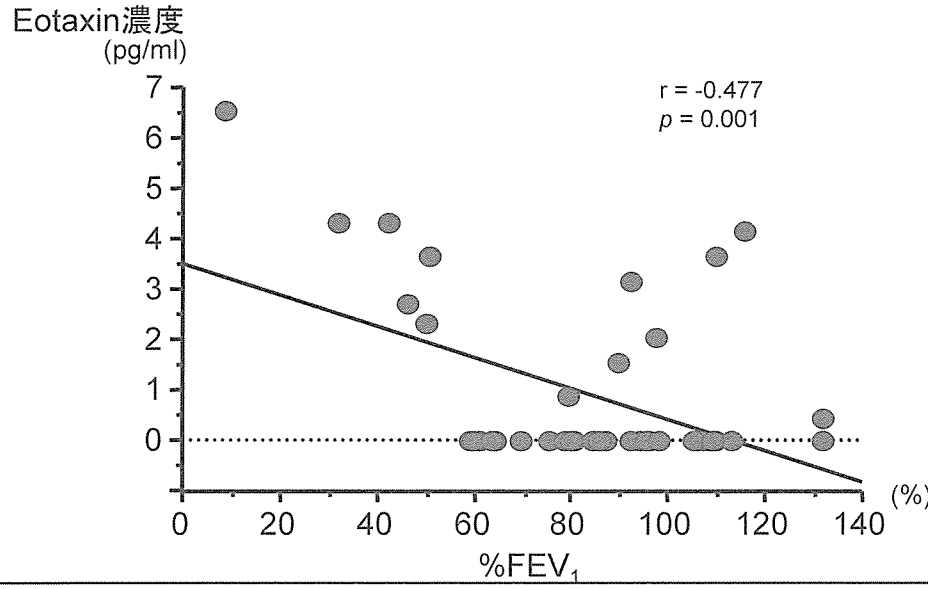
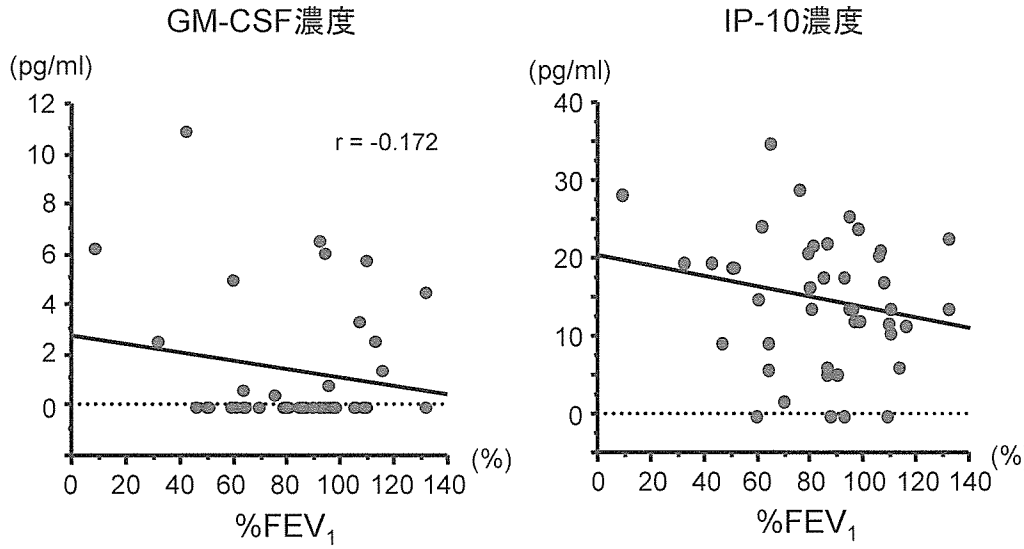
呼気凝縮液中のEotaxin濃度と閉塞性障害との相関（大田）



