

TABLE II. List of 40 examined molecules

	Cytokine	Sensitivity (pg/mL)		Cytokine	Sensitivity (pg/mL)
IL	IL-1α	1000	CXC-chemokine	IL-8	1
	IL-1β	100		Mig	1
	IL-2	25		IP-10	10
	IL-3	100	CC-Chemokine	I-309	1000
	IL-4	1		MIP-1α	20
	IL-6	1		MIP-1β	10
	IL-6sR	20		MIP-1δ	100
	IL-7	100		RANTES	2,000
	IL-10	10		MCP-1	3
	IL-11	10,000		MCP-2	100
	IL-12 p40	1000		Eotaxin-1	1
	IL-12 p70	10		Eotaxin-2	1
	IL-13	100	Colony-stimulating factor	G-CSF	2,000
	IL-15	100		GM-CSF	100
	IL-16	1		M-CSF	1
	IL-17	10	Growth factor	TGF-β	200
TNF	TNF-α	50		PDGF	1000
	TNF-β	1000	Others	TIMP-2	1
	sTNF RI	100		ICAM-1	50,000
	sTNF RI	10		IFN-γ	100

Mig, Monokine induced by IFN-γ; *IL-6sR*, IL-6 soluble receptor; *MCP*, Monocyte chemoattractant protein; *G-CSF*, granulocyte colony-stimulating factor; *M-CSF*, macrophage colony-stimulating factor; *PDGF*, platelet-derived growth factor; *TIMP-2*, tissue inhibitor of metalloprotease 2; *sTNF-R*, soluble TNF receptor; *ICAM-1*, intracellular adhesion molecule 1.

RESULTS

Reproducibility of measurements

Differences in the individual relative levels between the first and second EBC samples and the limits of agreement of each cytokine are shown in Fig 1 (n = 5). Within-subject reproducibility of the relative cytokine levels was expressed as the limit of agreement (mean difference ± 2 SDs of the differences).[17]

Cytokine expression in asthmatic airways

Selective upregulation of several molecules in EBC from both groups was detectable on the microarray membranes. The results of comparison analysis of the relative cytokine levels in 2 groups are summarized in Table III. The array analyses indicated that IL-4, IL-8, IL-17, TNF-α, RANTES, IFN-γ–inducible protein 10 (IP-10), TGF-β, macrophage inflammatory protein (MIP) 1α, and MIP-1β were the molecules with significantly upregulated expression in asthmatic airways compared with those of healthy subjects ($P < .01$).

Relationship between cytokine expression and pulmonary physiologic parameters

Among the upregulated molecules, correlations between the molecules and the physiologic properties of asthma, such as airway obstruction, airway lability, and AHR, were found (Table IV). The relative level of RANTES was significantly correlated with the percentage of FEV_1 ($r = -0.72$, $P < .01$ [Fig 2, *A*]) and Rrs values ($r = 0.53$, $P < .05$ [Fig 2, *B*]). In addition, the levels of both TNF-α and TGF-β were significantly correlated with the methacholine threshold ($r = -0.80$, $P < .01$ [Fig 3, *A*] and $r = -0.73$, $P < .01$ [Fig 3, *B*], respectively) and PEF variability for the week ($r = 0.75$, $P < 0.01$ [Fig 4, *A*] and $r = 0.66$, $P < .01$ [Fig 4, *B*], respectively).

DISCUSSION

In the present study the array analyses indicated that IL-4, IL-8, IL-17, TNF-α, RANTES, IP-10, TGF-β, MIP-1α, and MIP-1β were the molecules significantly upregulated in asthmatic airways compared with those of healthy subjects. Furthermore, we have shown that among the increased molecules the relative level of RANTES was significantly correlated with the parameters of airflow limitation. Both the TNF-α and TGF-β values were significantly correlated with the degree of airway responsiveness and airway lability.

A basic pathologic feature of asthma is airway inflammation, in which various inflammatory cells and inflammatory molecules produced from them are involved.[18] Both invasive (eg, bronchoalveolar lavage fluid) and semi-invasive (eg. induced sputum) methods have been used to quantify airway inflammatory molecules in many studies.[19,20] However, these relatively invasive approaches are unsuitable to monitor airway inflammation repeatedly.

