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ALLERGY

Review article

Monitoring the allergic inflammation

Individual symptoms of allergy such as asthma, dermatitis, rhinitis have many different underlying mechanisms. The detailed characterization of the inflammatory mechanisms underlying symptom development in the individual patient is important in order to optimally control treatment. Measurement of eosinophil cationic protein (ECP) in sputum or blood and eosinophil protein X/eosinophil derived neurotoxin (EPX/EDN) in urine may be used to read the involvement of the eosinophil granulocyte in the process. An important information as eosinophil dominated processes seem to be particularly sensitive to corticosteroid treatment. The possibilities to measure the involvement of other inflammatory cells exist today, but are only used to a small extent. The dream would be that every patient with an inflammatory disease is characterized with respect to the profile of involving cells and mediators. Such information would provide us with a unique understanding of the underlying mechanisms of the development of disease symptoms and the possibilities of treating these.

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Allergy is essentially an inflammatory disease (1). Our knowledge of the cells and mediators that are involved in the allergic inflammation has increased immensely during the last decade. This knowledge provides the basis of a more rational way for the development of therapeutic principles that may relieve or prevent the development of allergic symptoms. An important part in this development is the availability of diagnostic assays that clearly reflect the mechanisms underlying the symptom development in the allergic patient. This article will touch upon some of the possibilities that exist today that allow us to specifically measure various inflammatory components important for this development. Signs and symptoms of allergy are direct expressions of the activity of various inflammatory cells in the lung, nose, skin etc. The allergic inflammation involves a great number of cells; however, three cells seem to be of particular importance. These are the eosinophil granulocyte, the mast cell and the T-lymphocyte of the Th2-type (2–4). The eosinophils and mast cells are the major effector cells, whereas the Th2-cell seems to be the director of the process. The major consequences of the activation of mast cells are the release of histamine and other mediators responsible for the acute allergic reaction. Activation of eosinophils gives rise to the extracellular release of a number of potent cytotoxic proteins such as eosinophil cationic protein (ECP) (5), which are believed to play a role in the development of subacute and chronic symptoms of allergy. Other cells that may play major roles are neutrophil granulocytes, macrophages, epithelial cells and endothelial cells. Treatment of inflammatory diseases such as the allergic diseases is today often quite stereotyped based on national or international recommendations. In most cases the treatment works well, however, in many cases it is quite obvious that the treatment is less successful. The variability in treatment success has many reasons, but one important reason may be the fact that we do not treat our patients based on the knowledge of the underlying mechanisms of the individual patient. Thus, it is obvious that there is a great inter-individual variation as to the inflammatory cells and mediators that are involved in the disease process (6–8). Modern treatment of inflammatory disease should therefore not only consider the disease entity and symptoms, but also the underlying pathophysiology in order to be optimal to the patient (9).

Monitoring of inflammation

How can we best read the inflammatory process? When it comes to allergic diseases we know since long that we cannot rely on classic assays such as the erythrocyte sedimentation rate, plasma protein electrophoresis, C-reactive protein (CRP) or other so-called acute phase reactants, as the cytokines of allergic inflammation do not induce plasma protein synthesis by the liver. Making blood cell counts and differentials are other classic assays and we teach our students that the numbers of blood eosinophils typically are raised in allergy and even to some extent reflect the severity of disease such as in asthma (10). Counting the number of cells in the blood, although, provides us with limited understanding of the

