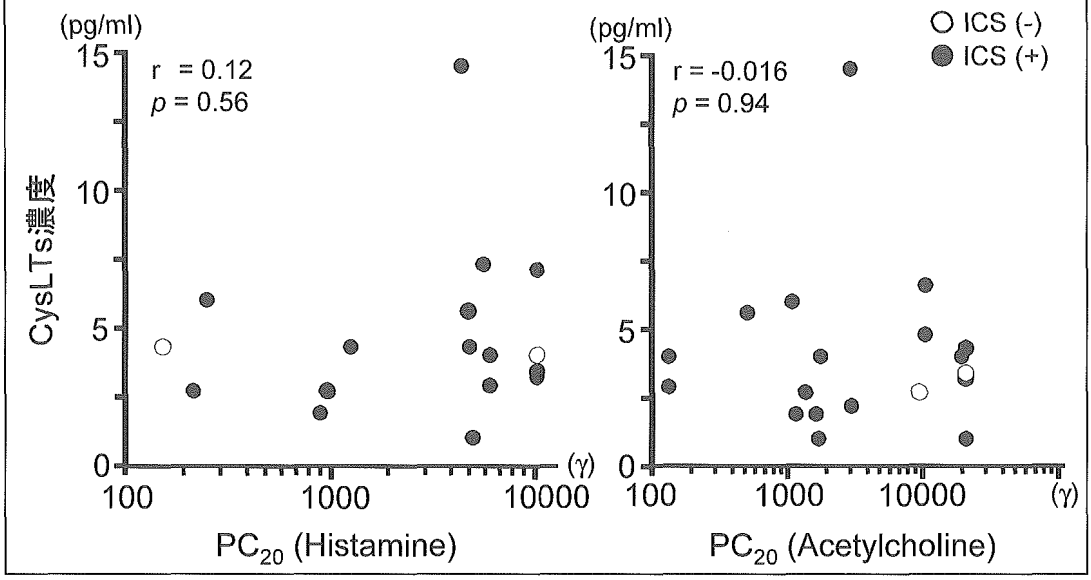
呼気凝縮液中CysLTs濃度と気道過敏性との相関

（秋山）



当該年度の研究成果

1. 呼気凝縮液中の炎症関連物質の検出と気管支喘息病態との関連についての検討　（一ノ瀬班、秋山班、大田班）
   - Cytokine array kitにより呼気凝縮液中の種々の炎症関連物質が再現性をもって測定可能で、気管支喘息患者では健常人に比べ、IL-4、IL-8、IL-17、TNF-α、IP-10、MIP-1α、MIP-1β、RANTES、TGF-β1の9つで有意な増加が認められた。
   - RANTES発現量は喘息患者の気道の閉塞性障害の程度と、有意な相関を認めた。
   - TNF-α、TGF-β1発現量は気道過敏性やPEFの変動性と有意な相関を認めた。
   - IGF-1やCysLTsも喘息で軽度の増加がみられたが、呼吸の生理学的パラメータとは関連性が無かった。

