

Inflammatory determinants of asthma severity: Mediator and cellular changes in bronchoalveolar lavage fluid of patients with severe asthma

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Cellular and mediator profiles in bronchoalveolar lavage have not been compared systematically between patients with asthma of different severities, mainly because the patients with more severe asthma have an increased need for antiinflammatory medication. Information is limited to comparisons of allergic and intrinsic asthma, which can be distinguished clinically. When patients from these two groups with similar degrees of bronchial hyperresponsiveness were compared, both groups showed increased numbers of activated T-helper lymphocytes; those in the allergic group expressed the IL-2 receptor (CD25+), whereas in patients with intrinsic asthma there was also an increased number of T-suppressor cells with the activation markers CD25, class II histocompatibility antigen, and very late activation antigen-1, as well as T-helper cells class II histocompatibility antigen and very late activation antigen-1. This pattern is compatible with a more chronic T-cell activation in patients with intrinsic asthma. In patients with allergic asthma the cytokine pattern is compatible with a pure T_{H2} response (elevated IL-4 and IL-5); however, intrinsic asthma is characterized by elevated IL-5 and IL-2 but not IL-4. Our own findings show similar concentrations of IL-1, IL-8, and granulocyte-macrophage colony-stimulating factor in bronchoalveolar lavage fluid of patients with allergic and intrinsic asthma, whereas IL-6 and interferon- γ tended to be higher in patients with intrinsic asthma. There are probably fundamental differences in the pathogenesis of allergic and intrinsic asthma. These findings suggest that asthma does not depend on the presence of IgE or IL-4, although both may contribute to the pathogenesis of atopic asthma. The only common pathway in the different presentations of asthma that has been related to clinical symptoms appears to be IL-5-mediated activation of eosinophils; therapies aimed at this mechanism may be promising. (J Allergy Clin Immunol 1996;98:S27-40.)

Key words: Allergic asthma, bronchoalveolar lavage, cytokines, intrinsic asthma, T-helper lymphocytes, T-suppressor lymphocytes

Symptoms of bronchial asthma and the resultant requirement for treatment vary spontaneously over hours, weeks, and months, as well as during the lifetime of the person. The current symptomatology and therefore the severity of asthma are influenced by a number of variables that are difficult to assess and control. These variables include low-level allergen exposure, respiratory tract infections

Abbreviations used

BAL:	Bronchoalveolar lavage
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
IFN- γ :	Interferon- γ

(which are often impossible to distinguish from spontaneous exacerbations of asthma), and exposure to various airway irritants such as cold temperature, noxious vapors, sidestream tobacco smoke, and air pollution (Table I). Asthma severity is poorly defined, as are exacerbations that can occur without any clinically obvious reason.¹ It is difficult to control for the influence of these exog-

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TABLE I. Determinants of asthma severity

Bronchial hyperreactivity
Pulmonary function
Reversibility of airflow obstruction
Extrinsic/intrinsic type asthma
Patient perception
Circadian variation
Symptoms
Infections
Age
Frequency of attacks
Allergen exposure
Duration
Medication

enous and endogenous variables in studies attempting to measure the effect of new therapies on the severity of asthma. Because of these problems, such studies, although very important, are also very rare. The recently published International Consensus Report² tried to establish the severity of asthma by a combination of requirement for medication (mainly bronchodilators) and symptoms. Several determinants of asthma severity have been linked to clinical and laboratory features. However, only eosinophilia of the peripheral blood, the bronchial mucosa, and the bronchoalveolar lavage (BAL) are consistently related to clinical parameters of asthma severity. Thus studies were able to link bronchial hyperreactivity and/or FEV₁ to circulating eosinophils,³ BAL eosinophilia,⁴ and levels of eosinophil-derived proteins such as eosinophil cationic protein in BAL fluid.⁵ IL-5 concentrations in BAL have also been correlated with BAL eosinophilia.⁶

Although BAL is a useful tool for investigating inflammatory cell profiles in various bronchopulmonary diseases, including asthma,^{7, 8} and has been performed in patients with asthma of varying severity,⁹ cellular and mediator profiles in BAL have not been compared systematically among different groups with varying degrees of clinically established severity, one of the major reasons being the increased need for medication and its propensity to influence inflammatory cell and mediator profile among patients with more severe asthma. Thus our information is currently limited to studies that have attempted to compare allergic and intrinsic bronchial asthma, which can be separated mainly by clinical features.¹⁰

CLINICAL FEATURES OF ALLERGIC VERSUS INTRINSIC ASTHMA

Allergic asthma usually has its onset in childhood and patients have a positive history of bronchoconstrictor responses after allergen inhalation, corresponding to a positive wheal and flare reaction to skin prick tests. Furthermore, elevated levels of total and allergen-specific IgE can be measured. Allergen-IgE interaction involving mast cells therefore seems to be a prerequisite for allergic asthma. Patients with allergic asthma have a strong family history of other allergic manifestations such as atopic dermatitis and allergic rhinitis, as well as asthma and asthmatic attacks, with early- and late-phase reactions after allergen provocation.

In contrast, intrinsic asthma usually begins at about the age of 40 years or even later, most often after a severe upper or lower respiratory tract infection. The patient has no history of allergen-induced bronchoconstriction and results of the patient's skin prick tests remain negative, even if large panels of allergens are used. Total IgE levels are in the normal range; specific IgE levels cannot be demonstrated. A positive family history for intrinsic asthma is rare.¹⁰ There is no challenge model for intrinsic asthma, which persists as a chronic continuing inflammation even in the absence of respiratory tract infections. Clinically, patients with intrinsic asthma are more difficult to treat and usually require higher doses of inhaled or systemic steroids; a large proportion of the patients who have so-called steroid-resistant asthma are from this group of patients with intrinsic asthma. Furthermore, eosinophilia is more prominent in intrinsic asthma than in allergic asthma. Thus patients with intrinsic asthma represent a group that, in clinical terms, has more severe disease and a worse prognosis. On the basis of these observations and recent studies with use of the BAL technique, different pathogenic mechanisms leading to the different clinical pictures and different degrees of severity have been postulated to be operative in allergic and intrinsic asthma.^{6, 11}

CELLULAR AND MEDIATOR PROFILES OF ALLERGIC AND INTRINSIC ASTHMA

Only a few studies have compared inflammatory mediators and cell profiles in patients with allergic and intrinsic asthma. In one study,¹² total protein content, albumin, fibronectin, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1 β , and IL-6 levels in BAL of patients with intrinsic asthma were significantly elevated when compared with that of healthy controls. Unfortunately, how-