extent to which the cells are involved in the local process nor does it provide us with any understanding of the activity of the cells, i.e. to what extent they have been affected by various mediators in the process, i.e. priming, or to what extent they are prone to release their own mediators. In order to be able to correctly evaluate the participation of the individual inflammatory cells we need to know, which are the participating cells and to what extent do they participate. During the last decades we and others have tried to identify secretory molecules of individual inflammatory cells and develop sensitive assays for these molecules with the aim of being able to measure the presence and activity of such individual inflammatory cells in inflammatory processes (11–16). Important in this development is the identification of unique molecules that are specific markers of individual inflammatory cells, i.e. when we see changes in the levels of the markers then they are signals of changes in the turnover and activity of the respective cell. Some of the markers that may currently be used to identify the activity and turnover of individual inflammatory cells are listed in Table 1. Some of these markers, although, are not entirely specific and may be produced to some extent by some other cells as well (16, 17). Among the products of eosinophils ECP (18) and eosinophil peroxidase (EPO) are the most cell-specific (19), as EPX/EDN (eosinophil protein X/eosinophil derived neurotoxin) is also produced in small amounts by neutrophils (20). Myeloperoxidase (MPO) and elastase are also produced in small amounts by monocytes (21–23). Lactoferrin is a unique marker of the neutrophil granulocyte when assayed in blood (24). In other body fluids it is not useful as a neutrophil marker, as exocrine glands are major producers of lactoferrin (25). Human neutrophil lipocalin (HNL) on the other hand seems to be an entirely specific marker of neutrophils (19, 26, 27). When it comes to monocytes/macrophages there is no unique marker available today. Lysozyme is also produced by neutrophils and when one measures lysozyme in blood the results may be difficult to interpret although roughly 90% of lysozyme in blood is derived from monocytes/macrophages (28–31). In other body fluids lysozyme is unsuitable as a monocyte/macrophage marker because of the secretion from exocrine glands (29). An alternative to lysozyme as a marker of monocyte/macrophage activity may the measurement of some cytokines in particular interleukin (IL)-6, as monocytes are major producers of this cytokine (32).The markers listed in Table 1 and illustrated in Fig. 1 have been analysed in allergic subjects as indices of inflammatory activity in several different body fluids (Table 2). It is a truism to say that measurement in local fluids better reflect local processes than measurements in blood. Measurements in blood, especially in serum, put special requirements on the handling of blood in order not to obtain false levels because of leakage from the leucocytes (33). The advantages with blood measurements, although, are the availability and sampling simplicity and as will be discussed below blood measurements may under certain circumstances provide us with important and useful information as to the inflammatory process. In addition to the cell-specific markers shown in Table 1, it is possible to measure a number of other inflammatory markers. These markers are among others the hormones of inflammation, i.e. cytokines and chemokines. Cytokines and chemokines are in general produced by many different cells and can therefore not be used to monitor the activity and turnover of individual cell populations. Alterations in the levels of cytokines and chemokines on the contrary may provide information of the mechanisms that are involved in the regulation of a particular inflammatory process. One dream is that we by means of the measurement of a panel of cytokines and chemokines will obtain individual profiles, which may help us classify and understand the process in addition to the knowledge we obtain by the measurement of the participation of individual cells. In allergic conditions the cytokines that reflect the Th2-driven process are of particular interest such as IL-4 and -5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (34). The assays which are commercially available today for the measurement of these cytokines are in most cases too insensitive and are only used for research purposes. Of chemokines eotaxin, IL-8, regulated on activation, normal T-cell expressed and secreted (RANTES) and others are of interest, but with the exception of IL-8, for which good assays are available (35, 36), the measurements of chemokines have limited clinical interest. Another inflammation marker, which is produced by many cells and is increasingly used as a tool to reflect inflammation in the airways is nitric oxide (NO) in exhaled air (37–40). Its position in the clinical routine is still uncertain mostly because of methodological problems and difficulties in the interpretation of the results (40–42).

Table 1. Inflammatory cells and some of their secretory products that may be used as markers of their activity and turnover

Eosinophils
Eosinophil cationic protein (ECP)*
Eosinophil peroxidase (EPO)*
Eosinophil protein X/eosinophil derived neurotoxin (EPX/EDN)
Mast cells
Tryptase*
Neutrophils
Elastase
Human neutrophil lipocalin (HNL)*
Lactoferrin
Myeloperoxidase (MPO)
Monocytes/macrophages
Lysozyme
Interleukin (IL)-6
T-lymphocytes
sIL2r
Endothelial cells
E-selectin*

Unique and cell-specific markers are marked with asterisk.