当該年度の各班の検討課題

1. 呼気凝縮液中の炎症関連物質の検出と気管支喘息病態との関連についての検討　（一ノ瀬班、秋山班、大田班）

2. 遺伝子操作動物モデルを用い、呼気凝縮液に応用可能な生化学的バイオマーカーの検索　（長瀬班）

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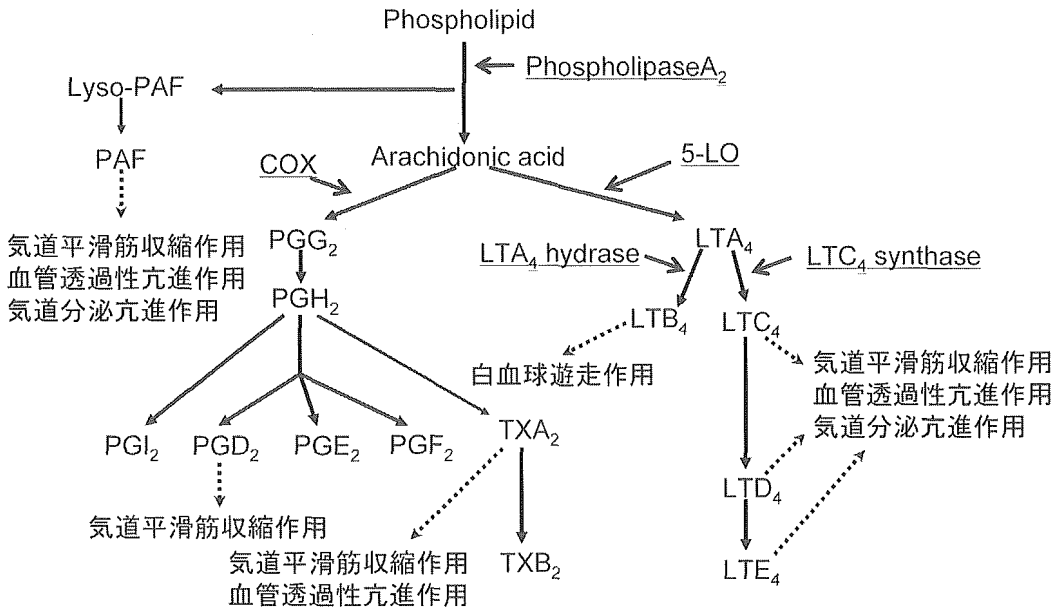
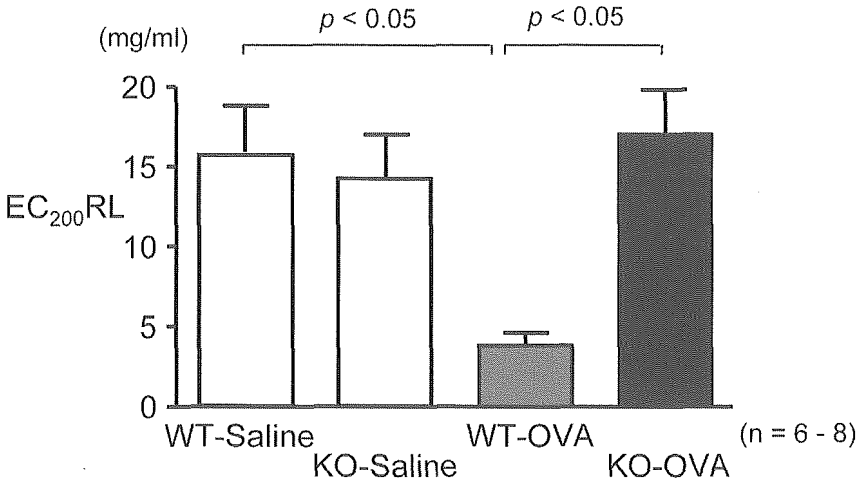


LTB_4受容体欠損によるアレルギー反応後の気道過敏性亢進の消失　（長瀬）



(Terawaki K, Nagase T,et al, *J Immunol* 175: 4217-25, 2005)

当該年度の研究成果

2. 遺伝子操作動物モデルを用い、呼気凝縮液に応用可能な生化学的バイオマーカーの検討　（長瀬班）

- LTB_4受容体のBLT-1欠損マウスでは、感作されたノックアウトマウス群は、野生型群と比べてメサコリンに対する気道反応性が低下していた。

今年度の成果に対する評価

1. 呼気凝縮液を用いて、喘息の気道炎症を簡便に評価しうるバイオマーカーを見出す。
   → 気管支喘息患者の呼気凝縮液中で9つの炎症物質の発現上昇を認めた。

2. バイオマーカーと生理学的パラメーターの関連性を検討し、臨床における有用性を検討する。
   → RANTESは閉塞性障害の程度と、TNF-αやTGF-β1は気道過敏性や気道内径の変動性の程度と有意な相関を認めた。

今後の課題

- 当該年度の検討で呼気凝縮液中に見出された種々の炎症関連物質の定量化を検討する。
  → 一般臨床応用へ向けた簡便性の追求。

- 呼気凝縮液中の各炎症関連物質と呼吸機能パラメータとの関連性を治療（ステロイドなど）前後でより詳細に検討する。
  → 炎症関連物質の喘息病態における特異度の追求。

V. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
T Yamagata, M Ichinose	Agents against cytokine synthesis or receptors	European Journal of Pharmacology	533	289-301	2006
Y Komaki, H Sugiura, A Koarai, M Tomaki, H Ogawa, T Akita, T Hattori, M Ichinose	Cytokine-mediated xanthine oxidase upregulation in chronic obstructive pulmonary disease's airways	Pulm Pharm Ther	18	297-302	2005
Y Minakata, M Nakanishi, T Hirano, K Matsunaga, T Yamagata, M Ichinose	Microvascular hyperpermeability in chronic obstructive pulmonary disease airways	Thorax	60	882-883	2005
Y Fukuchi, A Nagai, K Seyama, M Nishimura, K Hirata, K Kubo, M Ichinose, H Aizawa and the BAREC Research Group	Clinical Efficacy and Safety of Transdermal Tulobuterol in the Treatment of Stable COPD: An Open-Label Comparison with Inhaled Salmeterol	Treat Respir Med	4	447-455	2005
T Yamagata, Y Okamoto, Y Yamagata, M Nakanishi, K Matsunaga, Y Minakata, M Ichinose	Angioimmunoblastic lymphadenopathy with dysproteinaemia accompanied by pleural effusion	Respirology	10	124-127	2005
一ノ瀬正和	難治性喘息	喘息	18	76-80	2005
一ノ瀬正和	気管支喘息の病態理解と治療の実際	今月の治療	13	29-35	2005
山縣俊之，一ノ瀬正和	炎症性気道・肺疾患における呼気ガス分析	呼吸	24	694-699	2005
山縣俊之，一ノ瀬正和	誘発喀痰を用いた気道・肺の炎症の評価	呼吸	24	774-779	2005
山縣俊之，一ノ瀬正和	呼気温度測定の原理と有用性	呼吸	24	938-942	2005
南方良章，一ノ瀬正和	呼気ガス・呼気凝縮液による閉塞性肺疾患（COPD・喘息）の評価	Pharma Medica	23	23-27	2005
一ノ瀬正和	新規薬剤による治療の趨勢	臨床医	31	10-14	2005
松永和人，一ノ瀬正和	気管支喘息	呼吸と循環	53	907-914	2005
山縣俊之，一ノ瀬正和	咳喘息とその周辺疾患の治療	呼吸と循環	53	595-602	2005
山縣俊之，一ノ瀬正和	呼気と気道炎症のパラメーター	アレルギーの臨床	25	79-82	2005
松永和人，一ノ瀬正和	末梢気道炎症の治療吸入ステロイドデヴァイス	アレルギーの臨床	25	38-43	2005

VI.　研究成果の刊行物

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Review

Agents against cytokine synthesis or receptors

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Abstract

Various cytokines play a critical role in pathophysiology of chronic inflammatory lung diseases including asthma and chronic obstructive pulmonary disease (COPD). The increasing evidence of the involvement of these cytokines in the development of airway inflammation raises the possibility that these cytokines may become the novel promising therapeutic targets. Studies concerning the inhibition of interleukin (IL)-4 have been discontinued despite promising early results in asthma. Although blocking antibody against IL-5 markedly reduces the infiltration of eosinophils in peripheral blood and airway, it does not seem to be effective in symptomatic asthma, while blocking IL-13 might be more effective. On the contrary, anti-inflammatory cytokines themselves such as IL-10, IL-12, IL-18, IL-23 and interferon-γ may have a therapeutic potential. Inhibition of TNF-α may also be useful in severe asthma or COPD. Many chemokines are also involved in the inflammatory response of asthma and COPD through the recruitment of inflammatory cells. Several small molecule inhibitors of chemokine receptors are now in development for the treatment of asthma and COPD. Antibodies that block IL-8 reduce neutrophilic inflammation. Chemokine CC3 receptor antagonists, which block eosinophil chemotaxis, are now in clinical development for asthma therapy. As many cytokines are involved in the pathophysiology of inflammatory lung diseases, inhibitory agents of the synthesis of multiple cytokines may be more useful tools. Several such agents are now in clinical development.

Keywords: Asthma; Chronic obstructive pulmonary disease; Cytokine; Chemokine; Chemokine receptor

Contents

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1. Introduction

Although cytokines are important for maintaining homeostasis, these proteins also play critical roles in the development of chronic airway inflammation in all diseases, including asthma and chronic obstructive pulmonary disease (COPD). Indeed, it has been demonstrated that various cytokines and chemokines are involved in the pathophysiology of asthma (Barnes et al., 1998; Chung and Barnes, 1999; Miller and Lukacs, 2004) and COPD (Barnes, 2004a). These cytokines and chemokines exert their effect through G-protein coupling receptor expressed on inflammatory cell surface. Therefore, these cytokines and their receptors may be a useful therapeutic target for asthma and COPD. Although the precise involvement and interaction of these cytokines in the pathogenesis of asthma and COPD are still unclear, intensive investigation and several clinical trials for new therapies as specific targets against these cytokines and their receptors are now ongoing. These include blocking antibody of proper cytokines and chemokines, specific receptor antagonists and small molecular receptor inhibitors (Adcock and Caramori, 2004a; Barnes, 2001a, 2002a,b, 2004b; Barnes and Stockley, 2005; Belvisi et al., 2004; Garcia et al., 2005; Ichinose and Barnes, 2004). In addition, there are another therapeutic options including anti-inflammatory cytokines, inhibition or modifier of inflammatory cytokine synthesis, and blocking various intracellular signaling pathways (Barnes and Lim, 1998; Barnes, 2000, 2004b; Ichinose and Barnes, 2004). This review focuses on the recent development of cytokine-inhibiting therapy for asthma and COPD.