By contrast, collection of EBC samples is easy to perform, and because it is noninvasive, it can be done repeatedly. In the present study increased levels of several cytokines–chemokines–growth factors in EBC obtained from asthmatic subjects were demonstrated. The upregulation of these inflammatory molecules in asthmatic

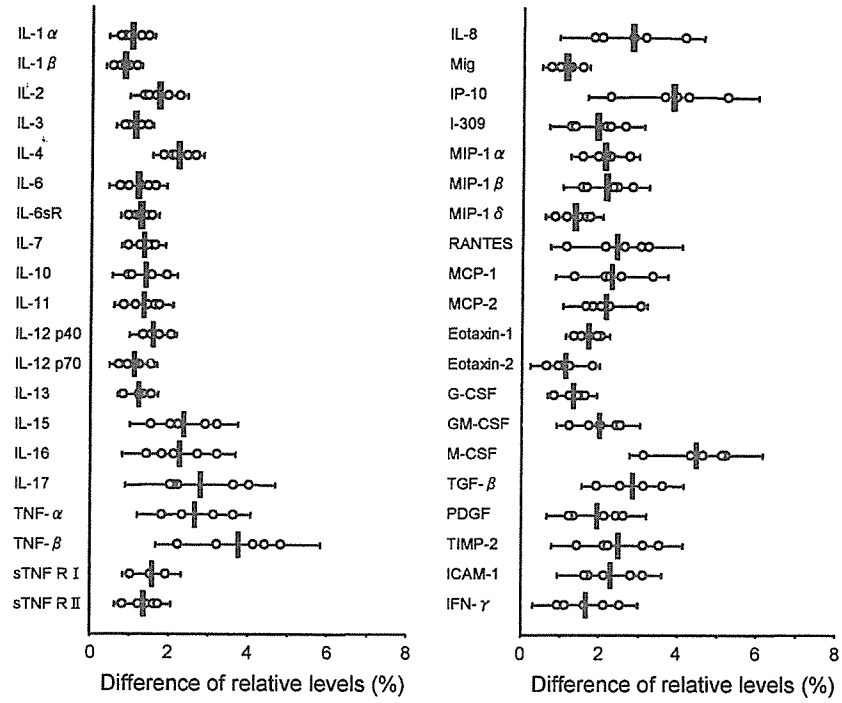

FIG 1. Within-subject reproducibility of relative cytokine levels. Data are presented as *bold vertical bars* and *whisker plots* showing the mean difference and the limit of agreement (±2 SDs of the differences). Differences in the individual relative levels between the first and second EBC samples (n = 5) are superimposed. *IL-6sR*, IL-6 soluble receptor; *sTNF-R*, soluble TNF receptor; *Mig*, monokine induced by IFN-γ; *MCP*, monocyte chemoattractant protein; *G-CSF*, granulocyte colony-stimulating factor; *M-CSF*, macrophage colony-stimulating factor; *PDGF*, platelet-derived growth factor; *TIMP-2*, tissue inhibitor of metalloprotease 2; *ICAM-1*, intercellular adhesion molecule 1.

TABLE III. Relative cytokine levels in EBC

Cytokine	Control subjects (%)[a]	Asthmatic subjects (%)[b]	Fold increase[b/a]	Cytokine	Control subjects (%)[a]	Asthmatic subjects (%)[b]	Fold increase[b/a]
IL-1α	4.0 ± 2.1	5.2 ± 1.3	1.30	IL-8	5.4 ± 2.1	8.3 ± 1.9*	1.52
IL-1β	4.6 ± 0.9	4.2 ± 2.0	0.92	Mig	4.2 ± 1.4	4.1 ± 1.5	0.97
IL-2	4.9 ± 1.7	4.1 ± 2.0	0.83	IP-10	8.4 ± 1.3	22.7 ± 6.4*	2.72
IL-3	5.7 ± 1.4	5.0 ± 2.0	0.88	I-309	3.5 ± 1.5	3.5 ± 2.2	1.00
IL-4	5.2 ± 1.7	8.2 ± 1.6*	1.56	MIP-1α	6.3 ± 1.3	9.2 ± 2.0*	1.47
IL-6	5.2 ± 1.2	4.7 ± 1.7	0.91	MIP-1β	6.5 ± 1.5	10.2 ± 3.7*	1.58
IL-6sR	5.1 ± 1.3	4.6 ± 1.8	0.91	MIP-1δ	3.7 ± 1.3	5.4 ± 2.9	1.45
IL-7	2.6 ± 0.8	3.2 ± 1.5	1.24	RANTES	6.2 ± 1.5	10.4 ± 2.5*	1.69
IL-10	5.4 ± 1.8	5.7 ± 1.6	1.04	MCP-1	6.5 ± 2.1	7.9 ± 2.2	1.20
IL-11	5.6 ± 1.8	5.2 ± 1.8	0.93	MCP-2	4.1 ± 1.7	4.3 ± 1.5	1.04
IL-12 p40	4.8 ± 1.4	4.2 ± 1.8	0.88	Eotaxin-1	4.6 ± 2.2	5.0 ± 2.3	1.09
IL-12 p70	2.8 ± 1.4	3.4 ± 2.1	1.24	Eotaxin-2	3.9 ± 1.7	4.3 ± 1.3	1.11
IL-13	4.0 ± 1.0	5.5 ± 2.3	1.37	G-CSF	3.6 ± 1.7	3.1 ± 1.5	0.88
IL-15	7.3 ± 2.8	7.4 ± 3.4	1.01	GM-CSF	3.8 ± 1.0	3.4 ± 1.6	0.92
IL-16	6.2 ± 1.8	6.5 ± 4.3	1.04	M-CSF	9.7 ± 3.4	9.4 ± 4.7	0.97
IL-17	8.6 ± 1.5	12.6 ± 4.1*	1.46	TGF-β	6.6 ± 1.2	11.6 ± 3.4*	1.69
TNF-α	7.0 ± 1.0	12.4 ± 3.8*	1.76	PDGF	6.8 ± 1.6	7.6 ± 1.8	1.12
TNF-β	27.7 ± 7.4	27.6 ± 8.3	1.00	TIMP-2	9.5 ± 2.9	9.0 ± 3.0	0.94
sTNF RI	4.8 ± 1.8	5.4 ± 1.4	1.13	ICAM-1	3.4 ± 0.8	3.4 ± 2.1	1.00
sTNF RII	5.1 ± 1.6	4.6 ± 1.5	0.90	IFN-γ	5.4 ± 2.2	5.5 ± 2.2	1.00