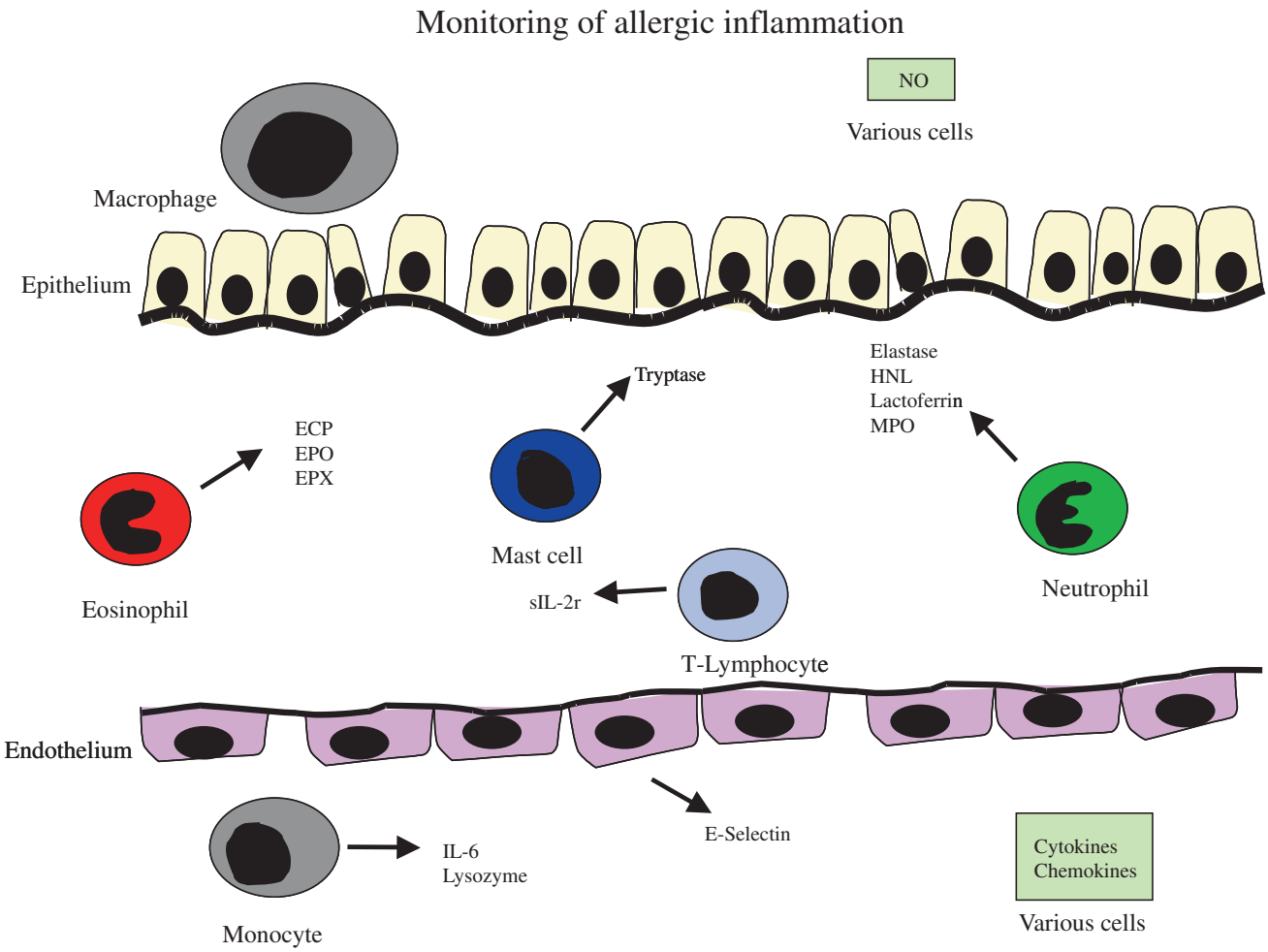

Figure 1. The figure illustrates some of the cells involved in the allergic inflammation and their secretory proteins that may be used to monitor these cells. It is also indicated that markers such as nitric oxide (NO) and cytokines and chemokines also may be measured as a reflection of the activity of the allergic inflammation although these compounds are not cell-specific, but produced by and secreted from many different cells.

Table 2. Biological fluids in which changes in inflammatory cell markers have been measured in allergic conditions

Serum
Plasma
Broncho-alveolar lavage fluid (BAL)
Nasal secretions
Tear fluid
Sputum
Urine
Skin blisters
Faeces

Monitoring eosinophil activity and turnover

The marker that so far has become most widely used in the everyday clinical routine of the allergist is ECP (5). Eosinophil cationic protein is an indicator of the activity and turnover of the eosinophil granulocyte. Currently it is measured in serum/plasma, but measurement in sputum seems to be an interesting alternative as ECP in sputum more accurately reflects the local process. Below I will discuss some of the advantages and disadvantages of measuring ECP in serum/plasma and sputum and also discuss the current evidence that ECP may be a useful addition to the diagnostic armamentarium for monitoring and characterization of disease activity in the allergic patient. I will also discuss the emerging evidence that seems to show that urine measurement of EPX/EDN may be an alternative to ECP measurements in blood as a reflection of eosinophil turnover and activity (43–49).