2. Cytokine directed therapies for asthma

2.1. Inhibition of cytokines

Cytokines derived from T helper 2 (Th2) lymphocytes play a key role in pathophysiology of asthma through the induction of eosinophilic airway inflammation. These cytokines include interleukin (IL) -4, IL-5, IL-9, IL-13 and IL-25. In addition, pro-inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α (TNF-α) may enhance the inflammatory response in asthma and may be linked to the disease severity. Therefore, blocking the release or effects of these cytokines may have therapeutic potential. This has been shown by several previous studies using animal models, including mice whose specific Th2 cytokine genes have been deleted.

On the other hand, there are several cytokines that suppress these inflammatory responses, which include IL-10, IL-12, IL-18, IL-23 and interferon-γ (IFN-γ). These cytokines per se may be useful therapeutic tools for asthma and COPD treatment (Barnes and Lim, 1998; Barnes, 2000, 2004b). Although its clinical benefits are still under investigation, it may be possible to develop drugs in the future that increase the release of these endogenous anti-inflammatory cytokines or activate their receptors and specific signal transduction pathways.

2.1.1. IL-1

IL-1 expression is increased in asthmatic airways (Sousa et al., 1996) and activates many inflammatory genes that are expressed in asthma. There are no small molecule inhibitors of IL-1, but a naturally occurring cytokine, IL-1 receptor antagonist, binds to IL-1 receptors to block the effects of IL-1 (Arend et al., 1998). In experimental animals IL-1 receptor antagonist reduced airway hyperresponsiveness induced by allergen. However, human recombinant IL-1 receptor antagonist does not appear to be effective in the treatment of asthma (Rosenwasser, 1998).

2.1.2. IL-4

IL-4 is critical for the synthesis of Immunoglobulin E (IgE) by B-lymphocytes and is also involved in eosinophil recruitment to the airways (Steinke and Borish, 2001). A unique function of IL-4 is to promote the differentiation of Th2 cells and therefore it acts at a proximal and critical site in the allergic response, making IL-4 an attractive target for inhibition.

IL-4 blocking antibodies inhibited allergen-induced airway hyperresponsiveness, goblet cell metaplasia and pulmonary eosinophilia in a murine model (Gavett et al., 1997). Inhibition of IL-4 may therefore be effective in treating allergic diseases, and soluble humanized IL-4 receptors have been tested in clinical trials. A single nebulized dose of soluble IL-4 receptor prevents the decrease in lung function induced by withdrawal of inhaled corticosteroids in patients with moderately severe asthma (Borish et al., 1999). In addition, weekly nebulization of soluble IL-4 receptor improved asthma control over a 12 week period (Borish et al., 2001). Subsequent studies in patients with milder asthma proved disappointing, however, and this

treatment has now been withdrawn. Another approach is blockade of IL-4 receptors with a mutated form of IL-4 (BAY 36-1677), which binds to and blocks IL-4 receptor α and IL-13 receptor α1, thus blocking both IL-4 and IL-13 (Shanafelt et al., 1998). However, because of its short duration of action, this treatment has also been withdrawn.

IL-4 and the closely related cytokine IL-13 signal through a shared surface receptor, IL-4 receptor α, which activates a specific transcription factor signal transducer and activator of transcription (STAT)-6 (Jiang et al., 2000). Deletion of the STAT-6 gene has an effect similar to that of IL-4 gene knockout (Foster, 1999). This has led to a search for inhibitors of STAT-6, and although peptide inhibitors that interfere with the interaction between STAT-6 and Janus kinases (JAK) linked to IL-4 receptor α have been discovered, it will be difficult to deliver these intracellularly. Thus, an endogenous inhibitor of STATs and suppressor of cytokine signaling (SOCS-1) that is a potent inhibitor of IL-4 signaling pathways may be a useful new therapeutic target (Jiang et al., 2000).

