In vitro and in vivo modulation of human T lymphocytes from allergic asthmatic subjects
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Introduction

Asthma – definition and clinical manifestations
In 1987 the American Thoracic Society defined asthma on the basis of clinical manifestations as a disease characterized by episodic, variable airflow obstruction and increased responsiveness of the airways to a variety of specific and non-specific stimuli [1]. More recently, new components were added to this definition, and asthma was described as a chronic inflammatory disorder of the airways [2]. In the Global Initiative for Asthma a proposal was formulated to define asthma severity in a range from Grade I (episodic) to Grade IV (severe) [3].

Allergy to inhalant allergens appears to trigger asthmatic airway inflammation, but in some asthmatic subjects no allergy can be demonstrated. Therefore, asthma was divided into an atopic (extrinsic) and a non-atopic (intrinsic) variant. This thesis is mainly about atopic asthma.

Studying inflammation in asthma
Ongoing (sub)mucosal inflammation is found in patients with asthma, even in the mildest forms of the disease. This inflammation is characterized by an infiltration and activation of cells, an increased leakage of plasma-proteins from the blood into the airway lumen, an abnormal local production and secretion of proteins, and airway-epithelial cell damage. Study of these inflammatory processes in the airways of asthmatic patients is possible via endobronchial biopsies, bronchoalveolar lavage (BAL) fluid, sputum, and postmortem analysis of airway tissue.

Histopathological findings in asthma
The airway epithelium of patients with asthma showed metaplasia and hyperplasia of Goblet cells [4,5], and thickening of the basement membrane due to deposition of collagen types II, III and V and fibronectin with, as a result, airway remodeling [6-8]. Shedding and damage of the epithelium was observed as well. The suggestion has been raised that this was the result of the sampling technique, but it probably reflected the fragility of the epithelium in asthma patients. Signs of increased metabolic activity [9-11] and increased expression of intercellular adhesion molecule-1 [12,13] were found in epithelial cells from asthma patients. Furthermore, increased numbers of inflammatory cells have been reported within the epithelium and below the basement membrane [14]. This cellular infiltrate consisted of mast cells, eosinophils, neutrophils and T lymphocytes, many of which showed
signs of activation [5,7,8,15-19]. Eosinophils are considered the most characteristic inflammatory cell in asthma. Numbers of activated T lymphocytes correlated with the total number of (activated) eosinophils in biopsies [15-17,20].

**BAL fluid findings in asthma**

It has been questioned whether BAL fluid accurately represents events in the airways. However, BAL fluid studies yielded similar results as biopsy studies, studying subjects with asthma, sarcoidosis, hypersensitivity pneumonitis, idiopathic pulmonary fibrosis or healthy subjects [21-24].

Increased numbers of epithelial cells were found in BAL fluid from asthma patients [7,25]. Airway epithelium of asthmatics appeared to be activated as concluded from increased mucus production [18,26] and high levels of lactoferrin and secretory IgA [27,28]. Increased BAL fluid/serum ratios of plasma proteins indicated that in asthma the epithelium of patients showed increased permeability which was related to bronchial hyperreactivity [29].

Elevated levels of the mast cell products histamine, tryptase and prostaglandins were detected in the BAL fluid of asthma patients pointing at increased mast cell degranulation [30-32]. Increased histamine levels were associated with increased bronchial hyperresponsiveness [30,31].

Increased numbers of eosinophils were found in asthma patients as compared to healthy subjects. Intensity of eosinophilia and severity of asthma were correlated [19,25,33,34]. Eosinophils in the airways were activated as concluded from the detection of hypodense eosinophils [35] and increased levels of eosinophilic proteins in the BAL fluid of patients [19,25,33,36].

T cells in the BAL fluid from asthma patients showed signs of activation [34,37-40]. Activation of T cells was correlated with eosinophilia and disease severity [34,39]. These T cells produced cytokines like IL-2, IL-4, IL-5, IL-13 and IFN-γ [40,41], that may play a pivotal role in airway inflammation [42].

