

## Review article

# Soluble markers of allergic inflammation

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The conventional way to assess and confirm an increased inflammatory activity in the body is to measure various acute-phase reactants. Of these, the erythrocyte sedimentation rate (ESR) is the most widely used, although there is a clear tendency today to replace the ESR by other more accurate methods such as measurement of the acute-phase proteins fibrinogen, haptoglobin, orosomucoid, and C-reactive protein (CRP). These methods are still indispensable in the estimation of inflammatory activity in large groups of patients. Except in a few cases, however, the measurements of these components provide little information on the type of inflammation and the kind of inflammatory cells involved. Thus, the pathophysiologic information gained from the measurement of these components is very limited. In addition, in many inflammatory diseases, none of these components react, and they are, in these instances, clinically useless. One large group of diseases for which this is particularly obvious is the allergic, of which asthma is the most notable, since asthma is an inflammatory disease which involves such a large organ as the lung.

Over the years, we have developed a number of assays for specific secretory proteins of various inflammatory cells such as the eosinophil, the neutro-

phil, and the monocyte/macrophage (3, 6, 7, 89). In addition, other researchers have developed assays specifically to reflect mast cell activity (3). The ultimate goal of this work is to obtain good, specific markers of most inflammatory cells, since, as we postulate, such markers would provide us with unique tools to estimate inflammatory events in the body more sensitively than those commonly used, and they would also provide us with the tools to define the pathophysiology of the given process. The advantages of such markers are quite obvious in the clinical management of patients with inflammatory disease, but they are most obvious in the management of those diseases for which no objective measures of inflammatory activity are available.

In which fluid should these markers be assayed? That depends wholly on what information one wants to obtain. Of course, the most direct way is to take measurements in local fluids close to the particular inflammatory process; i.e., in bronchoalveolar lavage (BAL) fluid, nasal lavage fluid, etc. (4, 5, 7, 9-12, 21-24, 41, 42, 48-50, 56, 57, 65, 75, 79, 91, 96, 97). Such measurements have been performed extensively and have provided us with important information on the pathophysiology of the inflammatory process. For practical reasons, however, this is sel-

dom possible in clinical practice where one is expected to assess blood or urine. In whatever material these markers are measured, it is of vital importance that the material be prepared with the utmost care to avoid unspecific leakage of the proteins because of maltreatment of the cells present. Thus, most important is the careful separation of the cells from the fluid.

In blood, the markers could be assayed either in plasma (EDTA) or in serum. When measured in plasma, the markers represent the circulating level of the marker. When measured in serum, the levels represent the plasma levels plus what is released from the cells during coagulation *in vitro*. Provided this latter procedure is strictly standardized, the serum levels give information of the activity of the given blood cell population. Thus, when we measure the serum levels, we measure the propensity of the cells to release their proteins as a response to the stimuli generated during the coagulation process. This propensity may be related to the extent to which the cells have been exposed to various priming agents, such as different cytokines, in the circulation. Clinically, the assay in serum of the different markers has usually proved to be the most discriminative and powerful.

## Eosinophil markers

The eosinophil granulocyte contains in its granules four major proteins, as listed in Table 1. These proteins are all released *in vitro* to the extracellular environment upon stimulation of the eosinophil by various secretagogues. These include particles opsonized with immunoglobulins of the IgG or IgA type, or with complement fragments such as C3b (14, 15, 27, 46, 80, 98). But soluble stimuli such as PAF (platelet-activating factor) (51, 52), GM-CSF (granulocyte macrophage-colony stimulating factor) (16), and IL-5 (interleukin-5) (47) also induce the extracellular release of these proteins. Even IgE-mediated stimuli induce the release of some of the proteins (45, 80). Some data indicate that the eosinophil may release its content differentially, depending on the stimulus exposed to the cells. Thus, when the receptors for IgG and C3b are involved preferentially, eosinophil cationic protein (ECP) is released and very little eosinophil peroxidase (EPO). This contrasts with the involvement of the IgE receptor, which preferentially gives rise to EPO release and

very little ECP. When the IgA receptors are involved, however, there is a parallel release of all proteins.

When measured in various body fluids, ECP and EPO are particularly good, specific indicators of eosinophil activity, since the content of these proteins in other cells is negligible. Thus, the content of ECP in a neutrophil granulocyte is less than 1% of the content of an eosinophil (1). Eosinophil protein x/eosinophil-derived neurotoxin (EPX/EDN) may also be present in neutrophils to a very small extent, i.e., 1–2% of the content of an eosinophil. Its identity with the liver ribonuclease (78), however, suggests that EPX/EDN during certain conditions may have other origins when measured in blood. Major basic protein (MBP) is also present in several other cells. These include basophils and certain placenta cells. Thus, during pregnancy, the blood level of MBP is highly elevated (2, 59).