2.1.3. IL-5

IL-5 plays an essential role in orchestrating the eosinophilic inflammation of asthma (Greenfeder et al., 2001). In IL-5 gene knockout mice the eosinophilic response to allergen and the subsequent airway hyperresponsiveness are markedly suppressed, and yet the animals exhibit normal survival, validating the strategy to inhibit IL-5. This has also been achieved using blocking antibodies that block IL-5. Blocking antibodies to IL-5 inhibit eosinophilic inflammation and airway hyperresponsiveness in animal models of asthma, including primates (Egan et al., 1996). This blocking effect may last for up to 3 months after a single intravenous injection of antibody in primates, making the treatment of chronic asthma with such a therapy a feasible proposition. Humanized monoclonal antibodies to IL-5 have been developed and a single intravenous infusion of one of these antibodies (mepolizumab) markedly reduces blood eosinophils for several weeks and prevents eosinophil recruitment into the airways after allergen challenge in patients with mild asthma (Leckie et al., 2000) (Fig. 1). However, this treatment has no significant effect on the early or late response to allergen challenge or on the baseline airway hyperresponsiveness, suggesting that eosinophils may not be of critical importance for these responses in humans (Fig. 2). A clinical study in patients with moderate to severe asthma who had not been controlled using inhaled corticosteroids therapy confirmed a profound reduction in circulating eosinophils, but no significant improvement in either asthma symptoms or lung function (Kips et al., 2000). In both of these studies it would be expected that high doses of corticosteroids would improve these functional parameters. These surprising results cast doubt on the critical role of eosinophils in asthma and indicate that other strategies aimed at inhibiting eosinophilic inflammation might not be effective. More recently, a biopsy study has demonstrated that anti-IL-5 antibody, while profoundly reducing eosinophils in the circulation (by over 95%), is less effective at reducing eosinophils in bronchial biopsies (by ~50%), which may explain why this treatment is not clinically effective (Flood-Page et al., 2003b). However, further study shows that anti-IL-5 therapy reduces the deposition of extracellular matrix protein that contributes to airway remodeling in the bronchial subepithelial basement membrane (Flood-Page et al., 2003a). This anti-IL-5 effect may be due to the capacity of IL-5 to drive epithelial and fibroblast responses. Nevertheless, these results suggest that blocking IL-5 is not likely to be a useful approach in asthma therapy.

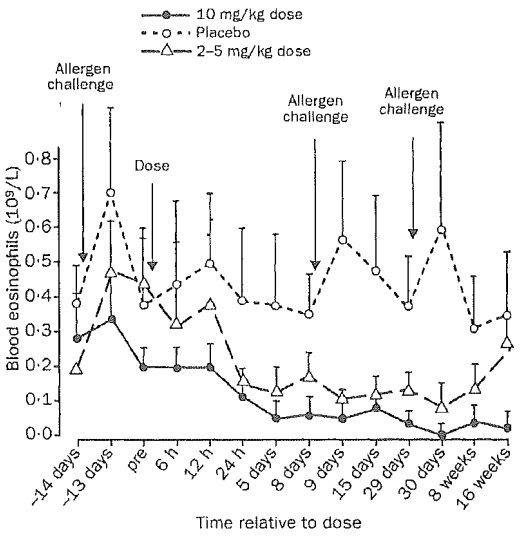

Fig. 1. The effect of a humanized monoclonal antibody against interleukin-5 (mepolizumab) on circulating eosinophils in patients with mild asthma, demonstrating a profound and very prolonged inhibitory effect. Reproduced from Leckie et al. (2000).

Somewhat similar findings have previously been reported in some studies in mice where anti-IL-5 antibodies reduced eosinophilic responses to allergen, but not airway hyperresponsiveness, whereas airway hyperresponsiveness was reduced by anti-CD4 antibody which depletes helper T cells (Hogan et al., 1998) suggesting that T cell derived cytokines other than IL-5 must be playing a more important role in airway hyperresponsiveness.

Non-peptidic IL-5 receptor antagonists would be an alternative strategy and there is a search for such compounds using molecular modeling of the IL-5 receptor α-chain and through large scale throughput screening. One such molecule, YM-90709, appears to be a relatively selective inhibitor of IL-5-receptors (Morokata et al., 2002). However, the lack of clinical benefit of anti-IL-5 antibodies has made this a less attractive approach. It is possible that eosinophils are associated with the more chronic aspects of asthma, such as airway remodeling, and in mice a blocking anti-IL-5 antibody prevented the increased collagen deposition in airways associated with repeated allergen exposure (Blyth et al., 2000). Eosinophils may be an important source of transforming growth factor-β in asthmatic airways, resulting in structural changes (Minshall et al., 1997). Indeed, more recently, it has been demonstrated that fibrotic lesions induced by antigen challenge are abolished in IL-5 receptor null mice, and that neutralizing anti-IL-5 antibody can almost completely prevent subepithelial and peribronchial fibrosis (Tanaka et al., 2004). Therefore, there is a possibility that IL-5