呼気凝縮液中のGM-CSF、IP-10濃度と閉塞性障害との相関（大田）



当該年度の各班の検討課題

1. 妥当性
   検体への唾液混入の可能性の検討　　（一ノ瀬班）

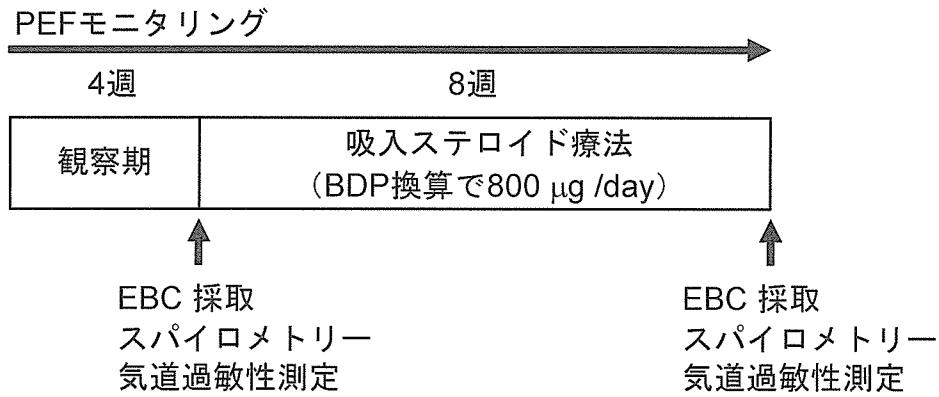
2. 有用性
   評価分子の検索　　（長瀬班、秋山班、大田班）
   ステロイド治療との関連性　　（一ノ瀬班）

3. 臨床応用
   評価分子の定量測定　　（大田班、一ノ瀬班）

吸入ステロイドによる呼吸機能の変化

プロトコール

対象：ステロイド未治療の喘息患者18例
(軽症または中等症持続型)

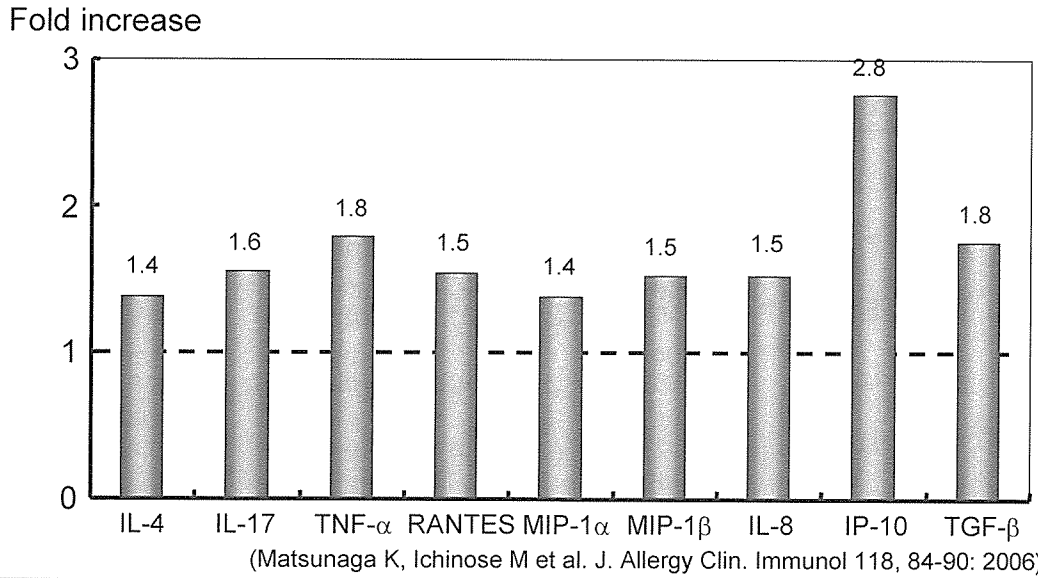


吸入ステロイドによる呼吸機能の変化

Number	18 (F/M = 12/6)	
Age (Years)	38.9±3.0	
	Pre-steroid	Post-steroid
FVC (L)	3.25±0.15	3.38±0.14
FEV_1 (L)	2.52±0.12	2.79±0.13
FEV_1/FVC (%)	78.1±2.4	83.0±2.1
FEV_1 %predicted (%)	92.6±2.9	103.4±3.2
Rrs (cmH_2O/L/s)	4.3±0.3	3.9±0.2
%PEF (%)	82.6±2.8	96.0±2.7
⊿PEF (%)	20.3±2.3	8.5±0.9
PD_{200} (mg/ml)	5.4±1.4	11.8±2.3