Relative cytokine levels to positive control in EBC obtained from either healthy subjects *(a)* or asthmatic subjects *(b)*.
Mig, Monokine induced by IFN-γ; *IL-6sR*, IL-6 soluble receptor; *MCP*, Monocyte chemoattractant protein; *G-CSF*, granulocyte colony-stimulating factor; *M-CSF*, macrophage colony-stimulating factor; *PDGF*, platelet-derived growth factor; *TIMP-2*, tissue inhibitor of metalloprotease 2; *sTNF-R*, soluble TNF receptor; *ICAM-1*, intracellular adhesion molecule 1.
*$P < .01$ compared with control subjects.

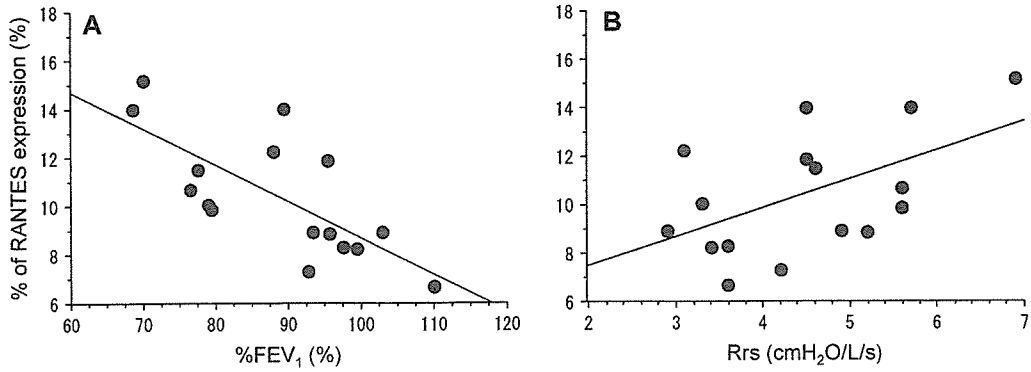

FIG 2. Relationship between the relative expression level of RANTES and parameters of airflow limitation: **A**, FEV_1 (percentage of predicted value; $r = -0.72$, $P < 0.01$); **B**, initial Rrs ($r = 0.53$, $P < .05$). The *lines* correspond to the fitted regression equation.

TABLE IV. Correlation between the molecules and airway physiologic parameters

Cytokine	% FEV_1	Rrs	PD_{200}	PEF variability
IL-4	$r = -0.20$, $P = .46$	$r = 0.12$, $P = .66$	$r = -0.22$, $P = .42$	$r = 0.35$, $P = .19$
IL-8	$r = 0.39$, $P = .14$	$r = 0.15$, $P = .58$	$r = 0.11$, $P = .68$	$r = -0.05$, $P = .86$
IL-17	$r = -0.40$, $P = .13$	$r = 0.49$, $P = .06$	$r = -0.16$, $P = .57$	$r = 0.15$, $P = .57$
TNF-α	$r = -0.35$, $P = .19$	$r = 0.30$, $P = .26$	$r = -0.80$, $P < .01$	$r = 0.75$, $P < .01$
RANTES	$r = -0.72$, $P < .01$	$r = 0.53$, $P < .05$	$r = 0.01$, $P = .96$	$r = -0.08$, $P = .79$
IP-10	$r = -0.24$, $P = 0.37$	$r = 0.45$, $P = .08$	$r = -0.28$, $P = .30$	$r = 0.31$, $P = .25$
TGF-β	$r = -0.38$, $P = .15$	$r = 0.36$, $P = .17$	$r = -0.73$, $P < .01$	$r = 0.66$, $P < .01$
MIP-1α	$r = 0.09$, $P = .75$	$r = 0.22$, $P = .41$	$r = 0.03$, $P = .92$	$r = -0.01$, $P = .96$
MIP-1β	$r = -0.16$, $P = .57$	$r = 0.29$, $P = .41$	$r = -0.21$, $P = .45$	$r = 0.16$, $P = .56$