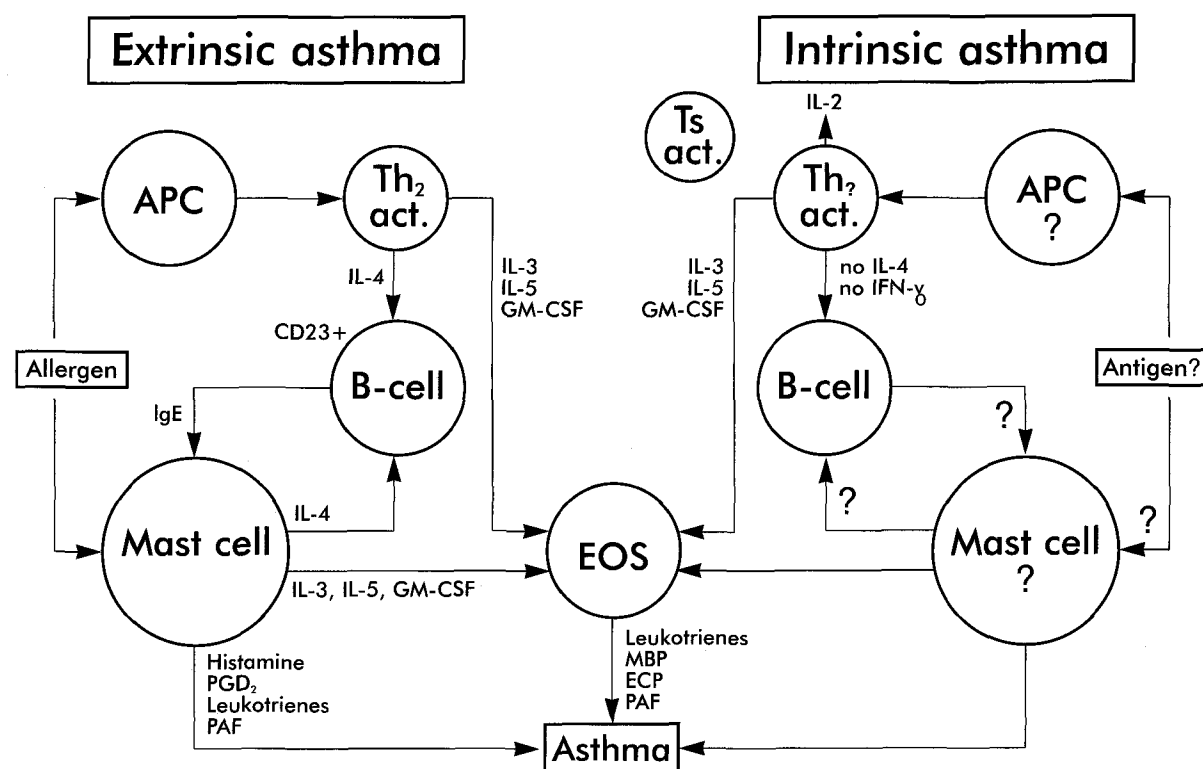


FIG. 1. Similarities and differences between extrinsic and intrinsic asthma. Many of the mechanisms operating in intrinsic asthma are unknown.

ever, patients with allergic asthma were not included as controls in this study. Another study that investigated cytokine profiles in BAL from patients with symptomatic asthma (mean FEV₁ 59% of predicted) reported significantly increased concentrations of tumor necrosis factor, GM-CSF, IL-1 β , IL-2, and IL-6 compared with that of patients with mild, asymptomatic asthma (mean FEV₁ 86% of predicted). IL-1 α and IL-4 concentrations were similar in both groups.¹³ However, the atopic status of these patients is not mentioned in the paper, and the patients with symptomatic asthma were pretreated with intravenous methylprednisolone. Recently we obtained similar results in BAL for IL-1, GM-CSF, IL-6, and tumor necrosis factor- α in patients with mild asthma 18 hours after endobronchial segmental allergen challenge.¹⁴ Thus the elevation in these cytokines is most likely a reflection of asthmatic inflammation common to allergic and to the more severe intrinsic asthma. No correlations of any of these variables with clinical parameters of asthma severity have been reported, and a direct comparison of these markers of inflammation in patients with mild allergic asthma and more severe allergic asthma, as well as intrinsic asthma, are still lacking.

Comparison of cellular profiles and cytokine content in patients with mild to moderate allergic and intrinsic asthma with similar degrees of bronchial hyperreactivity revealed elevated numbers of activated, CD25+ T-helper lymphocytes in the peripheral blood of patients in both the allergic and the intrinsic group. BAL fluid of patients with allergic asthma contained elevated numbers of activated T-helper lymphocytes expressing the IL-2 receptor (CD25).⁶ In contrast to patients with allergic asthma, however, BAL lymphocytes in patients with intrinsic asthma are characterized by elevated numbers not only of activated CD25+ T-helper cells but also of T-suppressor cells expressing the activation markers class II histocompatibility antigen and very late activation antigen-1.⁶ Thus in contrast to allergic asthma, intrinsic asthma is characterized by a pattern of T-lymphocyte activation compatible with a more chronic T-lymphocyte activation, as well as activated T-suppressor cells, which suggests chronic antigen or possibly autoantigen exposure.^{6,11} The role of these activated T-suppressor cells remains a matter of speculation.

Another study that compared cellular profiles and mRNA expression in patients with intrinsic