ECP in sputum and other secretions

There is an overwhelming literature (5) that shows that it is possible to measure reproducibly cells and mediators in

sputum and that sputum measurements have the potential of becoming clinical instruments for the characterization and monitoring of inflammatory processes in the airways (50, 51). This in particular has been shown for patients with asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis (8, 9, 19, 50, 52–69). The greatest problem with sputum measurement is to obtain sputum, as sputum production has to be induced in those subjects who are not spontaneous producers. When one collects sputum for the analysis of cells and mediators it has to be decided what the aim is. In order to analyse mediators released from inflammatory cells one needs to separate cells in the sputum from the supernatant, which is a relatively complicated procedure (51). If the only interest is the cellular composition of sputum you can either make smears and stain sputum in order to count the cells and their relative proportions or extract the entire sputum sample and measure the content of any cell-specific marker in the extract as an index of the number of the particular cell. The preparation of sputum for cell counting is technically difficult and only works well in laboratories with great experience. Extraction of the whole sputum sample and the measurement of its content of cell-specific markers have been proposed as a clinical routine assay because of its simplicity. The numbers of eosinophil granulocytes in sputum have been estimated in this way by the measurement of ECP in the extract and there is a large number of publications showing that the numbers of eosinophils measured in this way are well correlated to disease activity in asthma and how the numbers are reduced as a consequence of corticosteroid treatment. In patients with COPD it has been shown that treatment success with corticosteroids is related to the eosinophilic inflammation in the bronchi as measured by sputum ECP (60, 70). An interesting alternative to sputum could be the measurement in saliva, as we showed recently that asthmatics had significantly raised levels of ECP in saliva and that the levels were reduced by corticosteroid treatment (71). Still, however, we do not know what the ECP levels in saliva actually reflect. That is, do they reflect systemic or local eosinophil activity? Also the measurements of specific cell markers in nasal (72, 73) or ear secretions (74–76) have been performed quite extensively and clearly indicated the usefulness of such measurements in the understanding of cellular involvement. However, the clinical application is still not established.

ECP in serum/plasma

Eosinophil cationic protein may be measured in both serum and plasma (5, 33). If plasma is chosen the blood should be anticoagulated with ethylenediaminetetraacetic acid (EDTA) or citrate in order to prevent spontaneous extracellular release of ECP and interaction with heparin (33, 77). The levels of ECP in serum are consistently higher than in plasma, which is due to the fact that eosinophils in the test tube continue their extracellular release of ECP. This is an active process, i.e. both time- and temperature-dependent, which means that higher extracellular levels are achieved by time and at higher ambient temperature or vice versa. So, if one chooses to measure ECP in serum, strict standardization of the blood sampling procedure and the handling of the blood sample are vital in order to avoid artificial and unacceptable variations in the ECP levels. Our recommendation is that blood is taken in tubes with gel separator and that coagulation is allowed for 1 h at room temperature (+22°C) before centrifugation and separation of serum. Both plastic and glass tubes may be used. However, differences in the material and the inclusion of coagulation activators in the tubes may affect the levels. This means that normal ranges of ECP have to be prepared at each laboratory that does not follow the recommendation of the manufacturer. The levels of ECP in EDTA-plasma probably correctly reflect the circulating levels of ECP at the time of blood sampling. These levels are the consequences of production and elimination of ECP, i.e. local or systemic release of ECP to the circulation as well as variations in the turnover rate of ECP. Normally turnover is quite rapid with a $t_{1/2}$ of about 45 min (78). For several reasons we can assume that the turnover is more rapid in subjects with ongoing inflammation. This means that an increased release of ECP to the circulation in certain diseases does not always lead to the anticipated increase in plasma levels, as the increased release may be partly or fully counteracted by an increased elimination rate. This dynamics of counteracting principles may be the main explanation of the fact that the clinical information that is obtained by EDTA-plasma measurements of ECP in most cases is much less clear and useful than the information obtained by serum measurements. The serum levels of ECP reflect in addition to the circulating levels also the secretory activity of the eosinophil population in the blood and as the levels in serum are often five to 10 times those in plasma we may draw the conclusion that it is above all the secretory activity of the eosinophils that determines the levels. The secretory activity on the other hand is the result of the concentration of eosinophils in the blood and the propensity of the eosinophils to release ECP. My own interpretation of serum ECP levels is that they reflect the propensity of the eosinophil population to release ECP in the local process, e.g. in the lung of asthmatics. The higher this propensity the more damage is inflicted on the patient.