ECP has been measured in local fluids in a number of diseases and has clearly indicated the active involvement of the eosinophil in almost every inflammatory disease process; e.g., rheumatoid arthritis, psoriasis, inflammatory bowel diseases, bacterial meningitis, and adult respiratory distress syndrome (3, 17, 29–32, 34, 54, 60). In these diseases the presence of eosinophils is paralleled by the presence of neutrophils. In allergic disease, however, the increments in ECP are often isolated findings with no parallel increase of neutrophil activity as monitored by myeloperoxidase (MPO). The findings in local fluids are generally reflected in the blood levels. Thus, in the former diseases, we have observed increments in blood in both neutrophil and eosinophil markers, whereas in allergic diseases the increments in neutrophil markers are mostly absent (28). We have also observed a significant correlation between the ECP levels in BAL fluid and those in serum in asthmatic patients, an observation which clearly suggests that the serum levels reflect the local processes (5, 68). From a practical, clinical point of view, this is of interest, since these results justify the use of serum measurements to monitor the activity of the disease process.

Serum measurements of ECP in allergic disease include studies on asthma, rhinoconjunctivitis, and dermatitis. From the studies on asthma, it is concluded that the ECP levels in serum reflect the severity of the disease expressed as either lung function (PEF, FEV<sub>1</sub>), reactivity to histamine (PC<sub>20</sub>), or propensity to develop late asthmatic reaction (LAR) or exercise-induced asthma (28, 69, 83, 84, 86–88, 90). ECP levels may also be used to monitor antiasthma treatment with corticosteroids, and the levels seem to be a useful indicator of treatment efficacy, an indicator which is important as a means to avoid either over- or undertreatment with these drugs.

In one early study, a positive correlation was found

Table 1. Eosinophil granule proteins

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|                                                              |
|--------------------------------------------------------------|
| Eosinophil cationic protein (ECP)                            |
| Eosinophil peroxidase (EPO)                                  |
| Eosinophil protein x/eosinophil-derived neurotoxin (EPX/EDN) |
| Major basic protein (MBP)                                    |

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between the reactivity of the lung after challenge and serum levels of ECP and EPX/EDN, respectively (84, 86). It has even been suggested that serum measurements of ECP or EPX/EDN might be used to predict the occurrence of LAR after allergen inhalation challenge, since serum levels of these two proteins were significantly raised before challenge in those patients who developed a definite LAR but were normal in those who showed no LAR (84). Interestingly, the protein levels were significantly raised also in patients with an equivocal LAR, possibly indicating that ECP and EPX/EDN are more sensitive indicators of the processes going on in the lung than actual clinical indices of activity. In parallel with these findings, a highly significant linear correlation was found between the extent of exercise-induced asthma and serum ECP levels before exercise (90). Thus, one prerequisite for the development of either LAR after allergen challenge or asthma after exercise might be the presence of activated eosinophils in the circulation, as reflected by raised levels of these proteins. Alternatively, these data may indicate that the patients who responded to these challenges already before challenge had a certain degree of pulmonary inflammation in spite of the fact that they were seemingly asymptomatic.

A study of a group of asthmatic children (38) who were on daily treatment with inhaled budesonide showed that this group had elevated serum levels of ECP, despite seemingly efficacious medication. When these children's lung function was monitored closely, it was found that one group of patients did have a slight reduction in PEF, i.e., 80–95% of their optimal PEF, although the doctor and the parents believed that they were receiving efficacious treatment. When the ECP levels were evaluated in these two groups, it was obvious that these levels were much higher in the group with a slight reduction in lung function. Thus, the conclusion to be drawn from this study is that the serum measurement of ECP might be valuable in the evaluation of the treatment efficacy by steroids also in asthmatic children.