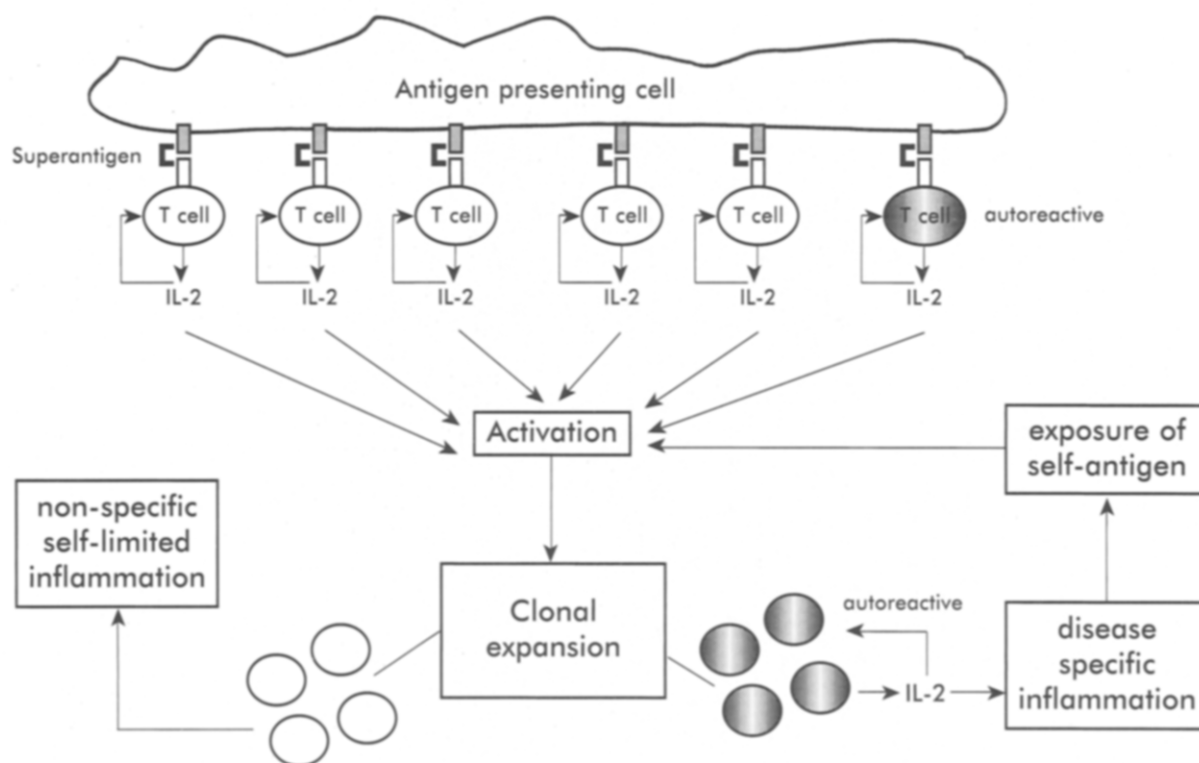


FIG. 2. A hypothesis for the pathogenesis of intrinsic asthma.

asthma with that of healthy controls also found increased numbers of eosinophils and activated T lymphocytes (CD25+) in the BAL of patients with intrinsic asthma.¹⁵

In BAL of patients with intrinsic asthma, a different pattern of cytokines has been described compared with that of patients with allergic asthma. Whereas elevated levels of IL-4 and IL-5, compatible with a T_H2 -type response, are present in concentrated BAL fluid of patients with allergic asthma, intrinsic asthma is characterized by elevated levels of IL-5 and IL-2 but not IL-4.⁶ Similarly, mRNA for IL-5, GM-CSF, and IL-6 was demonstrated in enriched T-lymphocyte preparations from patients with intrinsic asthma.¹⁵ mRNA for IL-2 and interferon- γ (IFN- γ) was demonstrated in some patients with intrinsic asthma; unfortunately, mRNA for IL-4 was not measured in this study.¹⁵ mRNA for IL-6, IL-1 β , and GM-CSF was expressed in monocyte preparations from patients with intrinsic asthma. Comparison of the levels of cytokines in BAL for patients with intrinsic and allergic asthma revealed similar concentrations for IL-1, IL-8, and GM-CSF, whereas IL-6 and IFN- γ tended to be higher in BAL fluid of patients with intrinsic asthma (Walker C, Virchow JC, Jr., unpublished results). Thus, in conclusion,

cytokine levels in BAL of patients with intrinsic asthma are not obviously compatible with a pure T_H2 type response, as has been suggested for extrinsic asthma.¹⁶ These results therefore suggest that the asthmatic condition does not depend on the presence of IgE or IL-4, although both might contribute to the pathogenesis of atopic asthma. Despite the fact that the underlying mechanisms are still unclear, these results suggest fundamental differences in the pathogenesis of allergic and intrinsic asthma (Fig. 1).

Mast cell-mediated inflammation triggered by the allergen-IgE-mast cell interaction has been postulated for allergic asthma. Although there is convincing evidence that the early asthmatic reaction is triggered by this interaction,¹⁷ the evidence for IgE-mediated, mast cell-dependent mechanisms in the late asthmatic reaction is circumstantial, even though it has been demonstrated that mast cells produce various proinflammatory cytokines.¹⁸⁻²⁰ Furthermore, it has not been conclusively demonstrated that the experimental model of the late asthmatic reaction is in fact responsible for the chronic inflammation of allergic asthma.

Thus the mechanisms leading to chronic symptomatic asthma in a patient who initially has only seasonal symptoms remain obscure. Our results on

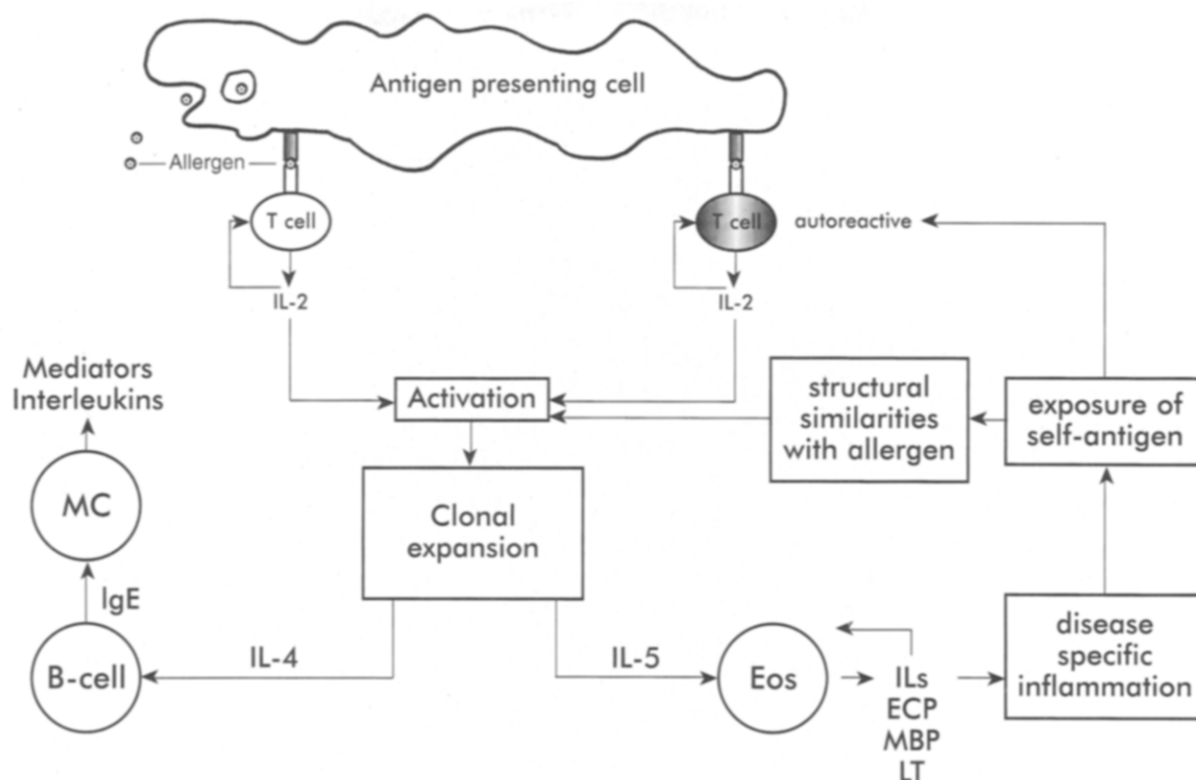


FIG. 3. A hypothesis for the pathogenesis of chronic allergic asthma.