Almost thousand papers have been published dealing with the relation between ECP levels and allergic or other inflammatory diseases (5). The majority of these publications have shown that ECP provides novel information about the process and that the information may be used in the monitoring and treatment of disease, as the ECP levels are closely related to exacerbation propensity and

severity in diseases such as asthma, atopic dermatitis. Several publications, although, have questioned and sometimes rejected ECP as a clinically useful marker. In many of the latter publications the authors have committed themselves to gross oversimplifications and sometimes serious misconceptions that partly are based on the simplified view that asthma is one disease and that the disease is caused by one cell, i.e. the eosinophil. This is obviously a reductionist view of the problem where one hopes that one marker will solve all clinical problems. As mentioned above we know today that the involvement of eosinophils in the asthmatic process is very variable between individuals (6) and the only thing we can hope to achieve by the measurements of ECP is to get a picture of the extent to which this particular cell is participating in the process. Another reason to the variations in the results is probably the unawareness of the importance of correct sample handling (33). Another possibility may be related to the new knowledge of several genetic variants of ECP and the fact that these variants are closely related to the expression of allergic symptoms (79).

EPX/EDN in urine

In a search for noninvasive means to monitor eosinophil involving inflammation urine measurement of EPX/EDN has emerged as a promising candidate in particular in children (44, 45, 48, 64). In order to minimize the influence of differences in water dilution of the urine the EPX/EDN levels have to be adjusted by the creatinine concentrations unless 24 h samplings are carried out. It is also useful to bear in mind that all eosinophil markers including the excretion of EPX/EDN show circadian rhythms with the highest levels occurring at night (80). Thus, for the sake of standardization blood or urine measurements of eosinophil markers sampling should be carried out at the same time of the day. Several studies have shown that EPX/EDN in urine is elevated in atopic dermatitis (47) and asthma and also shown reductions in relation to anti-inflammatory treatment, i.e. corticosteroid treatment (44). Elevated levels of EPX/EDN in urine in wheeze children also seem to predict the development of asthma (49).

References

1. Bousquet J, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I et al. Eosinophilic inflammation in asthma. N Engl J Med 1990;**323**: 1033–1039.
2. Holgate ST. The epidemic of allergy and asthma. Nature 1999;**402**:B2–B4.
3. Fireman P. Understanding asthma pathophysiology. Allergy Asthma Proc 2003;**24**:79–83.
4. Busse WW, Rosenwasser LJ. Mechanisms of asthma. J Allergy Clin Immunol 2003;**111**:S799–S804.
5. Venge P, Byström J, Carlson M, Håkansson L, Karawacjzyk M, Peterson C et al. Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. Clin Exp Allergy 1999;**29**: 1172–1186.
6. Amin K, Ludviksdottir D, Janson C, Nettelbladt O, Björnsson E, Roomans GM et al. Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. Am J Respir Crit Care Med 2000;**162**:2295–2301.
7. Amin K, Rinne J, Haahtela T, Simola M, Peterson CG, Roomans GM et al. Inflammatory cell and epithelial characteristics of perennial allergic and nonallergic rhinitis with a symptom history of 1 to 3 years' duration. J Allergy Clin Immunol 2001;**107**:249–257.
8. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma: evidence of neutrophilic inflammation and increased sputum interleukin-8. Chest 2001;**119**:1329–1336.
9. Haahtela T. Assessing airway inflammation: from guessing to quantitative measurements. Ann Med 2002;**34**:74–76.
10. Dahl R, Venge P, Fredens K. Eosinophils. In: Barnes PJ, Rodger IW, Thomson NCC, editors. Asthma. Basic mechanisms and clinical management. London: Academic Press, 1992:111–124.
11. Ackerman SJ, Loegering DA, Venge P, Olsson I, Harley JB, Fauci AS et al. Distinctive cationic proteins of the human eosinophil granule: major basic protein, eosinophil cationic protein and eosinophil-derived neurotoxin. J Immunol 1983;**131**:2977–2982.
12. Venge P. The monitoring of inflammation by specific cellular markers. Scand J Clin Lab Invest Suppl 1994;**219**:47–54.
13. Venge P, Roxin L-E, Olsson I. Radioimmunoassay of human eosinophil cationic protein. Br J Haematol 1977; **37**:331–335.
14. Venge P. Cell mediators as diagnostic tests in allergy. In: Wüthrich B, editor. Highlights in allergy and clinical immunology. Seattle, Toronto, Bern, Göttingen: Hoegrefe & Huber Publishers, 1992:279–284.
15. Schwartz LB, Yunginger JW, Miller J, Bokhari R, Dull D. Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. J Clin Invest 1989;**83**: 1551–1555.
16. Matsson P, Ahlstedt S, Venge P, Thorell J. Clinical impact of the monitoring of allergic inflammation. London: Academic Press Limited, 1991.
17. Venge P. Soluble markers of allergic inflammation. Allergy 1994;**5**:128–134.
18. Byström J, Garcia RC, Håkansson L, Karawajczyk M, Moberg L, Soukka J et al. Eosinophil cationic protein is stored in, but not produced by, peripheral blood neutrophils. Clin Exp Allergy 2002;**32**:1082–1091.
19. Metso T, Venge P, Haahtela T, Peterson CG, Seveus L. Cell specific markers for eosinophils and neutrophils in sputum and bronchoalveolar lavage fluid of patients with respiratory conditions and healthy subjects. Thorax 2002;**57**:449–451.
20. Abu Ghazaleh RI, Dunnette SL, Loegering DA, Checkel JL, Kita H, Thomas LL et al. Eosinophil granule proteins in peripheral blood granulocytes. J Leukocyte Biol 1992;**52**:611–618.