**Allergic asthmatic reactions**

In patients with allergic asthma an IgE-mediated allergy to inhalant allergens can be demonstrated. Mast cells play a major role in immediate allergic responses. They can bind allergen-specific IgE to their FcεRI, the high affinity receptor for IgE. Crosslinking of these receptors by allergen binding will lead to subsequent release of stored products [43] such as histamine, proteolytic enzymes, proteoglycans, and cytokines, so inducing acute airway
obstruction and inflammatory reactions. In asthmatic patients these reactions are characterized clinically by a drop in the forced expiratory volume in 1 second (FEV1) within 30 minutes after allergen inhalation. The immediate response is often followed by a second drop in FEV1, the so-called late phase response, that starts 3-4 hours after allergen exposure and reaches maximal intensity by 4-8 hours [44]. This late phase response is immunologically characterized by the infiltration of activated T lymphocytes, activated eosinophils, basophils and neutrophils, shown by studies on BAL fluid and bronchial biopsies [45-50]. The exact mechanism of induction of the late phase response remains to be elucidated.

Role of T lymphocytes in asthma

Human CD4+ T cells can differentiate into specialized cells with respect to cytokine production [51]. A diversity in cytokine production profiles has been found for individual T cells, forming a continuous spectrum. Th helper 1 (Th1) cells produce high amounts of IFN-γ, but no IL-4 and IL-5. Th2 cells produce high amounts of IL-4 and IL-5, but no IFN-γ. Cells producing intermediate levels of the mentioned cytokines are called Th0 cells.

In allergic asthma, T cells are involved in the regulation of the local inflammation in the lungs. T cells in the BAL fluid from patients with asthma showed signs of activation; the expression of IL-2R, HLA-DR and very late antigen-1 was found to be increased [40]. The activation of T cells correlated with disease severity [39]. Allergen-specific CD4+ T cells, cultured from the peripheral blood of subjects with allergic asthma, had predominantly a Th helper 2 phenotype, producing the cytokines IL-4 and IL-5 after in vitro stimulation [39,52]. In concentrated BAL fluid from allergic asthmatic subjects raised amounts of IL-4 and IL-5 were found, compared to healthy controls [40]. In the same study BAL fluid derived lymphocytes produced significantly more IL-4 whereas when compared to healthy controls IFN-γ production was not raised.

The Th2-cytokines IL-4, IL-5 and IL-13 may play a pivotal role in the pathophysiology of allergic asthma. IL-4 and IL-13 induce the switch to IgE production by B cells [53,54]. Furthermore, IL-4 activates endothelial cells and induces the expression of the vascular adhesion molecule-1, thereby facilitating the adhesion and influx of very late antigen-4 positive leukocytes, such as eosinophils and lymphocytes [55]. IL-5 promotes chemoattraction, differentiation and survival of eosinophils [56]. IL-5 mRNA and protein in biopsies of asthma patients were mainly found in T lymphocytes and correlated both with the number of activated T lymphocytes and eosinophils [57,58]. In patients with symptomatic asthma the numbers of cells in BAL fluid and biopsies expressing IL-4 or IL-5
mRNA were correlated with disease severity [59,60]. However, in patients with mild asthma or at early time points after allergen challenge, locally, predominance of Th2-cells could not be demonstrated. For example, no qualitative differences were found in the expression of cytokine mRNA when BAL fluid cells from mild asthmatic subjects were compared to healthy non-atopic controls [61]. Furthermore, T cell clones derived from BAL fluid 6 hours after allergen challenge of mild asthmatic subjects exhibited Th0-, Th1- and Th2-like cytokine profiles, with no dominance of either of the groups [62]. Krug et al. [63] observed in freshly isolated and stimulated BAL cells from asthmatic subjects a greatly increased percentage of IFN-γ producing cells as compared to atopic and non-atopic controls. The proportion of BAL cells producing IL-4 was small (range 0-7.8% in the asthmatic group). So, this study points at a more Th1 type of response in asthma. Summarizing, there is most probably not a fixed Th2 response in the asthmatic airways but local influences can direct Th1/Th2 responses. The shift to Th2 might correlate with disease severity.

**CD8⁺ T lymphocytes in asthma**

CD8⁺ T lymphocytes have long been considered to exert suppressive regulatory functions in allergic asthma. Several studies in mice and rats indicated a role for CD8⁺ T cells in the *in vivo* regulation of IgE production [64]. CD8⁺ T cells were able to inhibit IgE production by the production of substantial amounts of IFN-γ. In humans, it was reported that asthmatic subjects with markedly increased CD8⁺ T cell numbers were less likely to develop a late phase asthmatic reaction [65,66]. In atopic patients an increased capacity of CD8⁺ T cells to produce IFN-γ was detected [67]. These data may point at a suppressive role for this type of cell.