cellular profiles in patients with intrinsic asthma would favor a hypothesis in which autoreactive T-helper and possibly T-suppressor cells are operating (Figs. 2 and 3). Clinically, such a hypothesis is supported by the observation of severe respiratory tract infections preceding the onset of intrinsic asthma. The cytokine profile of intrinsic asthma, with elevated levels of IL-2, IL-5, and possibly IFN- γ , together with surface markers on T-helper and T-suppressor cells, would be compatible with persistent (auto) antigen exposure similar to that following superantigen exposure with persistent autoimmune disease, such as rheumatic fever in β -hemolytic streptococcal tonsillitis (Fig. 2). The causative agent(s) and/or superantigen(s) are unknown. However, the technique of BAL will be a helpful tool to further investigate this important question.

"MIXED" TYPE ASTHMA

Studies of allergic asthma have mainly included patients with mild, seasonal asthma that is in remission⁶ and after allergen inhalation²¹ and segmental allergen provocation.⁸ However, because of obvious methodologic difficulties, especially the use of medication (Table I), no

studies that have used BAL have tried to investigate whether there are differences between acute allergic asthma after allergen provocation and the chronic allergic asthma that continues despite allergen avoidance or only low-level allergen exposure. Chronic asthmatic symptoms develop in a large number of patients with allergic asthma during the course of their disease, whereas airway reactivity to the allergen to which skin test reactivity can still be demonstrated subsides gradually. These patients have been referred to as having "mixed" asthma.²² Until now, investigators have mainly focused on examining the two ends of the spectrum of clinical disease, namely seasonal allergic asthma and nonatopic intrinsic asthma. In one study, peripheral blood T-helper cell activation (CD25 expression) and eosinophilia were correlated in patients with allergic, mixed-type, and intrinsic asthma.²² To the best of our knowledge no studies have been reported that attempted to study this group of patients with use of BAL. In a small number of patients, T-cell activation markers and cytokine profiles in this patient group were similar to the changes observed in patients with intrinsic asthma (Virchow JC, Jr.,

Walker C, unpublished results). Hypothetically, structural similarities between autologous protein and allergen, as has been reported for profilin and birch-pollen allergen,²³ could activate autoaggressive T-helper and/or T-suppressor cells and lead to chronic asthmatic inflammation in allergic asthma in these patients (Fig. 3).

CONCLUDING REMARKS

Despite the fact that severity of asthma is poorly defined, there is a need for studies aimed at systematically investigating asthma of different severities. While in the future it will be helpful to investigate further the mechanisms causing acute allergic asthma (a condition that is easily treated), it seems to be even more important to establish the pathways leading to severe and persistent chronic asthma. The question of whether in fact there are any links between acute allergic asthma and chronic changes remains as yet unsolved. Furthermore, the pathogenesis of intrinsic asthma, which is usually more severe than allergic asthma and also poses a greater therapeutic challenge, requires further investigation.

Because the only common pathway of the different presentations of asthma that is related to clinical symptoms seems to be IL-5-mediated activation of eosinophils, therapeutic strategies aimed at this mechanism are most promising, whereas strategies aimed at reducing IL-4- and IgE-mediated mechanisms are unlikely to benefit all patients with asthma. The role of other cellular and/or mediator changes in BAL in the pathogenesis of mild and, especially, severe asthma requires further study.

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DISCUSSION

T. H. Lee. Looking at the pathology of early, mild, and severe asthma is very important because it teaches us something about the progression and chronicity of the disease. I would like to hear again from Lauri Laitinen his rationale for the definition of early asthma.

L. A. Laitinen. To be clearly diagnosed as having asthma or chronic asthma in Finland, the patient has to have hyperreactivity and clinical symptoms. There has previously been little interest in the patients who we describe as having "early" asthma, but they may provide the key to understanding how asthma develops, in the sense of determining the turning point for when something happens in the airways that changes a normal subject into someone who has asthma.

R. Pauwels. Lauri Laitinen said that his population of patients with early asthma includes subjects with chronic cough. How did he select these chronic coughers, and in what age group are they?

L. A. Laitinen. Those patients were selected in the lung clinic and had cough for at least 3 months without any other reasons that could be found by, for example, bronchoscopy and lung function studies. Peak flows were normal, although there may have been a small degree of variability. Airway reactivity was normal. The cough was a dry type, not productive. We do have experience with that type of cough leading to asthma-type symptoms later.

P. König. First, is there any difference in biopsy findings between early asthma and chronic asthma? Second, you implied that early asthma may be a reversible condition; do you have biopsy proof that it is actually reversible?

L. A. Laitinen. No to the last question. I divided early asthma into different types and therefore it is a little difficult to draw comparisons and generalize findings. If, however, I did try to generalize, my impression is that patients with early asthma tend to have less inflammation, with fewer inflammatory cells present and less of an increase in extracellular matrix proteins, compared with patients who have mild asthma.

S. Makino. Airway hyperresponsiveness is very important for the definition of bronchial asthma. In our center we have found that airway hyperresponsiveness is only elevated slightly in patients with the mildest asthma and rather more so in

patients with chronic asthma. From bronchial biopsy specimens we have seen that the density of eosinophils is the same, like your results, but the thickness of basement membrane is very different; in patients with early asthma the thickness of the basement membrane is almost normal, whereas in patients with chronic asthma it is thick. Thus we believe that the thickness of the basement membrane, with its implications for structural changes and remodeling of the airways, is extremely important for the development of bronchial hyperresponsiveness.

L. A. Laitinen. In addition to the groups we have been studying, we also have biopsy specimens from Olympic-class skiers, who are quite often hyperresponsive. Sixty percent of Olympic skiers in Norway, for example, are hyperreactive, and this gives us a special group in whom you can study inflammation and bronchial hyperreactivity at the same time. In that group, to our surprise, there was no correlation between inflammation, basement membrane constituents, and hyperreactivity. I cannot say if that is the same in asthma, but I am a little disappointed at the lack of correlation between inflammation and hyperreactivity.

R. J. Davies. Are there any changes in the morphology and structure of the epithelium in these patients with very early asthma? You started us all off a decade ago with your findings on the morphology of the epithelium and the changes that you saw.