PD_{200}, Cumulative provocative dose of methacholine causing a 100% increase in Rrs.

airways is in agreement with the findings of previous studies with bronchoalveolar lavage fluid,[21] supporting the hypothesis that a nonvolatile molecule in the airway-lining fluid can be transported in the form of aerosols in exhaled breath.[5]

The chemiluminescene-based cytokine array, a type of proteomics approach, is a simple and rapid method of analysis of multiple proteins. It has been confirmed that the amount of increase in protein expression agrees with the added protein amount in this method.[6-8] Furthermore, it has been shown that the relative levels obtained by using this method correlated well with the actual levels obtained by means of quantitative assays.[8,9] Additionally, in the present study the reproducibility of EBC analysis by means of protein array was expressed as the limits of agreement. Thus analysis of EBC by means of protein array would be a simple and useful monitoring system of airway inflammatory molecules.

Among the upregulated molecules, there was a striking difference between the IP-10 levels in EBC obtained from asthmatic patients and that from healthy subjects. IP-10 is regarded as a marker of T_H1 activity because its expression is induced by IFN-γ. However, in a mouse model of asthma, IP-10 expression increased after allergen challenge, and IP-10–transgenic mice experience a T_H2 inflammatory response and AHR.[22] A recent study indicated that IP-10 plays a key role in the migration of mast cells into the airway smooth muscle bundles in asthma.[23] In addition, several upregulated molecules, such as IL-4, IL-17, and IL-8, are regarded as principal molecules in the pathophysiology of asthma.[21] The present analytic system might be helpful to assess the potential role of these inflammatory molecules in asthma.

In the present study the RANTES level in EBC was significantly correlated with FEV_1 and Rrs, which are the indices of airflow limitation. The airflow limitation of asthma is multifactorial. The major cause is the contraction of smooth muscle provoked by mediators released from various inflammatory cells. This bronchoconstriction is exaggerated by thickening of the airway wall caused by mucosal edema, cellular infiltration, mucus plugging, and airway remodeling.[24] All of these features are related to the airway inflammation. RANTES, a member of the CC chemokines, is a powerful chemoattractant of eosinophils, T lymphocytes, and basophils.[21] It also activates these immune cells and induces the exocytosis of bronchoconstrictive mediators, such as histamine and cysteinyl leukotrienes from basophils and eosinophilic cationic protein from eosinophils.[21] Therefore RANTES might be involved in inflammatory cell recruitment and the induction of bronchoconstrictive mediators from cells, resulting in airflow limitation. A previous report has shown that RANTES-positive sputum eosinophils and the percentage of FEV_1 after allergen challenge are

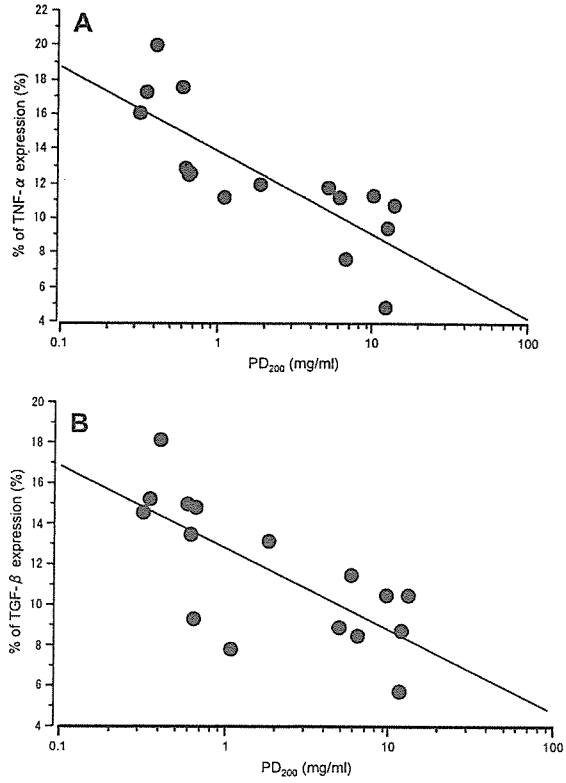

FIG 3. Relationship between airway responsiveness to methacholine (cumulative provocative dose of methacholine causing a 100% increase in Rrs *[PD_{200}]*) and the relative expression levels of TNF-α ($r = -0.80$, $P < .01$; **A**) and TGF-β ($r = -0.73$, $P < 0.01$; **B**). The *lines* correspond to the fitted regression equation.