More recently, it has become clear that under influence of IL-4, CD8⁺ T cells, like CD4⁺ T cells, can develop into Th2 type cells, producing IL-4 and IL-5 [68,69]. CD8⁺ T cells expressing mRNA and protein for IL-4 and IL-5 were found in bronchial biopsies of asthmatic subjects [70] and CD8⁺ T cells from the peripheral blood of asthmatics released IL-4 [71]. It is unknown whether CD8⁺ T cells significantly contribute to the allergic inflammatory reaction in the airways of asthma patients.

A still on-going discussion is whether or not CD8⁺ T cells are able to respond to allergen. Though it is believed that CD8⁺ T cells preferentially interact with peptides derived from intracellular antigens, in several model systems CD8⁺ T cells interacted with antigenic fragments from soluble antigens as well [72]. A recent study in a rat model, showed that antigen-primed CD8⁺ cells downregulated the late airway response [73].
Regulation of T lymphocyte functions

How T-cell reactions are regulated in vivo is still subject of ongoing studies. The type of antigen presenting cell (APC) and the type of stimulus may be important factors in dictating the phenotype of the T cells present. In vitro, the ratio between the release of IL-12 and PGE\(_2\) from APCs determined the cytokine profile of the T cells [74]. The IL-12/PGE\(_2\) ratio of APCs is, on its turn, highly influenced by series of autocrine and paracrine factors. The latter include factors produced by local accessory cells. Alveolar macrophages, mast cells and epithelial cells may be important local cells involved in the regulation of the activity of T cells in the airways.

Study of alveolar macrophages revealed that in asthma patients a considerable part of alveolar macrophages belongs to the type that may activate T cells, whereas in healthy subjects most of the alveolar macrophages belong to the type that suppresses T cell functions [75,76]. Thus, activation of macrophages by allergen may result in activation of T cells resulting in increased cytokine production in asthma patients.

The ability of sensitized mast cells to release cytokines and other products early upon allergen stimulation makes them important candidates for local immunoregulation as well.

Role of mast cells in T cell regulation

It has become more and more clear that mast cells are able to produce a wide range of mediators [77]. Those mediators can be divided into two groups: the preformed and the newly generated mediators. The preformed mediators are stored in secretory granules and can be released immediately after mast cell activation. To this group belong histamine, proteolytic enzymes (like tryptase and chymase), proteoglycans (like heparin), and cytokines. The presence of preformed cytokines, is still being explored. There is evidence for the production of TNF-\(\alpha\), IL-3, IL-4, IL-5, IL-6, IL-8, IL-13, IL-16 and GM-CSF. How fast these products are being released is still under investigation. Activation of mast cells also induces the formation of newly generated mediators which comprise lipids (prostaglandins and leukotrienes), and cytokines. Synthesis and secretion of all these mediators are upregulated after Fc\(\epsilon\)RI activation.

The release of those products in the microenvironment of the airways affects other cells and this may regulate allergic inflammation. Until now the immunoregulatory capacity of human mast cells has not been extensively studied. Because human mast cells express mRNA for IL-4 [78], and release of IL-4 protein was detected [79], they were thought to favour conversion of T cells to the Th2 phenotype [80]. However, human lung mast cells
did not induce the class switch to IgE in B-lymphocytes in the absence of exogenous IL-4 [81]. Another study reported mast cells' capacity to degrade IL-4 protein [82]. Thus, the in vivo relevance of IL-4 production by mast cells needs further investigation.

Another mast cell product that is relevant for the allergic reaction is histamine. Histamine was found to cause the release of preformed IL-16 (formerly Lymphocyte Chemoattractant Factor) by CD8+ T lymphocytes. IL-16 will be described in more detail below.

**Cell surface molecules on T-lymphocytes**

T-lymphocytes are equipped with cell surface molecules that allow interaction of the cells with the environment. Among them are antigen receptors, receptors for growth factors (e.g. CD25 and IL-16 ligand CD4), receptors involved in costimulation (e.g. CD27 and CD28) and cell adhesion receptors/homing receptors (CD103). CD25, CD27, CD28, and CD103 will be discussed in this paragraph.