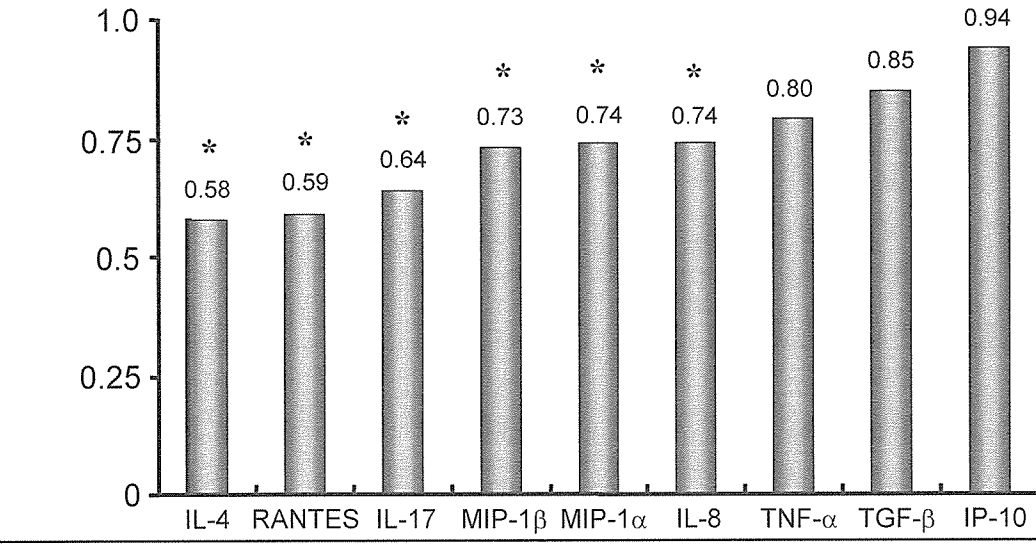
Mean±SE

喘息気道で発現が増加している物質



(Matsunaga K, Ichinose M et al. J. Allergy Clin. Immunol 118, 84-90: 2006)