L. A. Laitinen. The structure of the epithelium is fairly normal. We have not yet finished electron microscopy studies in all our patients, so I can only give a general impression at present, but it seems to be more normal than in patients with established asthma.

L. M. Fabbri. Lauri Laitinen showed us the data on cell counts, but were there differences in cell activation between the groups? He also described occupational asthma in aluminum workers, but in fact there is some doubt now whether this is a sensitizing-like asthma or an irritant-like asthma (Chan-Yeung M, Malo JL. *N Engl J Med* 1995; 333:107-12). There is some evidence from Boulet et al. (Gautrin D, et al. *J Allergy Clin Immunol* 1994;93:12-22) that the pathology of the airway mucosa of persons with "reactive airways dysfunction syndrome" is rather different from the pathology of asthma, and particularly that the subepithelial fibrosis is much more pronounced. Can he comment on this?

L. A. Laitinen. There were different types of occupational asthma in that group; of eight pa-

tients, maybe two had aluminum asthma and three had isocyanate asthma. I appreciate your point, however, and it may be that we cannot group together all types of occupational asthma and should look at them individually. To answer your first point, we are now doing the counting with use of specific antibodies such as EG1, EG2, CD3, and MAC3, and I think there may be something abnormal about the activity of the cells also.

K. F. Chung. One group that is really important, but nearly impossible to find, are the so-called preasthmatics. If one could pinpoint the people who are going to develop asthma within the next 6 months and look at their mucosal biopsy specimens, one could probably answer many questions about the relationship between the cells seen when they have early asthma compared with the situation before asthma developed. One way of finding this group might be to look at people who are atopic but do not yet have asthma symptoms. I would be particularly interested to hear, perhaps from Steve Holgate, whether he has any of this type of data, particularly in relation to the evidence concerning the mast cell-expressing cytokines and activation markers in established asthma.

S. T. Holgate. Ratko Djukanović has done some work counting eosinophils in atopic but nonasthmatic persons, but in terms of function, we found adenosine monophosphate (AMP) to be a very interesting challenge. We have compared normal subjects, atopic subjects, and atopic asthmatic subjects in terms of responsiveness to methacholine and AMP. We found that atopic asthmatic subjects have increased responsiveness to both AMP and methacholine. Atopic normal subjects have increased responsiveness to AMP, but no (or only a very slight) increase to methacholine, and the normal subjects have neither. We are increasingly interested in using AMP as a surrogate marker of mast cell activation, because this particular stimulant will activate the cells through the A2b cell receptor in mast cells that have been previously primed with cytokines. AMP (and adenosine) challenge may provide a closer surrogate marker of the inflammatory response. The problem with methacholine is that it is too far downstream to reflect the inflammation.

K. F. Chung. If I interpret this correctly, an increased reactivity of the mast cell occurs before the airway smooth muscle is altered?

S. T. Holgate. Yes. Simon Godfrey has produced a very nice paper showing that AMP is an earlier diagnostic test than methacholine in children (Thorax 1995;50:511-6).

R. Djukanović. The presentations have raised two important questions. The first, for Lauri Laitinen, is: What causes the early form of asthma? As far as I am aware, the house dust mite, certainly in the northern parts of Finland, is not really an issue, and the season for grass or tree pollen is of relatively short duration, so what mechanisms are responsible? We know that in other parts of the world allergen exposure is related to this. I would like to address the second question to Stephen Holgate and René Aalbers, in relation to what may determine the disease severity at some stage of the natural course of asthma; specifically, what are the factors that suddenly lead to this very severe form of asthma?

L. A. Laitinen. A typical patient in Finland who goes on to become asthmatic usually starts to show some common cold-type symptoms in April or May; these are usually taken care of by antibiotics, and the patients are fine by July. The next spring the same thing happens and the patients are all right by, let's say, September. In the third year the symptoms repeat and the patient becomes very concerned and goes to a specialist. Thus about 3 years have passed before the patient begins taking asthma-type medication, and in that time the main cause is pollen, birch, or another type of allergen. The second type of patient gets symptoms only when he or she has a viral infection and again the same thing happens. The third type of patient gets symptoms when he or she is in contact with cats or dogs. The problem is that it takes a long time for the patient to get the right type of medication, and it usually takes an equally long time before the patient turns out to be a real asthmatic. To do the type of study that Fan Chung referred to would be very difficult. It may take many years if you, like us, study the patients in the stage when they are starting to have asthma symptoms, and if they receive the appropriate treatment at that point, then it may take 10 to 15 years before they are "real" asthmatics.

W. J. Calhoun. Do you think that viruses are more important in these patients than allergen? Is it possible that viral infections precede sensitization, and do you have any indicators for that?

S. T. Holgate. There is little doubt that viruses play a key role in the acute asthma exacerbation. We now have some new data that confirms what we have believed clinically for some time, namely, that with use of polymerase chain reaction (PCR) technology one can identify the viruses with more confidence and security as to their causative link with the exacerbation (Johnston SL, personal com-

munication). The point underlying Dr. Djukanović's question is a rather more serious one that is becoming increasingly important for us to address; namely, do viruses in some way interact with the airway and maintain the chronic inflammatory state? That is the question that James Hogg and others are trying to answer with use of in situ hybridization and PCR technology. As far as I understand it, we have not made much progress in that area. I and quite a few of my colleagues are becoming increasingly disillusioned about the role of allergens in the chronicity of asthma. There can be no question about their importance in the induction of the asthma process, particularly in childhood, and maybe in the causation of acute exacerbations related to seasons and exposures, but the basic question is, what takes the asthma from the intermittent characteristics that Lauri Laitinen commented on a few moments ago into 40 years' worth of inflammation? The reason I say this is that in places like Calgary, where there is no house dust mite, there is more, not less, asthma. It is said that there must be other allergens from cats and dogs, but of course there are cats and dogs everywhere else as well. There is some difficulty in sustaining the view that house dust mite antigens drive the chronicity of asthma. I believe that it probably does not do so and that other factors are important, including T cells adopting an autoimmune response and the matrix cells—the epithelium, the endothelium, the fibroblasts, even the smooth muscle—taking over the cytokine repertoire that supports the eosinophil and mast cell-mediated inflammatory reaction and removing it from the external allergen stimulus.

T. H. Lee. I agree completely. We must devote much more attention to the structural cells in the airways and how they can influence the milieu in which inflammatory calls are found, because in many instances the models that we are studying reflect a very early phase, even though there may be late-phase reactions.