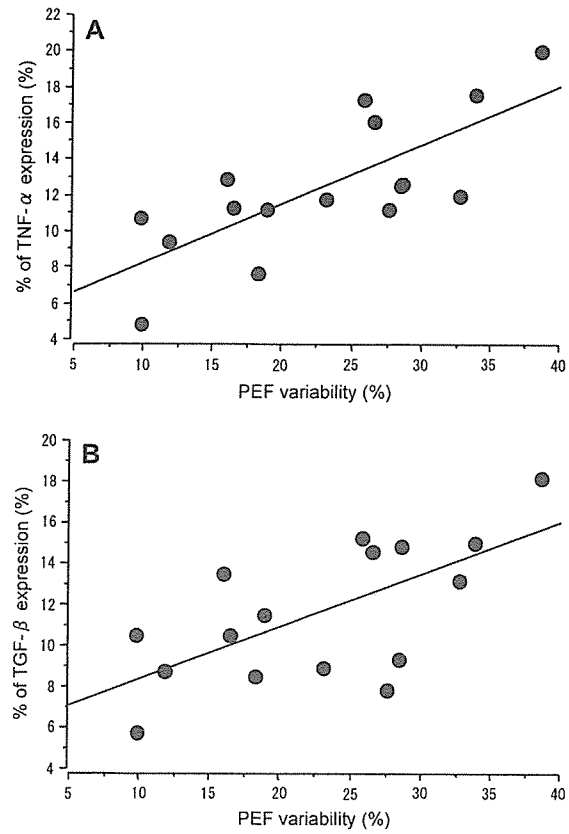

FIG 4. Relationship between PEF variability for the week and the relative expression levels of TNF-α ($r = 0.75$, $P < .01$; **A**) and TGF-β ($r = 0.66$, $P < 0.01$; **B**). The *lines* correspond to the fitted regression equation.

significantly correlated in asthmatic patients, which is compatible with our result.[25]

AHR is another important physiologic property of asthma.[1] Several mechanisms, such as airway inflammation, increased neural reflexes, airway geometric factors, and genetic factors, have been proposed to explain the AHR.[1,26] Among these mechanisms, airway inflammation has been reported to be a key factor, and it seems to cause AHR through 2 mechanisms.[26] One mechanism is active inflammation through the release of chemical mediators from immune cells, and another is modification of the airway resident cells through chronic inflammation, resulting in airway remodeling.

In the present study the degree of airway responsiveness correlated with both the TNF-α and TGF-β values in EBC. TNF-α is a proinflammatory cytokine produced by many cells and plays an important role in amplifying asthmatic inflammation.[21] TNF-α acts on epithelial cells to release a variety of molecules, including GM-CSF and RANTES, which then amplify the inflammatory response and lead to the influx of inflammatory cells.[21] In a previous study the inhalation of TNF-α increased AHR in human subjects.[27] Additionally, the possible effectiveness of TNF blockade with soluble TNF receptors for AHR in patients with severe asthma has been demonstrated.[28] The present result is in agreement with these reports.

TGF-β appears to play an integral role in promoting the structural changes of airway remodeling.[29,30] In asthma increased TGF-β mRNA expression in bronchial tissue is seen, and its level of expression correlates with the depth of subepithelial fibrosis.[29] In addition, the degree of thickening of the subepithelial layer is significantly correlated with the degree of airway responsiveness.[31] In contrast, it has been shown that there are no identifiable differences in collagen deposition or TGF-β–expressing cells in the large airways of patients with mild asthma when compared with those of patients with severe asthma.[32] Although the relationship between airway collagen deposition and physiologic parameters remains controversial, TGF-β might be involved in the mechanism of AHR through its promotion of airway remodeling.

The clinical consequences of AHR are an exaggerated variation in the airway caliber known as airway lability. Although the precise mechanism of airway lability in asthma is still unclear, it has been reported that the variability of PEF correlates better than any other indices with the degree of AHR.[1] In fact, in the present study both the TNF-α and TGF-β values were correlated with not only AHR but also the degree of PEF variability.

In conclusion, inflammatory molecule analysis with EBC appeared to be useful for monitoring the asthmatic airway condition and might be a promising approach to assess the efficacy of pharmacologic intervention and to investigate the pathophysiology of asthma.

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CASE REPORT

Two Cases of Asthma in Handicapped Elderly Persons in Which Assisted Inhalation Therapy Was Effective

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ABSTRACT

Background: Chronic airway inflammation is a basic pathology of bronchial asthma and it is important to control the inflammation by anti-inflammatory therapy mainly with steroids. However, in asthma in the elderly, there are cases where physicians hesitate to introduce the inhaled corticosteroid (ICS) therapy based on the diagnosis that the use of inhalants is difficult due to the existence of a functional lesion accompanying asthma.