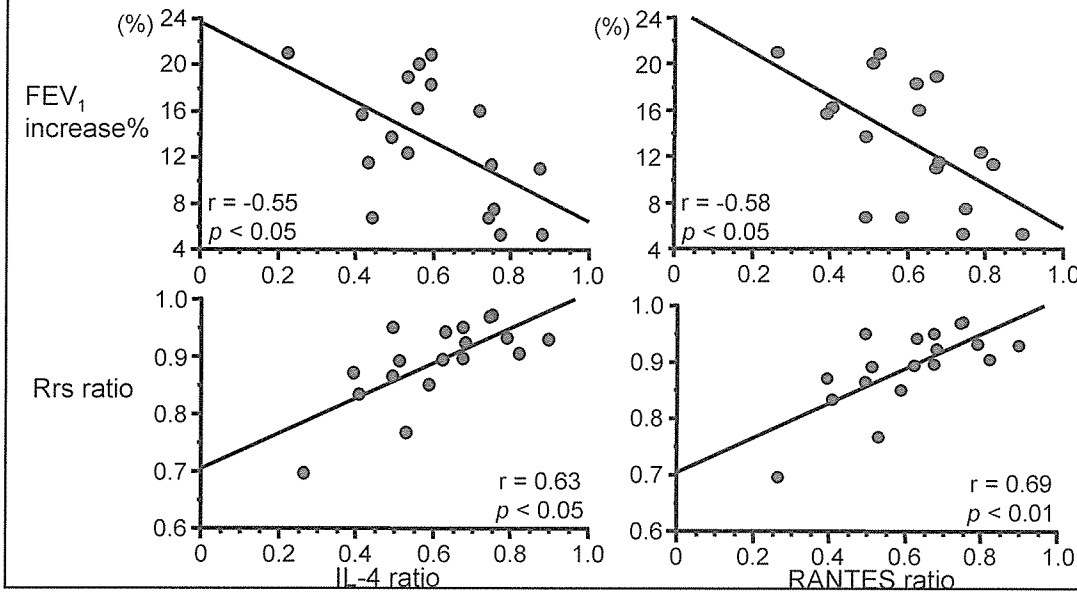
吸入ステロイドによる呼気凝縮液中サイトカイン発現の変化



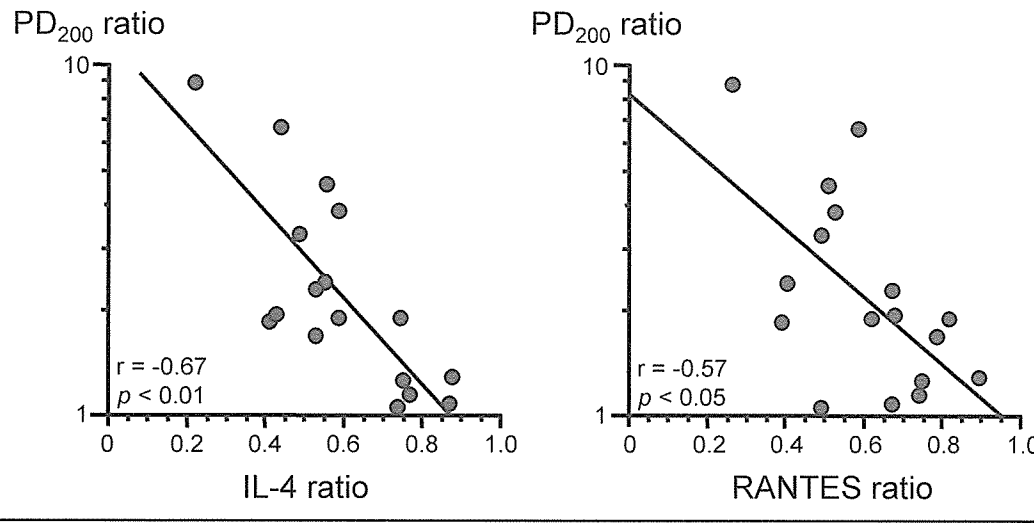
吸入ステロイドによるサイトカイン発現と呼吸機能の変化

Cytokine ratio	FEV_1 increase %	Rrs ratio	PD_{200} ratio	⊿PEF ratio
IL-4	r = -0.55 ($p < 0.05$)	r = 0.63 ($p < 0.05$)	r = -0.67 ($p < 0.01$)	r = 0.42 ($p = 0.08$)
IL-17	r = -0.34 ($p = 0.12$)	r = 0.45 ($p = 0.06$)	r = -0.47 ($p = 0.06$)	r = 0.12 ($p = 0.63$)
RANTES	r = -0.58 ($p < 0.05$)	r = 0.69 ($p < 0.01$)	r = -0.57 ($p < 0.05$)	r = 0.45 ($p = 0.06$)
MIP-1α	r = -0.04 ($p = 0.88$)	r = -0.27 ($p = 0.29$)	r = 0.46 ($p = 0.06$)	r = 0.04 ($p = 0.87$)
MIP-1β	r = -0.25 ($p = 0.32$)	r = 0.02 ($p = 0.94$)	r = -0.05 ($p = 0.86$)	r = 0.20 ($p = 0.44$)
IL-8	r = -0.25 ($p = 0.32$)	r = 0.06 ($p = 0.82$)	r = 0.06 ($p = 0.81$)	r = 0.02 ($p = 0.81$)