R. Aalbers. It is still unknown why patients progress from moderate to severe asthma. My clinical impression is that severe asthma can develop within a very short time period. During my presentation I described a patient who, despite receiving 20 mg of prednisone per day, had a very high peripheral blood eosinophilia and peak expiratory flow variability. It seems that in a very short period a relative "steroid resistance" develops in such patients. What causes this? As Professor Holgate has already said, it is probably not house dust mite, because our results are from Davos,

where house dust mite levels are extremely low. It could be that other allergens play an important role. Dr. Platts-Mills published a study some years ago in the *Lancet* showing that trichophyton interdigitalis could play a role in the causation of severe asthma. We looked for evidence of trichophyton interdigitalis in our patients but could not demonstrate specific IgE to this source of antigen by radioallergosorbent test. In the 1970s, Bert Roldaan, my successor in Davos, demonstrated that at high altitudes viral infections play an important role in causing exacerbations and could be an important factor in maintaining the chronicity of severe disease. Another possibility is a switch in T-cell activity leading to a type of autoimmune disease.

T. W. Higenbottam. When we are drawing these conclusions in terms of mechanisms and clinical pathologic correlations, I am a little unsure exactly what the definitions are of these different stages of the disease. In addition to symptomatology, we need to know more about the natural history of this early sort of mild asthma described by Dr. Laitinen. We have some clues from wheezy bronchitis in children that it may not be a prolonged illness, but I wonder if you could perhaps tease out the relationship between this sort of state and that which follows in everybody—an acute episode of upper respiratory tract viral infection, which does indeed alter reactivity. What happens over time to the natural history of the disorder: is it an on-off occurrence, does it recur every year, or does it last a lifetime?

L. A. Laitinen. If we take patients with seasonal asthma, it is continuous. If one has birch pollen asthma, as in Scandinavia, then there are problems each April and May. Medication can control the symptoms, but the problem will always occur at the same time. The problem is worse if a viral infection occurs at the same time. It is my clinical impression that this repeated exposure over time can turn the patient into a chronic asthmatic. If patients with occupational asthma are taken off work, many will eventually become free from symptoms over a period of time in proportion to the duration of exposure. It may take 1 year or more, or it may occur more rapidly if the patient receives the correct medication. Patients with chronic cough are very difficult to treat; we have tried different medications and yet they still cough.

S. T. Holgate. Dr. Higenbottam raised an important point. If one compares the effect of human rhinovirus on lower airway pathology in a normal subject and in a patient with asthma, in the normal

subjects there is a T-cell response that is similar to that in the patients with asthma. There is also a neutrophil and eosinophil response. The difference between the patient with asthma and the normal subject is not so much in the magnitude of the leukocyte infiltrate but the duration. Thus the T-cell and the eosinophil responses persist for several weeks after the virus exposure. Dr Johnston, working in our group, has shown that if human epithelial cells are infected with this virus there is increased transcription and secretion of chemokines, particularly IL8-1 and RANTES. We believe that in the patient with asthma the epithelium is again the target for this viral attack, which is just exaggerating an ongoing, maybe low-grade, inflammation initiated by allergen. The exciting work in children that Dr. Heaney and Ennis are doing in Belfast has shown that during the acute episodes of virus-induced wheezing in children, the lavage fluid (they have not done biopsies) shows predominance of neutrophils along with activated T cells, whereas in the children who had the persistent eosinophils during the virus episode there is a further increase in the eosinophils and very few neutrophils. It looks as if the character of the inflammation is quite different between the two.

W. J. Calhoun. Drs. Busse and Dick and I have worked with rhinovirus 16 (RV16), and there are two points I would like to mention. First, what do you think might be the influence of strain differences in the rhinoviruses? Second, I agree entirely that the persistent effect in atopics is fairly dramatic; in our work the effect of enhanced allergic reactions following allergen challenge persists in some persons for 6 months, despite the fact that we could not identify lower respiratory tract inflammation as a consequence of RV16, which contrasts with Stephen Holgate's experience. RV16 in particular proliferates most easily at 33° C as opposed to 37° C, so there does seem to be a tropism for the nose for that particular strain. We did not find lower respiratory tract inflammation either in our normal subjects or in our atopic subjects, but the persistence of the potentiated inflammatory response to allergen challenge was dramatically longer than we had anticipated.

S. T. Holgate. That is very interesting. We have shown with use of wild-type rhinovirus strains that there are tremendous differences in the affinity of the different subtypes of human rhinovirus to bind to soluble intercellular adhesion molecule-1 (ICAM-1), its cell surface receptor. Obviously that is just one marker, but there are up to three log differences between the different strains in the

binding constant between the cavern region of rhinovirus and soluble ICAM-1. Because ICAM-1 is the receptor through which the major class of rhinovirus is able to get into the epithelium, I suspect that there are important differences in pathogenicity that have not yet been investigated.

T. H. Lee. Sally Wenzel mentioned that it is important to try to correlate some of the BAL findings with biopsy. Can she comment further on that?

S. E. Wenzel. It is important to use as many tools as we have available to us. We are beginning to realize that asthma may not be only a disease of the large or small airways but that it extends into the interstitial spaces of the lung as well. Therefore, use of combined techniques of BAL, endobronchial biopsy, and, even more recently, transbronchial biopsy may be the way to go forward because they each sample different, specific portions of the airways.

T. H. Lee. Does anyone have any data comparing BAL and biopsy findings in the same persons, and can they tell us whether they show the same, complementary, or different information?

L. M. Fabbri. Comparing sputum, biopsy specimens, and BAL, the only cell that correlates in all three in persons with asthma and bronchitis is the eosinophil. We have no correlation for other cell types (Maestrelli et al. *Am J Respir Crit Care Med* [in press]). I also want to ask a question. We are assuming that severe asthma is an evolution of the same disease and that it is still an airway disease. However, looking at the lung pathology of subjects who have died of asthma, one is impressed by the massive infiltration of inflammatory cells in the adventitia and around the arteries (Saetta et al. *Am Rev Respir Dis* 1991;143:138-43). Can you give us more details of the clinical and imaging characteristics of these patients-high-resolution computed tomography (CT) scan or transthoracic tissue sampling? I believe we should adopt a more aggressive approach with patients with severe disease who are at risk of dying from asthma. They deserve the same diagnostic approach that we use in interstitial lung disease with alveolar involvement, as suggested by the pathology of people who have died of asthma (Saetta et al., as previously mentioned, and *Eur Respir J* 1989;2:1008-12).

S. E. Wenzel. We are starting to do that. We are doing transbronchial biopsies in our patients with severe asthma, and we are indeed finding changes all the way out to the alveoli.

T. H. Lee. Can we understand the reason for doing that?

S. E. Wenzel. Because it is a black box and no one has looked at that level of pathology in a patient with asthma before. It has just been assumed that it is an airway disease. It is important to get the complete picture. I agree with Leo Fabbri regarding the different ways we approach asthma and interstitial lung disease. If we see someone with a forced vital capacity (FVC) of 60% of predicted they automatically go to open lung biopsy. If we see a patient with asthma with an FEV₁ of 45% and an FVC of 60%, we give them more steroids. I have a problem with that.