21. Breton-Gorius J, Coquin Y, Guichard J. Cytochemical distinction between azurophils and catalase-containing granules in leukocytes. I. Studies in developing neutrophils and monocytes from patients with myeloperoxidase deficiency: comparison with peroxidase-deficient chicken heterophils. Lab Invest 1978;**38**:21–31.
22. Lehrer RI. The fungicidal mechanisms of human monocytes. I. Evidence for myeloperoxidase-linked and myeloperoxidase-independent candidacidal mechanisms. J Clin Invest 1975;**55**:338–346.
23. Kargi HA, Campbell EJ, Kuhn C. III. Elastase and cathepsin G of human monocytes: heterogeneity and subcellular localization to peroxidase-positive granules. J Histochem Cytochem 1990;**38**:1179–1186.
24. Bennett RM, Kokocinski T. Lactoferrin content of peripheral blood cells. Br J Haematol 1978;**39**:509–521.
25. Figarella C, Sarles H. Lactoferrin, a protein of human pancreatic external secretion. Scand J Gastroenterol 1975;**10**:449–451.
26. Xu S, Venge P. Lipocalins as biochemical markers of disease. Biochim Biophys Acta 2000;**1482**:298–307.
27. Seveus L, Amin K, Peterson CG, Roomans GM, Venge P. Human neutrophil lipocalin (HNL) is a specific granule constituent of the neutrophil granulocyte. Studies in bronchial and lung parenchymal tissue and peripheral blood cells. Histochem Cell Biol 1997;**107**:423–432.
28. Hansen NE, Karle H. Elevated plasma lysozyme in Hodgkin's disease. An indicator of increased macrophage activity. Scand J Haematol 1979;**22**:173–178.
29. Konstan MW, Chen PW, Sherman JM, Thomassen MJ, Wood RE, Boat TF. Human lung lysozyme: sources and properties. Am Rev Respir Dis 1981;**123**:120–124.
30. Torsteinsdottir I, Håkansson L, Hällgren R, Gudbjörnsson B, Arvidson NG, Venge P. Serum lysozyme: a potential marker of monocyte/macrophage activity in rheumatoid arthritis. Rheumatology (Oxford) 1999;**38**: 1249–1254.
31. Venge P. A critical look at blood samples in CB and COAD. In: Persson CGA, Brattsand R, Laitinen LA, Venge P, editors. Inflammatory indices in chronic bronchitis. Basel, Boston, Berlin: Birkauser Verlag, 1990:109–120.
32. Fuller GM, Bunzel RJ, Woloski BM, Nham SU. Isolation of hepatocyte stimulating factor from human monocytes. Biochem Biophys Res Commun 1987;**144**:1003–1009.
33. Björk A, Venge P, Peterson CG. Measurements of ECP in serum and the impact of plasma coagulation. Allergy 2000;**55**:442–448.
34. Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T cells, increased T_{H2}-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. J Allergy Clin Immunol 1993;**92**: 313–324.
35. Kanazawa H, Kurihara N, Otsuka T, Fujii T, Tanaka S, Kudoh S et al. Clinical significance of serum concentration of interleukin-8 in patients with bronchial asthma or chronic pulmonary emphysema. Respiration 1996;**63**: 236–240.
36. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor-α in induced sputum from patients with chronic obstructive pulmonary disease or asthma. Am J Respir Crit Care Med 1996;**153**:530–534.
37. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. Eur Respir J 1993;**6**:1368–1370.
38. Lundberg JO, Weitzberg E, Lundberg JM, Alving K. Nitric oxide in exhaled air. Eur Respir J 1996;**9**:2671–2680.
39. Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. Lancet 1994;**343**:133–135.
40. Kharitonov SA, Barnes PJ. Biomarkers of some pulmonary diseases in exhaled breath. Biomarkers 2002;**7**:1–32.
41. Pedroletti C, Zetterquist W, Nordvall L, Alving K. Evaluation of exhaled nitric oxide in schoolchildren at different exhalation flow rates. Pediatr Res 2002;**52**:393–398.
42. Paredi P, Kharitonov SA, Barnes PJ. Analysis of expired air for oxidation products. Am J Respir Crit Care Med 2002;**166**:S31–S37.
43. Reimert CM, Ouma JH, Mwanje MT, Magak P, Poulsen LK, Vennervald BJ et al. Indirect assessment of eosinophiluria in urinary schistosomiasis using eosinophil cationic protein (ECP) and eosinophil protein X (EPX). Acta Trop 1993;**54**:1–12.
44. Kristjánsson S, Strannegård IL, Strannegård Q, Peterson C, Enander I, Wennergren G. Urinary eosinophil protein X in children with atopic asthma: a useful marker of antiinflammatory treatment. J Allergy Clin Immunol 1996;**97**:1179–1187.
45. Wennergren G. Inflammatory mediators in blood and urine. Paediatr Respir Rev 2000;**1**:259–265.
46. Tauber E, Halmerbauer G, Frischer T, Gartner C, Horak F Jr, Veiter A et al. Urinary eosinophil protein X in children: the relationship to asthma and atopy and normal values. Allergy 2000;**55**:647–652.
47. Breuer K, Kapp A, Werfel T. Urine eosinophil protein X (EPX) is an *in vitro* parameter of inflammation in atopic dermatitis of the adult age. Allergy 2001;**56**:780–784.
48. Labbe A, Aublet-Cuvelier B, Jouaville L, Beaugeon G, Fiani L, Petit I et al. Prospective longitudinal study of urinary eosinophil protein X in children with asthma and chronic cough. Pediatr Pulmonol 2001;**31**:354–362.
49. Oymar K. High levels of urinary eosinophil protein X in young asthmatic children predict persistent atopic asthma. Pediatr Allergy Immunol 2001;**12**:312–317.
50. Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargreave FE et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax 1992;**47**:25–29.
51. Kelly MM, Keatings V, Leigh R, Peterson C, Shute J, Venge P et al. Analysis of fluid-phase mediators. Eur Respir J Suppl 2002;**37**:24s–39s.
52. Pin I, Freitag AP, O'Byrne PM, Girgis-Gabardo A, Watson RM, Dolovich J et al. Changes in the cellular profile of induced sputum after allergen-induced asthmatic responses. Am Rev Respir Dis 1992;**145**:1265–1269.
53. Virchow JC Jr, Kroegel C, Hage U, Kortsik C, Matthys H, Werner P. Comparison of sputum-ECP levels in bronchial asthma and chronic bronchitis. Allergy 1993;**48**:112–118.
54. Fahy JV, Liu J, Wong H, Boushey HA. Analysis of cellular and biochemical constituents of induced sputum after allergen challenge: a method for studying allergic airway inflammation. J Allergy Clin Immunol 1994;93:1031–1039.
55. Koller DY, Urbanek R, Götz M. Increased degranulation of eosinophil and neutrophil granulocytes in cystic fibrosis. Am J Respir Crit Care Med 1995;**152**:629–633.
56. Pizzichini E, Pizzichini MMM, Efthimiadis A, Evans S, Morris MM, Squillace D et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. Am J Respir Crit Care Med 1996;**154**:308–317.