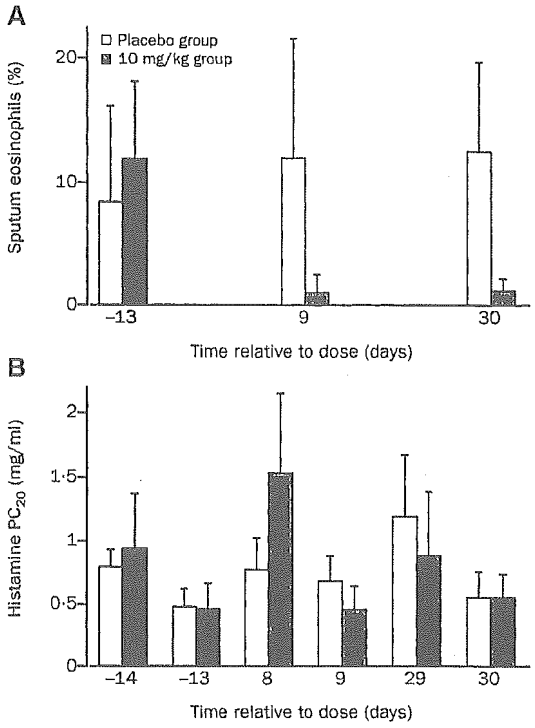

Fig. 2. Effect of a humanized monoclonal antibody against interleukin-5 (mepolizumab) on sputum eosinophils (A) and airway hyperresponsiveness (histamine PC_{20}) (B). Reproduced from Leckie et al. (2000).

may be a target for the more chronic asthmatic airway changes such as remodeling.

2.1.4. IL-9

IL-9 is a Th2 cytokine that may enhance Th2-driven inflammation and enhance mast cell mediator release and IgE production (Levitt et al., 1999). IL-9 may also enhance mucus hypersecretion (Longphre et al., 1999). IL-9 and its receptors show increased expression in asthmatic airways (Bhathena et al., 2000; Shimbara et al., 2000). A blocking antibody to IL-9 inhibited airway inflammation and airway hyperresponsiveness in a murine model of asthma (Cheng et al., 2002). Another study showed that anti-IL-9 antibody significantly reduced bone marrow eosinophilia, primarily by decreasing newly produced and mature eosinophils. In addition, in response to allergen, bone marrow cells over-express IL-9 (Sitkauskiene et al., 2005). These data suggest that IL-9 may participate in the regulation of eosinophils in allergic inflammation. Thus, IL-9 may be another therapeutic target for asthma. Strategies to block IL-9, including humanized blocking antibodies, are now in development (Zhou et al., 2001).

2.1.5. IL-10

IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of many inflammatory proteins, including cytokines (TNF-α, GM-CSF, IL-5, chemokines) and inflammatory enzymes (inducible nitric oxide synthase) that are over-expressed in asthma (Ichinose et al., 2000a; Pretolani and Goldman, 1997). Indeed, there may be a defect in IL-10 transcription and secretion from macrophages in asthma, suggesting that IL-10 might be defective in atopic diseases (Barnes, 2001b; Borish et al., 1996; John et al., 1998). In sensitized animals, IL-10 is effective in suppressing the inflammatory response to allergen (Zuany-Amorim et al., 1995) and CD4+ cells engineered to secrete IL-10 suppressed airway inflammation in a murine model of asthma (Oh et al., 2002). Specific allergen immunotherapy results in the increased production of IL-10 by T helper cells and this may contribute to the beneficial effects of immunotherapy (Akdis et al., 1998).

Recombinant human IL-10 has proven to be effective in controlling inflammatory bowel disease and psoriasis, where similar cytokines are expressed, and may be given as a weekly injection (Fedorak et al., 2000). Although IL-10 is reasonably well tolerated, there are hematological side effects. In the future, drugs that activate the unique signal transduction pathways activated by the IL-10 receptor or drugs that increase the endogenous production of IL-10 may be developed. In mice, drugs that elevate cyclic AMP increase the IL-10 production, but this does not appear to be the case in human cells (Seldon et al., 1998).

2.1.6. IL-12

IL-12 is the endogenous regulator of Th1 cell development and determines the balance between Th1 and Th2 cells (Gately et al., 1998). IL-12 administration to rats inhibits allergen-induced inflammation (Gavett et al., 1995) and inhibits sensitization to allergens. IL-12 induces IFN-γ release, but has additional effects on T cell differentiation. The IL-12 levels released from whole blood cells are lower in asthmatic patients, indicating a possible reduction in IL-12 secretion (van der Pouw Kraan et al., 1997).