T. W. Higebottom. Coming back to this comparison between tissue and lavage, I was very encouraged by Steve Holgate showing a transbronchial biopsy in patients with asthma. In an entirely different population of patients, after lung transplantation, we have looked in detail at the comparison between lavage and transbronchial biopsy findings in terms of distribution of certain cell types. In that setting, which is fairly comparable with what has been observed in sarcoidosis, the CD4 cells do not distribute into the airway and are not seen in the lavage during acute rejection episodes, whereas the CD8 cells distributed both in the tissue and lavage; I think this is because they were predominantly found in the subepithelium. Eosinophils and other cells, such as neutrophils, distributed quite effectively. What this raises is the difference between cell activation and their migratory capacity. I think we need to focus not only on simply finding the cell but also on why it has actually moved into the airway lumen.

R. Pauwels. I have a related comment. We seem to imply that the cell types differ between early, mild, and severe asthma. Is this a question of sampling or a question of the cells moving from a different place?

S. T. Holgate. Whether it be lavage or biopsy (apart from transbronchial biopsy), one only has the epithelium and the submucosa to sample. In the experience of many of us, there appears to be a gradient of response from mild to severe disease. As one moves towards the severe disease, there appears to be a stronger emphasis on the role of the T lymphocyte, and in the late-onset asthma that we have just heard about, which tends to be a more severe disease and more difficult to control than the allergic type, there also appears to be a strong T-cell signal. It is important to know whether one can attach a cell profile to different levels of disease activity, because if that is the case it has pharmacologic consequences.

R. Aalbers. As a follow-up to Leo Fabbri's earlier comment, I doubt that fatal asthma is the same as severe asthma. Most patients with severe asthma hardly ever die of severe asthma. There is a very strong morbidity, but mortality is mostly seen in patients who have a very poor compliance and either do not take their medication or take the wrong medication. We looked at many things in the patients with severe asthma, but we did not take transbronchial biopsy specimens. It was the first study of severe asthma in which we dared to take bronchial biopsy specimens, and we demonstrated that it is a safe procedure. Our ethical committee did not allow us to take transbronchial biopsy specimens, and I don't know whether others have been able to take such specimens from patients with severe asthma.

S. T. Holgate. Richard Martin and his colleagues in Denver have been looking at nocturnal asthma, which of course is a measure of asthma severity in its own right, and have been biopsying transbronchially at 4 AM and 4 PM. Their series of studies indicates that there is a very strong alveolar component to the inflammatory response that involves multiple cells, not just eosinophils. This is something we have not thought about previously, and thus I agree with Sally Wenzel and Leo Fabbri that we need to move to the periphery and examine what is happening there.

T. H. Lee. What sort of physiologic measurements are you going to make peripherally?

S. E. Wenzel. One of the issues that Dr. Monica Kraft is now working on is to measure peripheral airway resistance in patients with severe asthma. We have also done pressure-volume curves in most of these patients with severe asthma, and it is very interesting that in about 90% of them the curves are shifted to the left; they are not restrictive-looking curves but look much more like an emphysema curve (although these patients do not have emphysema). All this is fairly preliminary, but we have been impressed thus far, when comparing some of the nocturnal asthma transbronchials versus the patients with severe asthma who are receiving huge doses of steroids, to see that the inflammation is still there. There are eosinophils, mast cells, and increased lymphocytes in the patients with severe asthma despite their taking 30 to 40 mg of prednisone per day. Probably more important than that is the amount of structural changes that have occurred. The sub-basement membrane is markedly thickened, more so than in the patients with nocturnal asthma, and there appears to be a tremendous amount of collagen and elastin depo-

sition throughout the smooth muscle of these airways and out into the very small airways themselves.

J. M. FitzGerald. One of the problems is that much of what we have heard described today is very invasive and not practical for a population-based, prospective cohort study. I would like to ask Christian Virchow if he thinks that sputum induction as a noninvasive investigation in that setting with a large number of cohort patients may ultimately give us more answers than a lot of small studies using bronchial biopsies and bronchoalveolar lavage.

J. C. Virchow. I would truly agree, especially with the type of studies that are being done by the Hamilton group. With these types of studies you could probably address some of the questions with regard to when asthma starts and whether there are indices of inflammation, for instance, in induced sputum, that show if a patient might become an asthmatic. I want to raise a second point, which is that although we all know individually what we mean by mild, moderate, and severe asthma, the terms are rather ill-defined and might differ from country to country. My final point is that we need to remember the risks associated with transbronchial biopsy during bronchoscopy—severe bleeding and tension pneumothorax, for instance. In contrast to asthma, with interstitial lung disease the observations from transbronchial biopsy can at least influence our choice of therapy, but we have no idea if this is the case with asthma.

S. E. Dahlén. As Dr. Wenzel pointed out, the conclusions we can draw from measurements in various fluids and so forth, in particular at baseline, are quite limited. It is only when we use specific inhibitors of various pathways and when we can correlate changes with physiologic effects that we can draw firm conclusions. Dr. Wenzel also alluded to an effect of corticosteroids on eicosanoid pathways, but recent studies have suggested that these agents do not affect eicosanoid production *in vivo*. For example, a study from Oates' group (Dworski et al. *Am J Respir Crit Care Med* 1994;149:953-9) showed no change in BAL fluid eicosanoid mediators with high-dose oral prednisolone. Finally, I have a question to all the speakers here, which is how to overcome the confounding factor of differences in therapy when we are trying to compare the findings from different groups. The group described by Dr. Aalbers was treated with such heavy doses of steroids that one would expect the number of cells to be lower. How can we control for this?

S. T. Holgate. Coming back to the effect of steroid on the cyclooxygenase pathway, we have just completed a study with Julia Polak's group looking at *cox 1* and *cox 2* expression in the airway. We have been able to demonstrate in the bronchial epithelium, and in other cells, that 6 weeks' treatment with inhaled steroids reduces the expression of *cox 2*, in terms of the product measured by cytochemistry and also by *in situ* hybridization looking at the mRNA. This sort of work puts lavage into something of a bad light when one considers how carefully mapped and designed the airway structures are. Unless mediators are very elevated in lavage fluid I think they are of dubious significance in terms of elucidating mechanisms.

S. E. Wenzel. Although numerous studies have suggested that steroids have no effect on eicosanoids, a few have suggested they do. We published a paper last year showing that *LTB₄* levels from BAL cells in patients with nocturnal asthma appeared to decrease after 50 mg of prednisone, so I think it is still a controversial issue. I agree strongly that there needs to be a high starting point in lavage fluid to be able to see an effect. At low starting levels there may be an effect, but it is just not measurable.