CD25 is the α-chain of the IL-2 receptor. IL-2 is a growth factor for T-cells. T-cells with increased expression of CD25 were found in bronchial biopsies and BAL fluid of patients with allergic and non-allergic asthma [16,34,37].

CD27 is a membrane surface protein on T cells which is upregulated on naive T cells after first contact with antigen via antigen presenting cells [83]. At the same time the cells are induced to express high levels of the ligand CD70. Subsequently, the CD27/CD70 pair will support clonal expansion and cytokine production by specific T lymphocytes [84]. After repeated antigenic stimulation of the TCR/CD3 complex the CD27 molecule is shed [85]. Thus CD27+ T cells and CD27 T cells are considered to be naive T cells and memory-type T cells activated by antigen, respectively. In a house dust mite-reactive donor the allergen-reactive T cells in the blood were exclusively found within the CD27- population [86]. Shed CD27 can be detected in serum.

CD28 is a major costimulatory signal receptor for T cells. The CD28/B7 costimulatory pathway enhances secretion of cytokines [87], whereas CTLA-4 may downregulate T cell activity [88]. CD8+ T cells can be functionally divided as naive, memory and memory-effector cells on the basis of the surface marker phenotypes CD45RA+CD27+CD28+, CD45RO+CD27+CD28+ and CD45RA+CD27CD28+, respectively [89]. Cytolytic function of CD8+ cells has been linked to cells with a CD28 phenotype [90].

CD103 is a membrane surface molecule that is predominantly expressed on mucosal leukocytes [91]. It specifically binds to E-cadherin expressed on the basolateral surface of epithelial cells [92]. Furthermore, it is described as an activation antigen which can in vitro
be upregulated by mitogens, phorbolesters, antigens and IL-2 [93,94]. The percentage T cells that express CD103 is larger in BAL fluid than in blood [95].

**IL-16**

As stated before, patients with asthma have a chronic airway inflammation which is characterised by the presence of increased numbers of activated inflammatory cells in the airway mucosa. Activated mast cells, eosinophilic granulocytes and T lymphocytes are most prominent. It is important to know the factors that attract these cells into the airways and that may activate them. Interleukin (IL)-16 was found to be one of the chemoattractants for lymphocytes, monocytes and eosinophils. Other chemokines able to attract lymphocytes, monocytes, and/or eosinophils are eotaxin, MCP-1, MCP-3, MCP-4, MDC/TARC, I-309, IL-2, IL-8, RANTES, MIP1α and MIP1β [42,96,97]. It is still not clear whether all of these chemokines contribute in a major way to in vivo chemotaxis. For the lymphocytes, IL-16 and MIP1α represented the major chemoattractant activity in BAL fluid at 6 h after allergen challenge of asthmatic subjects [97].

IL-16 was first described in 1982 when it was named Lymphocyte Chemoattractant Factor [98]. Recombinant IL-16 produced in E. coli forms homotetramers composed of 14-17 kDa chains. The tetrameric structure appeared to be an absolute requirement for IL-16 bioactivity [99]. In 1997 the occurrence of two forms of pro-IL-16 (80 kDa and 60 kDa) was reported [100]. These could serve as functional precursors. Pro-IL-16 was subject to proteolytic processing when incubated with cell lysates from CD8+ cells [101]. The ligand for IL-16 is the CD4 molecule. IL-16 functions as a chemoattractant for CD4+ T-lymphocytes, monocytes and eosinophils [102]. In addition, it may further activate cells, induce cell adhesion and protect cells against antigen-induced apoptosis [102-104]. Another function which was raised recently, is that IL-16 might prevent antigen-specific T-cell activation [104].

The first identified source of IL-16 was the CD8+ T lymphocyte. In this cell IL-16 was constitutively synthesized and CD8+ T cells contained stored bioactive protein. IL-16 is released from these cells in response to histamine [105]. Since mast cells are the most important source of histamine, IL-16 may play an important role in the sequence of events leading from activation of airway-tissue mast cells to infiltration of inflammatory leukocytes into the airways, characteristic for allergic asthma. CD4+ T lymphocytes contained constitutive IL-16 mRNA and the 80 kDa precursor but no preformed bioactive protein [106,107]. In addition to its presence in T lymphocytes, IL-16 protein has been detected in eosinophils [108], human lung mast cells [109] and in airway epithelial cells of asthmatic
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subjects [110]. Release from airway epithelial cells occurred following stimulation with histamine. Airway epithelial cells from normal subjects did not contain T-cell chemotactic activity [111]. Thus, the sources of IL-16 in vivo may be T lymphocytes, bronchial epithelial cells, mast cells and eosinophils.