Recombinant human IL-12 has been administered to humans and has several toxic effects that are diminished by slow escalation of the dose (Leonard et al., 1997). In patients with mild asthma, weekly infusions of human recombinant IL-12 in escalating doses over 4 weeks caused a progressive fall in circulating eosinophils, and a reduction in the normal rise in circulating eosinophils after allergen challenge (Bryan et al., 2000) (Fig. 3). There was a concomitant reduction in eosinophils in induced sputum. However, there was no reduction in either the early or late response to inhaled allergen challenge or any reduction in airway hyperresponsiveness (as with anti-IL-5 therapy). Furthermore, most of the patients suffered from malaise and one out of the 12 subjects had an episode of cardiac arrhythmia, suggesting that IL-12 may not be a suitable treatment for asthma. In mice, administration of an IL-12-allergen fusion protein resulted in the development of a specific Th1 response to the allergen, with increased production of the allergen-specific IgG2, rather than the normal Th2 response with IgE formation (Kim et al., 1997). This indicates the possibility of using local IL-12 together with specific allergens to provide a more specific immunotherapy, which might even be curative if applied early in the course of the atopic disease.

2.1.7. IL-13

There is increasing evidence that IL-13 in mice mimics many of the features of asthma, including airway hyperresponsiveness,

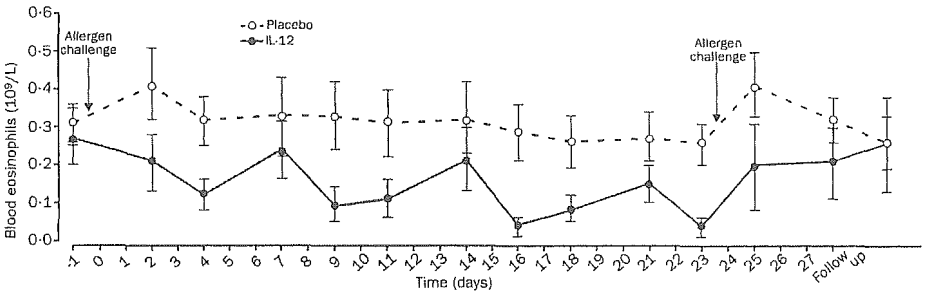

Fig. 3. Effect of interleukin-12 on peripheral blood eosinophils in patients with mild asthma. IL-12 was given in progressively increasing doses as an intravenous injection. Reproduced from Bryan et al., 2000.

mucus hypersecretion and airway fibrosis, independently of eosinophilic inflammation (Wills-Karp and Chiaramonte, 2003). It potently induces the secretion of eotaxin from airway epithelial cells (Li et al., 1999) and transforms airway epithelium into a secretory phenotype (Danahay et al., 2002). Knocking out the IL-13, but not the IL-4, gene in mice prevents the development of airway hyperresponsiveness after allergen challenge, despite a vigorous eosinophilic response (Walter et al., 2001), and the increase in airway hyperresponsiveness induced by IL-13 is only seen when the expression of STAT6 is lost in airway epithelial cells (Kuperman et al., 2002). IL-13 signals through the IL-4 receptor α, but may also activate different intracellular pathways via the activation of IL-13 receptor α1 (Jiang et al., 2000), so that it may be an important target for the development of new therapies. A second specific IL-13 receptor α2 exists in soluble form and has a high affinity for IL-13, thus acting as a decoy receptor for secreted IL-13. Soluble IL-13 receptor α2 is effective in blocking the actions of IL-13, including IgE generation, pulmonary eosinophilia and airway hyperresponsiveness in mice (Wills-Karp et al., 1998). In a murine model IL-13 receptor α2 is more effective than IL-4-blocking antibodies, highlighting the potential importance of IL-13 as a mediator of allergic inflammation. Blocking IL-13 may be more important in established asthma where the concentrations of IL-13 are much higher than those of IL-4.

Recently, it has been shown that neutralizing anti-IL-13 monoclonal antibody significantly suppresses airway hyperresponsiveness, eosinophil infiltration, the production of pro-inflammatory cytokines, serum IgE and airway remodeling induced by ovalbumin challenge in mice (Yang et al., 2004, 2005). In addition, a soluble murine anti-IL-13 receptor fusion protein that specifically binds to and neutralizes IL-13 has been demonstrated to prevent airway hyperresponsiveness induced by allergen challenge in mice (Leigh et al., 2004). These results confirm that IL-13 is critical for the development of airway hyperresponsiveness induced by allergen exposure, and that anti-human IL-13 treatment such as anti-IL-13 antibody or humanized IL-13 receptor α2 might be an effective therapeutic approach for asthma.

2.1.8. IL-18

IL-18 was originally described as an IFN-γ releasing factor, but has a different mechanism of action than IL-12 (Dinarello, 2000). IL-12 and IL-18 appear to have a synergistic effect on inducing IFN-γ release and for inhibiting IL-4-dependent IgE production and airway hyperresponsiveness (Hofstra et al., 1998), but no clinical studies have so far been reported. On the other hand, recent studies have shown that IL-18 can also promote Th2 cytokine production from T cells, NK cells, basophils, and mast cells (Nakanishi et al., 2001; Sugimoto et al., 2004). Thus, it is important to determine the precise role of IL-18 in bronchial asthma before considering its use as a relevant therapeutic target.