IL-4、RANTESの減少は閉塞性障害の改善と相関する



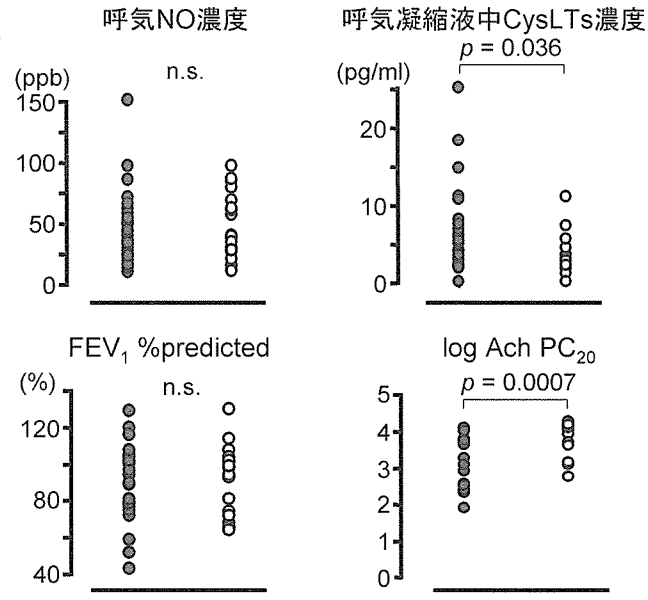
IL-4、RANTESの減少は気道過敏性の改善と相関する



気管支喘息の発作の予測因子　（秋山）

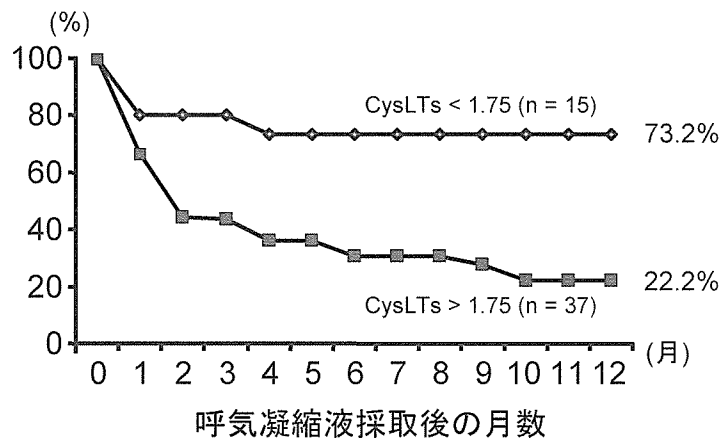
各指標の測定後一年間の
発作の有無を検討

● 発作群　(n = 33)
● 非発作群 (n = 19)



呼気凝縮液中CysLTs濃度と喘息発作との関連　（秋山）

発作を起さない
症例の割合



呼気凝縮液採取後の月数

2．有用性に関する研究の成果

- 基礎検討でLTB_4の喘息病態への関与が示唆されたが、呼気凝縮液中LTB_4と重症度や呼気NO濃度に関連は認められなかった。
- 呼気凝縮液中eotaxin濃度は閉塞性障害との関連が示唆された。
- 呼気凝縮液中IL-4、IL-8 、IL-17、RANTES、MIP-1α、MIP1βはステロイド反応性で、IL-4、RANTESの減少の程度は閉塞性障害や気道過敏性の改善の程度と有意な相関を認めた。
- ステロイド抵抗性分子はTNF-α, TGF-β, IP-10であった。
- 呼気凝縮液中CysLTs濃度は発作の予測因子となる可能性が示された。