Bioactive IL-16 was found in BAL fluid of asthmatic subjects 6 h after allergen and histamine challenge [97,112]. No IL-16 bioactivity was found in BAL fluid from challenged normal subjects or from atopic non-asthmatic subjects.

In summary, IL-16 is a chemoattractant factor that may play an important role in regulating the inflammation in allergic asthma. CD8+ T cells might be an important source of this mediator.

Therapeutic intervention in asthma

Patients with asthma are frequently treated with the inhalation of β2-agonists and/or glucocorticosteroids. Corticosteroids are effective in controlling asthma symptoms, reducing airway obstruction and bronchial hyperresponsiveness [113]. Their effects are attributed to their anti-inflammatory action [114]. Corticosteroids interact with a specific intracellular cytoplasmic receptor that modulates transcription of genes under the control of a corticosteroid responsive element [115]. Furthermore, corticosteroids affect the function of transcription factors either directly or by the induction of inhibitory factors as I-κB [116,117]. Oral corticosteroids were reported to affect activation and expression of cytokine mRNA by CD4+ T lymphocytes. Both in bronchial biopsies [118] and in BAL fluid [119] of asthmatic subjects a decrease of IL-4 and IL-5 and an increase of IFN mRNA positive cells were detected after oral corticosteroid treatment. In peripheral blood of asthmatic subjects treatment with oral corticosteroids resulted in a decrease of IL-3, IL-5 and GM-CSF mRNA positive cells [120]. High dose inhaled fluticasone resulted in a reduction of the proportion of lymphocytes in BAL fluid and a reduction of lymphocyte activation as measured by HLA-DR [121], but low dose fluticasone did not affect total numbers and BAL cell differentials [122]. Inhaled beclomethasone dipropionate (dose varying from intermediate to low) did not alter BAL cell differentials but did reduce T-cell activation as measured by CD25 and HLA-DR [123]. In bronchial biopsies a reduction of CD3+, CD45RO+, CD4+, CD8+, and CD25+ cells was found in the lamina propria of patients who were treated with inhaled corticosteroids [114,121,124-126].

Until now the available literature described the effects of glucocorticosteroids on total lymphocyte subpopulations, and markers for T cell activation which do not discriminate between polyclonal and antigen specific activation.
Chapter 1

Segmental allergen challenge in allergic subjects

Local deposition of allergen in sensitized subjects has been used as an experimental model to study pathophysiologic responses and development of inflammatory reactions in the airways. Segmental challenge technique allows the evaluation of inflammatory reactions in BAL fluid at several time points early after challenge. The late phase asthmatic response (LAR) is also considered a model for the chronic inflammation in asthma.

It can be hypothesized that T lymphocytes play a role in the onset of the LAR. Then one would expect to see changes in their numbers and/or activation state before and at the early onset of the LAR. Indeed, a fall has been shown in the proportion of CD3⁺ lymphocytes in the BAL fluid of patients with allergic asthma within 10 min after allergen exposure [127]. Furthermore, increased CD3⁺ lymphocytes counts were detected in the mucosa at 6 h after allergen exposure [128] and also an increased HLA-DR expression by T lymphocytes in the BAL fluid, suggestive for activation of the cells [129]. At 12 to 48 h after allergen challenge, T lymphocytes were clearly increased and they had acquired an activated state as concluded from increased percentages of CD25⁺ T cells and FAS-L positive cells [39,130-134], and from increased percentages of T cells expressing cytokine mRNA and protein [70,135,136]. Also soluble products from T cells are present in increased amounts in BAL fluid at those later time points after allergen challenge [132,135,137]. As stated before, bioactive IL-16 was found in BAL fluid of asthmatic subjects already at 6 h after allergen and histamine challenge [97,112].

Evidence is still growing that activated T cells play an important role in the regulation of both the late asthmatic response and the chronic inflammation in allergic asthma. Effects of allergen challenge on antigen-specific and mucosal cells have not been studied.