Methods & Results: In cases where self-administrated inhalation therapy is difficult to execute due to the accompaniment of a functional lesion and in cases where sufficient curative effects of steroids are not produced in self-inhalation, administration of assisted inhalation resulted in improvement of clinical symptoms and pulmonary function and was proven effective.

Conclusions: Assisted inhalation therapy is expected to be useful in general and also in terms of expanding the application of ICS in the asthma in the elderly.

KEY WORDS

assistance, bronchial asthma, elderly persons, handicapped persons, inhaled corticosteroid

INTRODUCTION

A basic pathology in bronchial asthma is chronic airway inflammation, in which various cells such as eosinophils, lymphocytes, mast cells, and airway epithelial cells and various cytokines, chemokines, and inflammatory mediators produced from them are involved.[1] In addition, chronic airway inflammation is also involved in the enhancement of airway hyperresponsiveness, which is another pathology of bronchial asthma.[2,3] Therefore the basics of the therapy against bronchial asthma is to control inflammation caused by these wide range of cells and the drug of first alternative at this point is inhaled corticosteroids (ICS). ICS improves enhancement of airway hyperresponsiveness as well as controls airway inflammation by their powerful anti-inflammation action.[3] ICS are positioned as the drug of first choice for asthma controllers in the 2002 GINA Guidelines as well.[4]

Devices for inhalants are developed assuming that the circumstances where patients use inhalants by themselves. In actual clinical conditions, however, there are cases where physicians hesitate to introduce ICS therapy because of the diagnosis that the use of inhalants is difficult due to the accompaniment of a functional lesion, and asthma cases where sufficient curative effects of steroids are not produced in self- inhalation mostly among the elderly. Even in such cases, however, we experience cases where asthma control is improved with execution of accurately manipulated ICS therapy assisted by a caregiver for a part of the inhalation procedure (Fig. 1). Such being the case, this paper presents handicapped elderly asthma cases where clinical symptoms and pulmonary functions were improved by providing assistance for inhalation to discuss problems in inhalation therapy against asthma in the elderly. Written informed consent was obtained from

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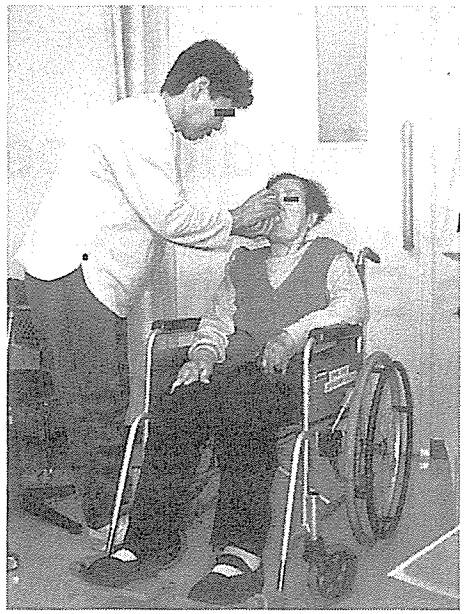

Fig. 1 Actual Assisted Inhalation Therapy. An asthma case where self-inhalation is impossible due to finger deformation associated with rheumatoid arthritis. A caregiver will spray the inhalant in a timely manner for the patient's inspiration and make sure of the inhalation condition.

both patients.

CLINICAL SUMMARY

CASE 1

Case: 81-year-old, woman

Chief complaints: Wheezing, coughing, sputum

Past history: She suffered from infantile asthma until nine years of age and has been suffering from rheumatoid arthritis since the age of 50.

Personal history: She has no smoking history, and occasionally drinks alcohol.

History of the present illness: The patient has been aware of coughing, sputum, and wheezing developing mostly during the night and continuing to the early morning for approximately four years and was treated with cough suppressants after being diagnosed as suffering from chronic bronchitis. After being admitted to our hospital for work-up of low back pain and rehabilitation, the patient was referred to our department for work-up of coughing.

Laboratory findings: FVC (forced vital capacity) 1.49 (L), $FEV_{1.0}$ (forced expiratory volume in one second) 1.05 (L), $FEV_{1.0}$% 70.5 (%), %$FEV_{1.0}$ 62.5 (%), %PEF (Peak expiratory flow) 50.2 (%)

Clinical course: With bronchial asthma suspected from the clinical history, administration of inhalational β_2 stimulants and monitoring of PEF under assistance started. We used the predicted PEF values in the Japanese report by Tsukioka *et al.*[5] After administration of inhalational β_2 stimulants, %PEF value reversed by approximately 20% and symptoms improved with no other cardiopulmonary diseases accompanied, the patient was diagnosed as suffering from bronchial asthma. Thus, self-administrated ICS therapy with 400 μg / day of hydrofluoroalkane-beclometasone (HFA-BDP) was started. With no improvement recognized both in the subjective symptoms and in the %PEF, which remained on the order of 59.1%, after eight weeks passed since the introduction of the ICS therapy, inhalation guidance was provided again, where it was found that inhalation was conducted with an inaccurate technique. Because it was considered difficult for the patient to manipulate inhaled steroids on her own due to a swan-neck deformity resulting from rheumatoid arthritis recognized in the inter-phalangeal joints, we introduced administration of assistance for inhalation with the cooperation of the patient's family. After administration of assistance for eight weeks, the clinical symptoms disappeared almost completely and the %PEF relative to the predictive values improved from approximately 59% to 90% (Fig. 2).