2.1.9. IL-23

IL-23, which is mainly expressed in dendritic cells, is structurally related to IL-12 and shares some of its biological effects, so should have a protective function in asthma (Oppmann et al., 2000). Although IL-23 induces the proliferation of memory T-cells and the secretion of IFN-γ, its precise clinical potential and role have not yet been examined.

2.1.10. IL-25

IL-25 is a recently described Th2 cell-derived cytokine that belongs to the IL-17 family and induces the production of IL-4, IL-5, IL-13 and eotaxin in the lung (Hurst et al., 2002). Transgenic over-expression of IL-25 results in the induction of airway hyperresponsiveness, airway eosinophilia and an increase in the serum levels of IL-5, IL-13, and IgE (Kim et al., 2002; Pan et al., 2001). These results suggest that IL-25 may play a role in allergic inflammation. It is released from mast cells via an IgE-dependent mechanism and is therefore a possible target for the treatment of asthma (Ikeda et al., 2003).

2.1.11. TNF-α

TNF-α is expressed in asthmatic airways and may play a key role in amplifying asthmatic inflammation through the activation of nuclear factor-κB (NF-κB), activator protein-1 (AP-1) and other transcription factors (Kips et al., 1993).

In rheumatoid arthritis and inflammatory bowel disease a humanized blocking monoclonal antibody to TNF-α (infliximab) and soluble TNF receptors (etanercept) have produced remarkable clinical responses, even in patients who are relatively unresponsive to steroids (Markham and Lamb, 2000; Jarvis and Faulds, 1999). Such TNF inhibitors are a logical approach to asthma therapy, particularly in patients with severe disease, and clinical trials are now underway.

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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Cytokine-mediated xanthine oxidase upregulation in chronic obstructive pulmonary disease's airways

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Abstract

Reactive oxygen species have been reported to be involved in the airway inflammatory process of chronic obstructive pulmonary disease (COPD). The aim of this study was to quantify the activity of xanthine oxidase (XO), which generates a potent radical superoxide anion in COPD airways.

Thirteen stable COPD patients and 10 healthy subjects participated in this study. We collected the epithelial lining fluid using a newly developed microsampling technique, and quantified of cytokines responsible for the XO gene upregulation.

The XO activity was significantly increased in COPD patients compared with that in healthy subjects. A significant negative correlation was found between the XO activity and the $\%FEV_1$ values. The level of tumor necrosis factor-α, interleukin-1β, and interferon-γ in COPD patients was significantly higher than that in healthy subjects. Both the amount of tumor necrosis factor-α and interleukin-1β were significantly correlated with the degree of XO activity.

These results suggest that the XO activity is increased in COPD airways, possibly due to its gene upregulation by proinflammatory cytokines. Because the XO activity was significantly correlated with the degree of airway obstruction, these cytokine–XO production pathways may play a key role in the inflammation of COPD.

Keywords: Oxidative stress; Superoxide; TNF-α; IL-1β; IFN-γ

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a condition characterized by airway inflammation and airflow limitation that is progressive and largely irreversible [1–3]. Although COPD is a major cause of morbidity and mortality in the world [4], the pathogenesis of this disease has not yet been fully elucidated.

There is increasing evidence that an oxidant/antioxidant imbalance occurs in COPD [5–7]. Oxidants including superoxide anion (O_2^-), hydrogen peroxide and peroxynitrite cause airway inflammation by means of tissue injury [5], the activation of matrix metalloproteinases [8], the inactivation of α_1-antitrypsin [9], and enhanced production of the potent neutrophil chemoattractant interleukin (IL)-8 [10]. Therefore, these oxidants appear to be involved in the pathophysiology of the airway inflammation in COPD patients. Recently, endogenously generated oxidants have been thought to be important in COPD [5,6]. Among these, O_2^- is the most important molecule since other oxidants are derived from this molecule.

Xanthine oxidoreductase (XOR) is a rate-limiting enzyme of purine catabolism that exists in two forms, as xanthine dehydrogenase (XD) and as xanthine oxidase (XO) [11,12]. XOR, particularly in the XO form, generates reactive oxygen species such as O_2^-, hydroxyl radicals and hydrogen peroxide. It has been reported that XO was enhanced in an animal model of asthma [13] and in virus-induced pneumonia in mice [14]. These studies showed that O_2^- mediated by XO caused both airway and lung parenchymal inflammation. Previously, we have reported that the XO activity in sputum from patients with COPD

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