当該年度の各班の検討課題

1. 妥当性
   検体への唾液混入の可能性の検討　（一ノ瀬班）

2. 有用性
   評価分子の検索　（秋山班、大田班）
   ステロイド治療との関連性　（一ノ瀬班）

3. 臨床応用
   評価分子の定量測定　（大田班、一ノ瀬班）

Multiplex Immunoassay、Luminex System

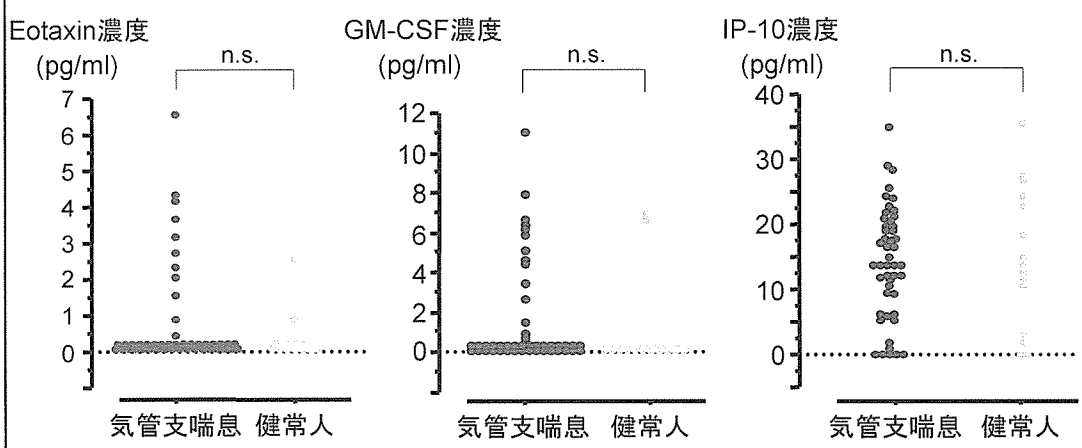
測定原理

- 別々のプローブを固定したLuminexビーズのセットを用意する。
- このビーズセットと蛍光物質で標識したサンプル中のターゲットとをチューブの中で反応させる。
- 反応後、Luminexビーズのセットをフローサイトメトリーで流し、赤色レーザでビーズの直径とビーズ番号の識別を、緑色レーザでビーズ表面の蛍光物質量を測定する。

今回の測定項目

- IL-2、IL-4、IL-5、IL-6、IL-8、IL-9、IL-10、IL-12 p70、IL-13、IL-15、IL-17、GM-CSF、IFN-γ、TNF-α、Eotaxin、RANTES、MCP-1、MIP-1α、MIP-1β、IP-10

呼気凝縮液中のEotaxin、GM-CSF、IP-10濃度（大田）



Cytometric Beads Array System (CBA)

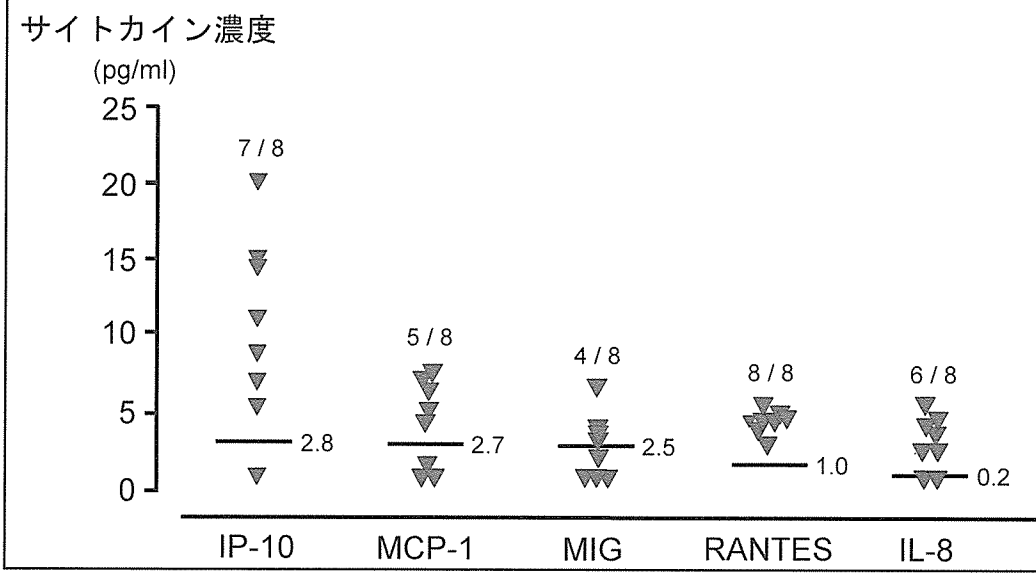
CBAの測定原理

- 蛍光強度が異なるビーズに、標的物質のcapture抗体がcoatingされている。
- サンプルおよびdetection用抗体を反応させる。
- Flow cytometryで、ビーズの蛍光強度により抗原を検出する
- 抗体の蛍光量により抗原量を同時測定する。