Aims and outline of this thesis

From the literature it can be concluded that T lymphocytes play a key role in the regulation of the chronic inflammation in the airways of asthma patients. The T cells in the airways show signs of activation and produce a variety of cytokines and other mediators which can induce inflammatory responses. Many studies describe the importance of CD4⁺ T cells in this respect. We extended former studies by our group [62,138,139-141] by studying CD4⁺ T cells in more detail, and by analyzing several characteristics of CD8⁺ T cells, thereby focussing on T cells from the airway compartment. We addressed the following main themes:

1) characteristics of local T cells *in vitro* by assessing clonal cytokine production;
modulation of local T cells \textit{in vitro} by studying effects of mast cells and products of mast cells on local T cells;

3) modulation of local T cells \textit{in vivo}:
   a. effects of glucocorticosteroid therapy on T cell activation and inflammation;
   b. effects of segmental allergen challenge of the airways on T cell activation and release of IL-16.

Chapter 2 is a review about the role of T lymphocytes and mast cells in asthma. The review describes a possible link between the early mast-cell activation and CD4$^+$ and CD8$^+$ T lymphocyte stimulation.

To study possible effects of mast cells on T lymphocytes we exposed T lymphocyte-lines and -clones to (products from) Human Mast Cell Line-1 mast cells. We studied T-lymphocyte proliferation and the production of the cytokines IFN-$\gamma$, IL-4 and IL-5. Preliminary results are described in chapter 3, while a more detailed study is described in chapter 4.

Chapter 5 is about IL-16. IL-16 release was studied in the bronchoalveolar lavage fluid of allergic asthmatic and control subjects before and after \textit{in vivo} challenge of the airways with allergen. Additionally, IL-16 release by local CD4$^+$ and CD8$^+$ T lymphocytes was studied more extensively \textit{in vitro}.

In chapter 6 we discuss the possible role of CD8$^+$ T cell modulation for the therapy of asthma and COPD.

Glucocorticoids are effective in the treatment of asthma. In chapter 7 we describe the effects of fluticasone propionate on CD4$^+$ and CD8$^+$ T lymphocyte activation in bronchoalveolar lavage fluid and peripheral blood of allergic asthmatic subjects who completed a double blind randomized trial. As activation markers, cell surface membrane molecules were studied.

In chapter 8 a newly developed immunocytochemical technique is described which allows detection of intracellular cytokines in bronchoalveolar lavage fluid and peripheral blood CD4$^+$ and CD8$^+$ T lymphocytes, without the need of prior \textit{in vitro} stimulation.

In chapter 9 are the effects of segmental allergen challenge of the airways of allergic asthmatics and controls on CD4$^+$ and CD8$^+$ T lymphocyte activation. Described are the effects on the expression in CD4$^+$ and CD8$^+$ T cells of cell surface membrane molecules and intracellular cytokines. We measured the expression of the activation marker CD27, the activation of mucosal T cells by analysing CD103 expression (together with CD27) and the expression of intracellular cytokine proteins in bronchoalveolar lavage fluid T cells.

Chapter 10 contains a summary of the results in english and dutch.
Chapter 1

References


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50. Dupuis R, Collins DS, Koh YY, Pollice M, Albertine KH, Fish JE, Peters SP. Effect of antigen dose on the recruitment of inflammatory cells to the lung by...
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65. Gonzalez MC, Diaz P, Galleguillos FR, Ancic P, Cromwell O, Kay AB. Allergen-induced recruitment of bronchoalveolar helper (OKT4) and suppressor (OKT8) T-
cells in asthma; relative increases in OKT8 cells in single early responders compared with those in late-phase responders. Am Rev Respir Dis 1987;136:600-604.


70. Stanciu LA, Shute J, Promwong C, Holgate ST, Dijukanovic R. Increased levels of IL-4 in CD8+ T cells in atopic asthma. J Allergy Clin Immunol 1997;100:373-378.


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Cruikshank W, and Center DM.
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128. Montefort S, Gratziou C, Goulding D, Polosa R, Haskard DO, Howarth PH, Holgate ST, Carroll MP. Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte-endothelial cell adhesion molecules 6 hours after local allergen challenge of sensitized asthmatic