PATHOLOGICAL FINDINGS

CASE 2

Case: 82-year-old, woman

Chief complaints: Wheezing, dyspnea, insomnia

Past history: She suffered from asthma since the age of 70 and has been suffering from vascular dementia since the age of 76.

Personal history: She has no smoking history, and occasionally drinks alcohol.

History of the present illness: The patient became aware of paroxysmal dyspnea since the 70 years of age. Diagnosed as suffering from asthma by a general physician, the patient was receiving treatment with a theophylline drug. From approximately six months prior to admission, the frequency of wheezing and paroxysmal dyspnea increased and the patient became aware of insomnia due to nighttime asthma symptoms on several occasions a week. The patient was additionally administered with a patch-type β_2 stimulant but no sufficient improvement was noted in the asthma symptoms. The patient was referred to our hospital for the purpose of controlling the asthma.

Laboratory findings: FVC 1.48 (L), $FEV_{1.0}$ 1.12 (L), $FEV_{1.0}$% 75.7 (%), %$FEV_{1.0}$ 59.1 (%), %PEF 40.2 (%)

Clinical course: After administration of inhalational β_2 stimulants, $FEV_{1.0}$ improved by approximately 280 ml with no simultaneous onset of other cardiopulmonary diseases recognized in blood tests, thoracic radiographs, and electrocardiography. From the above findings, the asthma was diagnosed as being under control. With no problem in the patient's short term memory notwithstanding the accompaniment of vascular dementia, self-administrated ICS therapy

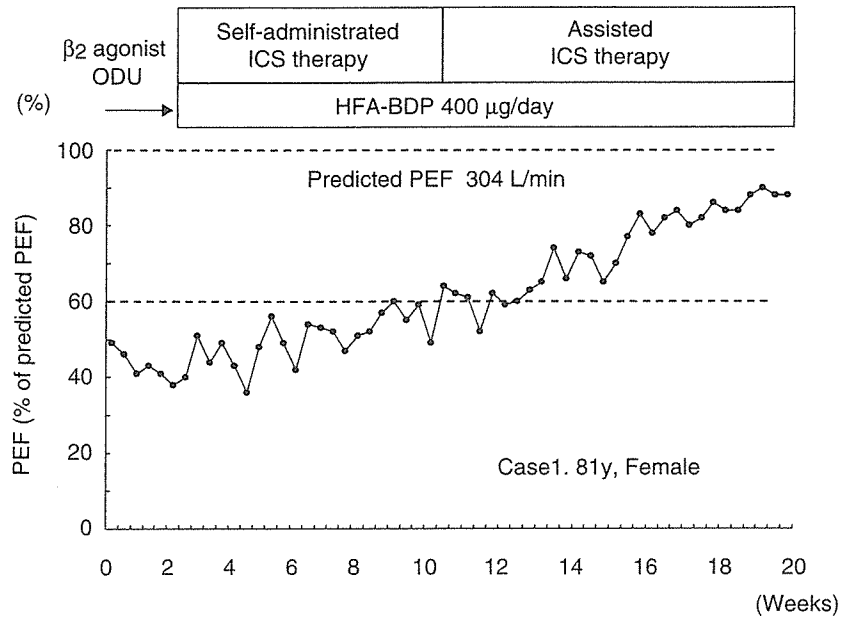

Fig. 2 Clinical Course of Case 1. PEF: peak expiratory flow; ODU: on demand use; ICS: inhaled corticosteroid; HFA-BDP: hydrofluoroalkane-beclometasone

was diagnosed as possible in inhalation guidance by a physician and ICS therapy with 400 μg/day of fluticasone propionate (FP) was introduced. In order to monitor the pulmonary function, the PEF values were measured by the physician three times from 9:00 to 11:00 in the morning and the highest value indicated in the measurement was regarded as the PEF value at that point. No improvements were recognized both in the subjective symptoms and in the %PEF, which remained on the order of 39.1%, after eight weeks passed since the introduction of the self-administrated ICS therapy. Inhalation guidance was provided again, where it was found that inhalation was conducted not only with an inaccurate technique but also in poor compliance with the prescription. To cope with the situation, assisted ICS therapy with a FP introduced under the cooperation of her family was administered with the objective of continuing inhalation with an accurate technique. After eight weeks of assisted administration of FP, insomnia associated with the asthma symptoms developing during the night time as well as the wheezing and dyspnea disappeared. The %PEF relative to the predictive value also improved from approximately 42% to 76% (Fig. 3).