CBAの特徴

- 少量のサンプル (50 μL) で多種類の物質を、同時に測定することができる。
- 測定に要する時間は約4時間と、従来のELISA法の半分程度である。
- 感度は従来のELISA法とほぼ同等である。
- 専用のソフトウェアで自動解析が可能である。

CBAを用いた喘息患者呼気凝縮液中のサイトカイン定量測定



3．臨床応用に関する研究の成果

- Cytometric Beads Arrayを用いて呼気凝縮液中の炎症物質を定量測定した結果、IP-10、MCP-1、MIG、RANTES、IL-8が測定可能であった。
- Luminex Systemを用いたMultiplex Immunoassayでは、eotaxin、GM-SCF、IP-10が測定可能であったが、それ以外の物質は測定限界以下であった。

今年度（2年目）の成果のまとめ

1. 妥当性
   - 唾液成分の呼気凝縮液解析に及ぼす影響は無視しうる。
2. 有用性

   評価分子の検索
   - CysLTs濃度は発作の予測因子となる可能性が示された。
   - 生理パラメータとの関連では、RANTES, IL-4, TNF-α, TGF-βを凌駕するものは無かった。

   ステロイド治療との関連性
   - ステロイド抵抗性喘息の検知にTNF-α, TGF-β, IP-10が有用。
3. 臨床応用
   - Cytometric Beads ArrayによりIP-10、MCP-1、MIG、RANTES、IL-8 が定量化できた。
   - Multiplex Immunoassayでは、eotaxin、GM-SCF、IP-10が定量化できた。

最終年度（3年目）の課題

■ 臨床応用へ向けた定量化及び臨床的意義のさらなる検討
（長瀬、秋山、大田、一ノ瀬すべての班で）

- 症例の集積による治療の予測因子としての有用性の確立
  - 吸入ステロイド治療のガイド（用量設定、ディバイス選択）
  - 難治性（ステロイド抵抗性）喘息群の選別
  - 発作の予測
  - テーラーメイド治療への可能性
- 物質の定量化の精度の上昇：測定法の改良、濃縮
- カットオフ値の設定

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	Eur J Pharma	533	289-301	2006
K Matsunaga, M Ichinose, et al:	Airway cytokine expression measured by means of protein array in exhaled breath condensate: Correlation with physiologic properties in asthmatic patients	J Allergy Clin Immunol	118	84-90	2006
K Matsunaga, M Ichinose, et al:	Two cases of asthma in handicapped elderly persons in which assisted inhalation therapy was effective	Allergology International	55	347-351	2006
T Hirano, M Ichinose, et al:	Inhibition of reactive nitrogen species production in COPD airways: comparison of inhaled corticosteroid and oral theophylline	Thorax	61	761-766	2006
K Matsunaga, M Ichinose, et al:	Importance of assistance by caregivers for inhaled corticosteroid therapy in elderly patients with asthma	J Am Geriatric Soc	54	1626-1627	2006
松永和人、 一ノ瀬正和	鼻炎合併喘息の治療とロイコトリエン受容体拮抗薬	喘息	19	2-6	2006
南方良章、 一ノ瀬正和	気道炎症の評価	内科	97	249-254	2006
松永和人、 一ノ瀬正和、他:	気道過敏性の簡便な臨床指標の検討	呼吸	25	41-42	2006
市川朋宏、 一ノ瀬正和	喘息の発症機序に関わる因子－候補遺伝子も含めて－	Medical Practice	23	267-269	2006
一ノ瀬正和	成人気管支喘息・COPD 治療におけるテオフィリンの位置づけ	Medical Postgraduates	44	1-6	2006
一ノ瀬正和	喘息治療の進歩と新しい管理目標	日本呼吸管理学会誌	15	357-363	2006

山縣俊之、 一ノ瀬正和	呼気ガス分析	呼吸と循環	54	591-598	2006
山縣俊之、 一ノ瀬正和	気管支喘息	図解　呼吸器 内科学テキスト		149-152	2006
一ノ瀬正和	COPDと気管支喘息	呼吸	25	827-835	2006

VI. 研究成果の刊行物

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Review

Agents against cytokine synthesis or receptors

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Available online 2 February 2006

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.

