

Role of ECP in Monitoring Inflammatory Conditions

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Introduction

Eosinophils appear in large numbers at inflammation sites and in response to certain parasitic infections. Cytoplasmic granules containing positively charged proteins characterize the cells. The granule proteins have pI's greater than 10 and avidly bind acidic dyes, especially the red-orange dye eosin for which the cell is named. (See Figure 1.) Four highly basic proteins—eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), eosinophil peroxidase (EPO), and major basic protein (MBP)—enter surrounding tissues when activated eosinophils degranulate. The granule proteins can kill certain parasites and some mammalian cells, and they might cause the tissue damage associated with asthma and other inflammatory diseases. One of the proteins, ECP (Figure 2), has proven to be useful in monitoring many active inflammatory diseases.¹

Eosinophils participate in the complex regulatory system that mediates inflammatory responses. Originating from bone-marrow stem cells, the mature leukocytes reside mostly in tissues. About one percent of the eosinophil population circulates in the blood. Cytokines interleukin-3 (IL-3), IL-5, and granulocyte macrophage colony stimulating factor (GM-CSF) promote eosinophil development and activation. Chemotactic factors such as eotaxin, leukotrienes, and histamine recruit eosinophils to inflammation sites. Eosinophil activation accompanies a wide range of inflammatory conditions, including bronchial asthma, atopic dermatitis, rhinitis, allergic eye inflammation, allergic middle ear effusion, parasitic and bacterial infections, autoimmune diseases, and chronic fatigue syndrome.¹ Studies show that ECP circulating levels often reflect the status of inflammatory conditions.

ECP Levels Related to Inflammatory Conditions

Eosinophil granule proteins function in the host defense system against parasitic infections by forming lethal outer membrane pores in some

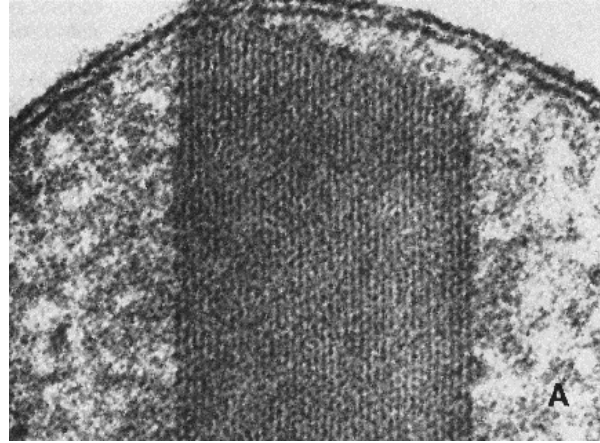


Figure 1. Secondary granules contain four basic proteins: eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), eosinophil peroxidase (EPO), and major basic protein (MBP). MBP forms a dense, crystalline array that appears as a dark block in an electron micrograph. Reproduced from *J Cell Biol* 1966;31:349 by copyright permission of The Rockefeller University Press.



Figure 2. ECP shares high primary sequence homology with EDN and ribonuclease A. All three proteins adopt the same three-dimensional fold, represented by the ribbon diagram based on bovine pancreatic ribonuclease A coordinates. (Diagram based on protein sequence information by Wlodawer et al.²)

parasites. ECP, produced by eosinophils exclusively, is toxic to neurons, some epithelial cell lines and isolated myocardial cells.³ Since only activated eosinophils release granular contents, researchers suspect that circulating levels of these proteins may be more specific markers for active inflammatory conditions than eosinophil counts alone. Although circulating ECP levels can vary widely among patients, some studies point to the utility of serum ECP measurements as an inflammation marker. ECP concentrations in plasma and other body fluids increase during inflammatory reactions marked by activated eosinophils.⁴⁻⁸

ECP in Asthmatic Patients

In industrialized countries, about 5 percent of the population suffers from asthma, and the incidence continues to rise. Asthma costs millions of dollars per year in hospitalization, medication and lost work days; it can be fatal in serious cases. Several mechanisms can combine to generate an asthma attack. Specific IgE antibodies, activated inflammatory cells, neurogenic mechanisms, hyperresponsiveness and individual hormonal imbalances can contribute to this response in varying degrees. Allergic reactions in the lung typically have two phases. In the early phase, occurring within minutes of allergen exposure, mast cells release histamine, tryptase and leukotrienes. During the late phase, typically occurring several hours after exposure, eosinophils accumulate in the bronchus and release their granule proteins, causing bronchial irritability.