R. Aalbers. I would like to go back to the remarks made by Dr. Dahlén concerning the limitations of the study in severe asthma. We still give high doses of inhaled or oral corticosteroids to patients whom we consider to have severe disease without knowing whether it is helpful. In patients with moderate and mild asthma, beautiful clinical effects have been shown, and by Dr. Laitinen and Dr. Djukanović in biopsies as well, but in severe asthma we have hardly any markers to see whether patients improve with therapy. You cannot measure *PC₂₀* methacholine, and the measurement of *FEV₁* is not always reliable in these patients. All you have are their complaints and their peak flow variability. If a patient receives 40 mg of prednisone per day and still has complaints, such as not being able to go to the toilet without being short of breath, what parameter can you use to tell us if we need to taper down or increase the medication? When we took biopsy specimens from these patients, mast cells and eosinophils and an increased expression of *CD25+* cells were still present, and the patients were taking high doses of steroids, so it seems that either steroids do not affect those cells or that the dose is still too low.

L. A. Laitinen. It is very important to emphasize the effect of characteristics of patients and even more important to describe the medication very

carefully to patients. There is also a need for guidelines. One of the definitions of severe asthma is that FEV₁ is under 60%, although when I look at René Aalbers' patients, I see that half of them had FEV₁ values of more than 60%, so there appears to be a need for a unified acceptance of the term "severe asthma," for example.

R. Aalbers. I totally agree. I wonder whether FEV₁ is such a good parameter in asthma. It is useful in chronic obstructive pulmonary disease and in emphysema, but, for instance, patients with very severe brittle asthma and patients with fatal asthma can have FEV₁ values of more than 100% of predicted while they can also have very severe attacks that may be even fatal.

R. Pauwels. When we drew up the guidelines we had many discussions about defining the severity of asthma, and it is indeed impossible to define the severity of asthma on a single characteristic. An FEV₁ of less than 60%, for example, is only one of the characteristics for severe asthma. If you look at the different characteristics that are used in classifying severity, they are not all obligatory and they are not all exclusive, so this classification has been set up to help people understand how to classify, mainly from a practical point of view. I believe it is extremely important in studies that we clearly describe what we define as severe. Although we may differ one from another in our criteria for severe asthma, as long as they are clearly defined then the data can be interpreted.

R. J. Davies. One of the things we all do clinically is to judge the severity of asthma on the outcome of treatment. We go down this spectrum until we come to Dr. Aalbers's group of patients, who are terribly difficult to treat with anything at all. I am wondering whether you have to have even higher and higher doses of steroids. Is there any evidence from biopsy studies that either high-dose inhaled corticosteroids or oral corticosteroids actually influence activated CD25 lymphocytes in a properly controlled study?

R. Aalbers. As far as I know there are no studies available on the effects of steroids on CD25+ cells in good clinical studies with use of biopsies. We started a study in patients with severe asthma with use of human immunoglobulins as a steroid-sparing therapy. These are very preliminary results, but in a few patients we have seen a huge clinical improvement in addition to a marked decrease in CD25+ T cells, and also a marked decrease in IL-8 in peripheral blood. We do not know how the human immunoglobulins are having this effect, but we see it.

K. F. Chung. The question that comes to my mind is whether severe asthma is more difficult because the localization of inflammation is much wider, extending much more peripherally into the bronchioles. We have heard some evidence for this. Following on the studies from Montpellier, we have also been doing some studies with CT scans in patients with asthma. We have studied the patients with the most severe asthma in our clinic and performed inspiratory and expiratory CT scans of their lungs. One very often sees either focal areas that do not deflate on expiration or the entire lung not deflating on expiration as it should. This could have been diagnosed just by looking at the patient's chest! This suggests peripheral airways obstruction and presumably ongoing bronchiolar inflammation. The second thing is whether severe asthma is a different type of inflammation; certainly there appear to be more CD25+ cells, but this returns to the question as to whether this is because these cells do not respond to steroids. Finally, this leads me to the question of how far we should go on investigating difficult asthma and the dangers of doing transbronchial lung biopsies to assess peripheral airway inflammation in asthma. There is another possibility of using video-assisted lung biopsies, as we do with lung fibrosis; we know what the morbidity and perhaps mortality is of that procedure. In patients in whom, at least on CT scan, there is evidence of peripheral bronchial disease, I wonder whether we should not go ahead and do that anyway to confirm this, because you are going to give those patients drugs that will themselves increase morbidity—high doses of steroids or cyclosporin and so on. It would also advance our understanding of the problem and occasionally give us an alternative diagnosis, just as in one case that we had recently of a steroid-resistant "asthmatic" who bronchodilated to β -agonists, but who turned out in the end to have a completely different pathology.

R. Rodriguez-Roisin. Concerning the role of FEV₁ or other lung function tests, it is important to keep in mind that FEV₁ does not tell us very much. For instance, we have compared patients with chronic severe asthma under stable conditions who had an FEV₁ of 35% with patients who had acute severe asthma, who also had FEV₁ values of 35% within the first or second day of hospitalization: two groups with the same lung volumes and spirometries and yet from a completely different clinical viewpoint. The degree of hypoxic pulmonary vasoconstriction resulting from alveolar hypoxia and probably the pulmonary circulation also

are completely different, with PaO_2 values of 50 and 90 in the two groups. This type of information about what is happening in peripheral airways could be important as a noninvasive tool. I also wonder if it would be worth trying bronchial biopsies in patients with acute severe asthma who need to go to intensive care. Although we do not always know the cause, we know that by 6 to 10 days these patients can go home and can be followed-up as an outpatient on a routine basis. Sequential biopsies might provide useful information to differentiate the patterns of asthma in different populations.

L. A. Laitinen. The problem is that people are speaking about asthma as one disease, and it has been shown very clearly that it is not one disease, and therefore it is very important that we have lots of studies in different groups in different parts of the airways and try to describe the patients as well as possible, as Romain Pauwels emphasized.

J. C. Virchow. Does anybody here believe that severity might be induced by treatment? I am

thinking of the younger, usually female patients who, probably because they want to function normally in society, will not take on the hassle of inhalation therapy and low doses of whatever, are usually started very early on high doses of steroids, and turn out to have this most dreadful steroid-resistant asthma or asthma that requires high doses of steroids. It seems also very important that we try to dissect as far as possible the results that we get in terms of what the treatment effect is on one side and what is caused by the severity of asthma on the other side.

T. H. Lee. That question is very interesting. There are actually *in vivo* data demonstrating that incubation of cells and tissues in the presence of corticosteroids downregulates steroid receptors, so it is indeed very possible that chronic use of corticosteroids might actually induce a situation of resistance, but resistance is a very complicated issue that we will not have time to address today.