57. Pizzichini MMM, Pizzichini E, Clelland L, Efthimiadis A, Mahony J, Dolovich J et al. Sputum in severe exacerbations of asthma – kinetics of inflammatory indices after prednisone treatment. Am J Respir Crit Care Med 1997;**155**:1501–1508.
58. Koller DY, Nething I, Otto J, Urbanek R, Eichler I. Cytokine concentrations in sputum from patients with cystic fibrosis and their relation to eosinophil activity. Am J Respir Crit Care Med 1997;**155**:1050–1054.
59. Keatings VM, Barnes PJ. Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. Am J Respir Crit Care Med 1997;**155**:449–453.
60. Keatings VM, Jatakanon A, Worsdell YM, Barnes PJ. Effects of inhaled and oral glucocorticoids on inflammatory indices in asthma and COPD. Am J Respir Crit Care Med 1997;**155**: 542–548.
61. Fujimoto K, Kubo K, Matsuzawa Y, Sekiguchi M. Eosinophil cationic protein levels in induced sputum correlate with the severity of bronchial asthma. Chest 1997;**112**:1241–1247.
62. Sorva R, Metso T, Turpeinen M, Juntunen-Backman K, Björksten F, Haahtela T. Eosinophil cationic protein in induced sputum as a marker of inflammation in asthmatic children. Pediatr Allergy Immunol 1997;**8**:45–50.
63. Shoji S, Kanazawa H, Hirata K, Kurihara N, Yoshikawa J. Clinical implication of protein levels of IL-5 in induced sputum in asthmatic patients. J Asthma 1998;**35**:243–249.
64. Koller DY, Nilsson M, Enander I, Venge P, Eichler I. Serum eosinophil cationic protein, eosinophil protein X and eosinophil peroxidase in relation to pulmonary function in cystic fibrosis. Clin Exp Allergy 1998;**28**:241–248.
65. Dahlen I, Janson C, Björnsson E, Stålenheim G, Peterson CG, Venge P. Inflammatory markers in acute exacerbations of obstructive pulmonary disease: predictive value in relation to smoking history. Respir Med 1999;**93**:744–751.
66. Hargreave FE, Leigh R. Induced sputum, eosinophilic bronchitis, and chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999;**160**: S53–S57.
67. Maestrelli P, Richeldi L, Moretti M, Fabbri LM. Analysis of sputum in COPD. Thorax 2001;**56**:420–422.
68. Woodruff PG, Khashayar R, Lazarus SC, Janson S, Avila P, Boushey HA et al. Relationship between airway inflammation, hyperresponsiveness, and obstruction in asthma. J Allergy Clin Immunol 2001;**108**:753–758.
69. Wilson NM, James A, Uasuf C, Payne DN, Hablas H, Agrofioti C et al. Asthma severity and inflammation markers in children. Pediatr Allergy Immunol 2001;**12**:125–132.
70. Dahlen I, Janson C, Björnsson E, Stålenheim G, Peterson CG, Venge P. Changes in inflammatory markers following treatment of acute exacerbations of obstructive pulmonary disease. Respir Med 2001;**95**:891–897.
71. Schmekel B, Ahlner J, Malmstrom M, Venge P. Eosinophil cationic protein (ECP) in saliva: a new marker of disease activity in bronchial asthma. Respir Med 2001;**95**:670–675.
72. Rasp G, Hochstrasser K. Tryptase in nasal fluid is a useful marker of allergic rhinitis. Allergy 1993;**48**:72–74.
73. Rasp G, Thomas PA, Bujia J. Eosinophil inflammation of the nasal mucosa in allergic and non-allergic rhinitis measured by eosinophil cationic protein levels in native nasal fluid and serum. Clin Exp Allergy 1994;**24**:1151–1156.
74. Hurst DS, Venge P. Levels of eosinophil cationic protein and myeloperoxidase from chronic middle ear effusion in patients with allergy and/or acute infection. Otolaryngol Head Neck Surg 1996;**114**:531–544.
75. Hurst DS, Amin K, Seveus L, Venge P. Mast cells and tryptase in the middle ear of children with otitis media with effusion. Int J Pediatr Otorhinolaryngol 1999;**49**:S315–S319.
76. Hurst DS, Venge P. Evidence of eosinophil, neutrophil, and mast-cell mediators in the effusion of OME patients with and without atopy. Allergy 2000;**55**: 435–441.
77. Reimert CM, Poulsen LK, Bindslev-Jensen C, Kharazmi A, Bendtzen K. Measurement of eosinophil cationic protein (ECP) and eosinophil protein X/ eosinophil derived neurotoxin (EPX/ EDN). J Immunol Methods 1993;**166**:183–190.
78. Venge P, Nilsson L, Nyström S-O, Åberg T. Serum and plasma measurements of neutrophil granule proteins during cardiopulmonary bypass. A model to estimate human turnover of lactoferrin and myeloperoxidase. Eur J Haematol 1987;**39**:339–345.
79. Jönsson UB, Byström J, Stålenheim G, Venge P. Polymorphism of the eosinophil cationic protein-gene is related to the expression of allergic symptoms. Clin Exp Allergy 2002;**32**:1092–1095.
80. Wolthers OD, Heuck C. Circadian variations in serum eosinophil cationic protein, and serum and urine eosinophil protein X. Pediatr Allergy Immunol 2003;**14**:130–133.