At natural allergen exposure, the ECP level in serum increases as a sign of an increased activity of the circulating eosinophil population (15, 69). When patients were treated with hyposensitization, this rise was eliminated in parallel to a reduced need of anti-allergy medication. However, in another group of children that was exposed to mite allergen, the ECP levels increased in spite of seemingly efficient treatment with sodium cromoglycate (Boner & Venge, submitted). In both groups, the production of chemotactic activity was completely inhibited, which may help to explain the seeming paradox. Thus, according to our simplified working hypothesis, two things are required to achieve an accumulation of activated eosinophils and create the necessary conditions for

the development of asthma. One is an activated population of eosinophils, and the other is the production of eosinophil chemotactic activities in the lung, which attract the activated eosinophils. In the hyposensitization study, both these factors are inhibited, whereas in the study on the mite-exposed children, sodium cromoglycate treatment prevented only the production of the chemotactic activities. But since in neither study were the two necessary conditions fully operative, no symptoms developed.

Studies on patients with atopic dermatitis have clearly indicated that ECP measurements may be of clinical value in the evaluation of the disease activity of the patient. This has been reviewed very recently in this journal, and the reader is referred to this review and other studies for further details (19, 43, 44).

## Neutrophil markers

The neutrophil granulocyte contains several granule proteins, which are secreted in the extracellular environment and may therefore serve as markers of neutrophil activity *in vivo* (Table 2) (82). These proteins are contained in different subpopulations of granules and may therefore reflect slightly different aspects of neutrophil activation. Thus, the secondary granule proteins are released much more readily and as a response to much lower secretagogue concentrations than the primary granules and their proteins. A cytoplasmic protein L1, or calprotectin, has also been used as a marker of neutrophil activity (25), but no data exist on comparison of the levels of this marker with the levels of a secretory granule protein.

Two proteins have been widely used as markers of primary granule protein secretion: elastase and MPO. Elastase is measured as its complex with  $\alpha_1$ -proteinase inhibitor, and that may be one reason that elastase levels often show less impressive increments than MPO. Thus, in our experience, MPO is a more

Table 2. Some neutrophil granule proteins

|                           |
|---------------------------|
| <i>Primary granules</i>   |
| Cathepsin G               |
| Elastase                  |
| Proteinase 3              |
| Defensins                 |
| Myeloperoxidase (MPO)     |
| Azurocidin                |
| Lysozyme                  |
| <i>Secondary granules</i> |
| Lactoferrin               |
| 40-kDa protein            |
| Gelatinase                |

sensitive and better marker to monitor primary granule secretion *in vivo* (6, 17, 20, 34, 39, 60–65, 73–75, 93). As a marker of secondary granule secretion, lactoferrin has been used in several studies. Lactoferrin is an even more sensitive marker of neutrophil activation than any of the primary granule proteins. However, lactoferrin suffers from the drawback that measurements are unsuitable in materials other than blood, since lactoferrin, in addition, is produced by most exocrine glands (35, 36, 53, 55, 70, 99). To circumvent this problem, we have recently purified a new protein from the secondary granules of human neutrophils; i.e., the 40-kDa protein, which seems to be entirely specific to the neutrophil (Xu & Venge, submitted). In preliminary clinical studies, the 40-kDa protein showed results very similar to those of lactoferrin in blood. The 40-kDa protein may therefore be an ideal marker of neutrophil activation in any biologic material from man.

Measurements of MPO in local fluid from the lung and from the nose of patients with allergy have shown variable levels with no clear relation to the clinical situation of the patients (11, 50, 57). Overall, the levels are higher the higher in the respiratory tract they are measured. The highest levels are seen in the nose. When measured in the blood of asthmatic patients, the levels of MPO seemed unaltered except in patients who have experienced some kind of infection (Formgren & Venge, unpublished). These data have led to the conclusion that the neutrophil is not a major factor in allergic inflammation and that the measurement of these markers is of value only in the detection of concurrent infections.

## Mast cell markers

Histamine has been used in many studies to reflect mast cell activity. In this regard, however, histamine suffers from the drawback that it is not a specific marker of the mast cell, since the basophil is also a rich source of histamine. When measured in blood, for technical reasons, most histamine may in fact originate from basophils. The mast cells contain in their secretory granules several proteins, some of which have been purified and characterized (Table 3). One of these is tryptase, which seems to be exclusively found in the mast cell (3). Thus, measurements of tryptase in any biologic material signal the activation of the mast cell population. In the lung, the local tryptase levels in some studies on asthma have been shown to relate to the histamine reactiv-

ity, whereas in others no such relationship was discernible (8, 11–13, 81, 97). In adults, no relationship between tryptase levels in BAL fluid and asthma severity was found (9, 11), prompting the conclusion that the mast cell is active in asthmatic inflammation, but is not the cell that actually causes asthma. This contrasted with a study in children where a close relationship was found between tryptase levels and lung function (26). Thus, the mechanisms operative in asthma may differ between adults and children, with a larger contribution of the mast cell in children.