The goal in treating asthma is to maintain a relatively symptom-free condition and, above all, to avoid debilitating asthma attacks. Serum ECP levels may provide a useful, objective measure of asthma severity. Physician evaluations of asthma severity and medication requirements often rely on subjective indices reported by patients, including daily symptom scores and use of inhaled asthma medication. These measures are prone to inconsistencies due to variations in investigator and patient assessments. They can also be difficult to obtain, especially in young

children, and most of them imperfectly reflect physiologic alterations involved with asthma. Patient symptoms may subside despite the presence of residual disease in the form of reduced lung capacity and bronchial hyperreactivity. Patients with decreased pulmonary function who remain asymptomatic may later experience shortness of breath caused by severely restricted lung capacity.

Studies point to ECP's clinical utility as an indicator of asthma severity and as a monitor for asthma therapy. Several studies report high individual and group correlations between ECP levels and other indicators of clinical asthma, such as peak expiratory flow (PEF) measurements, airway responsiveness, number of inhaler puffs needed and patient symptom scores.^{4,5} Circulating ECP levels increase in patients after they have been exposed to specific allergens, demonstrating the involvement of ECP in active inflammatory conditions. Atopic serum samples often have higher ECP levels than nonatopic control samples, even when the circulating-eosinophil count remains within the normal range.⁶ Increased ECP levels correspond to symptom onset; in seasonal asthmatic patients, ECP measurements reflected changes in disease activity throughout the year.^{4,6} Roquet et al. reported significant correlations between ECP levels and bronchial hyperreactivity in mildly asthmatic patients.⁷ Tomassini et al. showed that serum ECP concentrations exceeded normal control levels in both IgE-mediated and non-IgE-mediated atopic conditions.⁶

ECP in Inflammatory Skin Conditions

ECP's neuronal toxicity might contribute to itching disorders, and circulating ECP concentrations can indicate the severity of certain skin disorders. Patients with papular erythematous eruptions and prurigo nodularis displayed increased serum ECP, which normalized when the conditions healed.^{3,9} Several groups found that serum ECP concentrations reflected atopic dermatitis (AD) activity.^{8,9} The commonly used clinical scoring system for atopic dermatitis records lichenification, loss of sleep, erythema, papules, pruritus and excoriations. Czech et al.

showed that ECP correlates with each of these symptoms and most highly correlates with the total clinical score.⁸ Although altered immunological parameters such as serum total IgE accompany atopic dermatitis, serum total IgE levels did not show the same degree of correlation with some of the clinical symptoms as did ECP levels.

ECP Assays

Accurate ECP measurements require consistent sample processing procedures to produce measurements of optimal value. Blood sample collection parameters, including the type of collection tube, the time and temperature of blood clotting and centrifugation, and the storage conditions, impact measured ECP concentrations. Eosinophils degranulate and release ECP in vitro by a temperature-dependent mechanism that remains unclear. Blood clotting at temperatures of 37°C causes higher measured ECP levels than clotting at 22°C.¹⁰ EDTA appears to inhibit in vitro eosinophil degranulation and effectively decreases the serum ECP level.

Conclusion

ECP has proven to be useful in monitoring many active inflammatory diseases. It has shown value for assessing asthma severity, monitoring therapy, and indicating the severity of certain inflammatory skin conditions. The measurement of serum ECP presents an advantage over subjective clinical measures, which are prone to inconsistencies due to the broad variability of individual investigator and patient assessments.

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