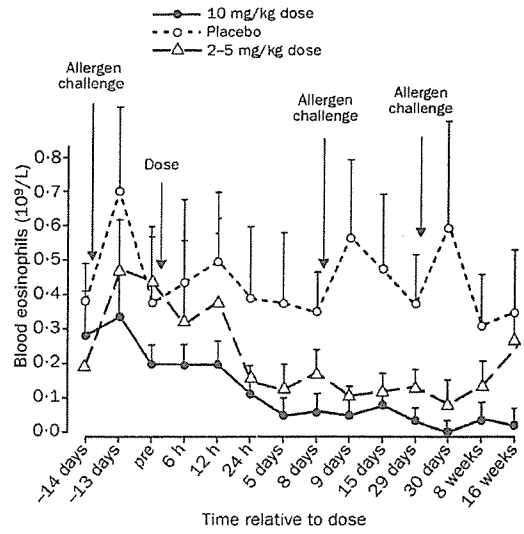

Fig. 1. The effect of a humanized monoclonal antibody against interleukin-5 (mepolizumab) on circulating eosinophils in patients with mild asthma, demonstrating a profound and very prolonged inhibitory effect. Reproduced from Leckie et al. (2000).

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

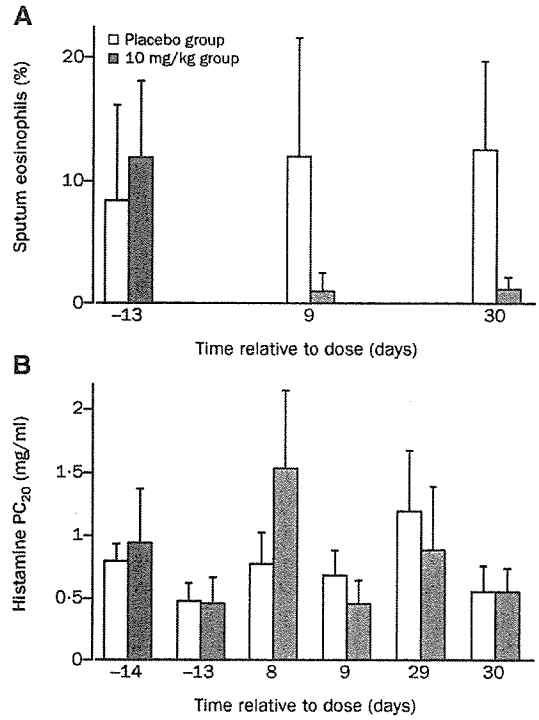

Fig. 2. Effect of a humanized monoclonal antibody against interleukin-5 (mepolizumab) on sputum eosinophils (A) and airway hyperresponsiveness (histamine PC_{20}) (B). Reproduced from Leckie et al. (2000).

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,

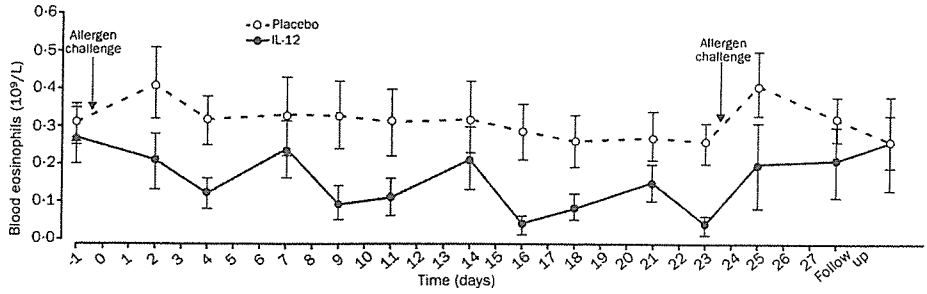

Fig. 3. Effect of interleukin-12 on peripheral blood eosinophils in patients with mild asthma. IL-12 was given in progressively increasing doses as an intravenous injection. Reproduced from Bryan et al., 2000.

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.