DISCUSSION

In a case where self inhalation therapy is difficult to perform due to motor dysfunction associated with rheumatoid arthritis, and in a case where a problem was recognized in the inhalation technique and in the compliance to the prescription due to intellectual disability associated with vascular dementia, execution of assisted ICS therapy resulted in improvement in the clinical symptoms and the pulmonary function parameters.

It has been proven in many clinical studies that periodical use of ICS for persistent asthma results in improvement in pulmonary function, improvement in airway hyperresponsiveness, improvement in asthma symptoms, improvement in frequency and severity of attacks, and improvement in QOL.[6-9] In order to produce sufficient curative effects of ICS therapy, inhalation by an accurate technique is imperative.[4] It is often experienced, however, that complications accompanying asthma inhibit the inhalation technique in cases of asthma mostly in the elderly. While intellectual disability is a complication causing lack of understanding about the inhalation technique, motor dysfunction is a complication resulting in impairment of the inhalation technique. An inaccurate inhalation technique will not bring about improvement in asthma control because it will not produce sufficient curative effects of inhaled steroids, which may also result in lowering of compliance to an inhalation prescription. Assisted inhalation therapy is "an inhalation therapy performed with the assistance of caregivers for patients with diseases for which the therapy is indicated, in cases that the patients have difficulty in inhalation by themselves due to some functional disorders". Introduction of assisted ICS therapy is expected to be useful for improvement of asthma control in handicapped persons, in cases where self inhalation therapy is difficult to execute due to accompani-

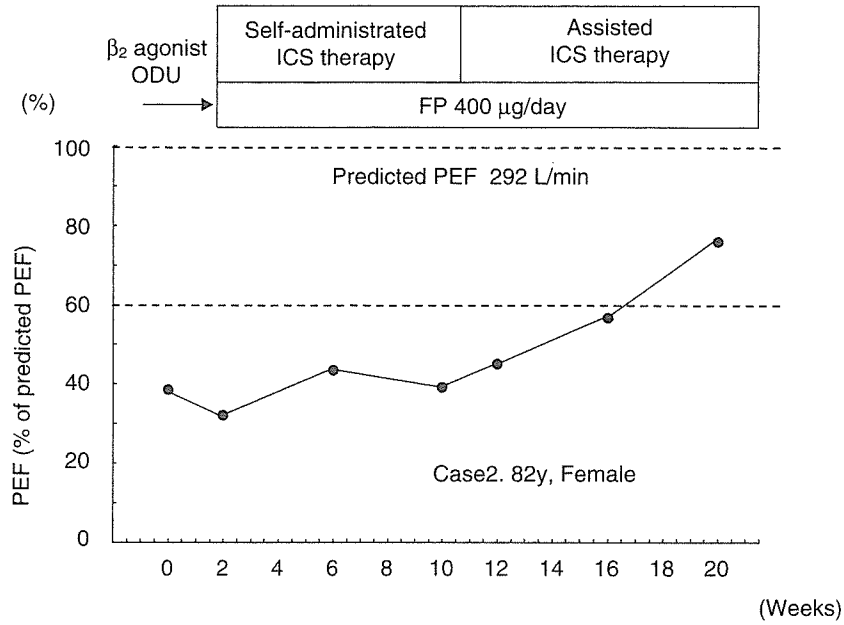

Fig. 3 Clinical Course of Case 2. PEF: peak expiratory flow; ODU: on demand use; ICS: inhaled corticosteroid; FP: fluticasone propionate

ment of a functional lesion or in cases where sufficient curative effects of steroids are not produced in self- inhalation, as presented this time. In the future, more handicapped asthma cases, for which the assisted ICS therapy is applicable, need to be accumulated to prove the clinical utility of assistance for inhalation.

The asthma mortality in the Japanese population, which continued to be on the order of approximately 6,000 people per annum in the early 1990s, has improved to less than 4,000 people per annum in the recent years.[10] The prevalence of anti-inflammatory therapy mostly with ICS is considered to have made a substantial contribution to the decrease in the asthma mortality.[11] On the contrary, according to a study on the change of asthma mortalities by generation over the years, however, the asthma mortality in the elderly aged 80 or higher has been increasing.[12] Chronic airway inflammation is a basic pathology of asthma in the elderly too and therefore it is important to control the inflammation by a therapy centering on ICS. However, it has been pointed out that the usage of ICS is extremely low in asthma in the elderly.[13,14] Assisted inhalation therapy is expected to be useful also in terms of expanding the application of ICS in the asthma in the elderly.

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