Normal blood and blood from unchallenged allergic patients do not contain measurable levels of tryptase. After allergen challenge, however, considerable increments are sometimes seen. The most impressive tryptase levels, though, are seen in patients with anaphylaxis and mastocytosis (3, 77). As a tool to distinguish between different causes of hypotensive crisis, tryptase measurements seem to be, clinically, a unique and very valuable tool.

## Other soluble markers of allergic inflammation

Assays for most cytokines and soluble adhesion molecules have become available during 1992. Many of these are of great potential interest in the description of allergic inflammation in man. However, few reports have so far been published. The soluble receptor IL-2r has been assayed in BAL fluid and in blood in patients with asthma, as a reflection of T-cell activity (18). The levels were raised in asthmatic patients, as compared with controls, as a sign of involvement of T cells. IL-4, which is believed to regulate IgE production, was shown to be raised in allergic asthma, as opposed to nonallergic (94). Lysozyme, which is a good marker of the activity of the macrophage population, was shown to be correlated to the generation of neutrophil chemotactic activity after allergen challenge (85). These data suggest that the macrophage population is affected by allergen exposure. The involvement of platelets in the allergic type of inflammation is still controversial. However, the measurement of specific marker proteins for this cell, i.e., beta-thromboglobulin and platelet factor IV, has suggested that this cell is indeed activated after allergen challenge of asthmatic patients (40).

IL-5 is probably the most important cytokine in relation to eosinophil growth and activation (71). Several studies indicate that T cells in the asthmatic lung express and secrete raised amounts of IL-5 (37). This may explain the eosinophilia seen in asthmatic patients and the increased propensity of these cells to secrete their granule proteins and to respond to chemotactic and adhesive stimuli (16, 95). Commer-

Table 3. Mast cell granule proteins

|          |
|----------|
| Chymase  |
| Tryptase |

Table 4. Some soluble markers of inflammatory cells in blood

| Marker                                                       | Cellular origin  |
|--------------------------------------------------------------|------------------|
| Eosinophil cationic protein (ECP)                            | Eosinophil       |
| Eosinophil peroxidase (EPO)                                  |                  |
| Eosinophil protein x/eosinophil-derived neurotoxin (EPX/EDN) |                  |
| 40-kDa protein                                               | Neutrophil       |
| Elastase                                                     |                  |
| Lactoferrin                                                  |                  |
| Myeloperoxidase (MPO)                                        |                  |
| Tryptase                                                     | Mast cell        |
| IL-2r                                                        | T lymphocyte     |
| Lysozyme                                                     | Macrophage       |
| Beta <sub>2</sub> -thromboglobulin                           | Platelet         |
| Endothelial-leukocyte adhesion molecule (ELAM-1)             | Endothelial cell |

cial assays for IL-5 will probably soon be available and will be of particular interest as tools in the monitoring of allergic inflammation. Another group of molecules of particular interest to measure in patients with asthma and allergy are the adhesion molecules. In particular, ELAM-1 (endothelial-leukocyte adhesion molecule) and VCAM-1 (vascular cell adhesion molecule) may be of interest, since ELAM-1 seems to be the only specific marker of endothelial cell activity available, and VCAM-1 is involved in the selective accumulation of eosinophils at sites of inflammation (72).

## Conclusion

The concept of evaluating the activity of inflammatory cells, and not just their number, has proved to be a useful approach to monitor any inflammatory process and has required the development of assays for unique secretory proteins of the individual inflammatory cells. This approach not only provides information about the activity of the process in quantitative terms, but also provides useful information on the quality of the process; i.e., on which cells are involved in the specific process. It is postulated that assays which fulfill the requirements of specifically reflecting a given inflammatory cell are of clinical value in the refined monitoring of the treatment of many inflammatory diseases such as the allergic type, including asthma, dermatitis, etc. At the moment, specific markers of eosinophils, mast cells, neutrophils, and platelets are generally available (Table 4). As a marker of T-cell activity, the IL-2r assay may be used, although some IL-2r may originate from other cells as well. Likewise, blood measurement of lysozyme may be used as a marker of the activity of the macrophage population, although a minor part of lysozyme also originates from monocytes and neutrophils. ELAM-1 may be used as a specific

marker of endothelial cell activity. Specific markers of monocytes, basophils, etc. are, however, still lacking. As a complement to these specific cell markers, it is likely that the assay of some cytokines and some other soluble adhesion molecules will provide even deeper understanding of the process, all to the benefit of the management of our patients.

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