

## Eosinophilic Cationic Protein (ECP) in Skin Disorders

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Eosinophil cationic protein (ECP) is exclusively secreted only by the eosinophilic leukocyte. In this study the ECP concentration in the serum was measured in patients (n=155) with various skin disorders and compared with the number of circulating eosinophils. The presence of activated eosinophils in the skin was also studied immunohistochemically using the monoclonal antibody EG-2, which recognizes both the eosinophil protein X (EPX/EDN) and ECP. EG-2 distinctly revealed these proteins in the eosinophils and their granules. Non-activated eosinophils were studied with the monoclonal antibody EG-1. In most cases this did not disclose any more eosinophils and often it was located more diffusely and not seldom on collagen fibers.

Elevated serum ECP but normal numbers of circulating eosinophils were found in half of the patients with progressive plaque psoriasis and long-standing daily chronic urticaria. In patients with prurigo nodularis, papular erythematous eruptions, vasculitis, purpura and toxic drug reactions, Wells' syndrome, porphyria cutanea tarda and persistent light reaction the serum ECP was increased, although in some cases the number of circulating eosinophils was normal. In these disorders an increased number of activated eosinophils was found in the skin. Both serum ECP and the number of activated eosinophils normalized when the patients' condition improved. In atopic dermatitis the serum ECP and the number of activated eosinophils in the skin were increased only during exacerbation of the disease.

High serum levels of ECP and activated eosinophils in the skin are frequent findings in many skin disorders in spite of often normal blood eosinophil counts. The specific pathophysiologic role of the eosinophils in these disorders is unknown but the finding of activated eosinophils in itching dermatoses in particular, may suggest a specific pathogenetic role. The measurement of serum ECP may be of clinical value in certain skin disorders.

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The human eosinophil granules contain several basic proteins, including major basic protein (MBP), the eosinophilic cationic protein (ECP), the eosinophilic protein X (the eosinophil-derived neurotoxin) (EPX/EDN) and eosinophil peroxidase (1). Both ECP and EPX/EDN have ribonuclease activity and augment the inflammatory response. Both ECP and EPX/EDN affect nerves and might therefore be involved in itching conditions. The eosinophil, which once was mainly regarded as a scavenger cell cleaning up after the reaction between mast cells and antigens, has now been re-evaluated and is considered to be a more aggressive cell, and it seems to take an active part in certain inflammatory reactions. An increase in the number of eosinophilic leukocytes in the blood and/or dermis is common in several skin disorders, such as atopic dermatitis, pemphigoid, toxic erythema of the newborn, and hypersensitivity reactions (1).

This study was undertaken to assess the diagnostic value of ECP examination in skin disorders. In order further to elucidate the role of ECP, which unlike MBP has only been found in eosinophils, we have compared the number of eosinophils with the levels of circulating ECP in certain disorders and have also studied the presence of secreted ECP and EPX/EDN in the tissues immunohistochemically, using the monoclonal antibody EG-2.

### METHODS

#### *Eosinophil counts*

The total number of eosinophils in the blood was counted by a chamber method (2), but in some cases it was calculated from the total number of white cells and the percentage number of eosinophils obtained by an automated system counting 2000 cells.

#### *ECP concentration in the blood*

ECP was initially assayed by the method described by Venge et al. (3). The range for healthy subjects with this method is 5.5-54 µg/l. More recently the kit for determination of ECP produced by Pharmacia Diagnostics, Uppsala, Sweden has been used, according to the instructions (4). The range for normal values with this method has been

Table I. Number of activated eosinophils (EG-2-positive cells) in the skin (mean from three sections), serum ECP ( $\mu\text{g/l}$ ) and number of circulating eosinophils ( $\text{mm}^3$ ) in various patients. The figures indicate numbers of patients.

Diagnosis	EG-2 positive cells in dermis				ECP		Eosinophils	
	0-3	4-10	11-30	>30	0-16	>16	0-470	>470
Chronic urticaria	8	3	2	2	11	9	20	-
Pressure urticaria	-	-	-	3	2	1	3	-
Cold urticaria	5	-	-	-	5	-	5	-
Dermographic urticaria	4	-	-	-	4	-	-	-
Cholinergic urticaria	2	-	-	-	2	-	2	-
Psoriasis	19	1	1	-	8	8	16	-
Atopic dermatitis	4	1	1	-	3	5	4	4
Papular erythematous eruption	-	2	-	3	-	8	2	6
Nummular eczema	8	1	1	-	4	-	3	1
Prurigo nodularis	-	1	4	3	0	8	2	6
Vasculitis-Purpura	-	1	3	1	-	3	1	2
Toxic reaction to drug	-	1	3	-	-	4	-	4
Wells' syndrome	-	-	-	3	-	3	-	3
Kimura's disease	-	-	-	1	1	0	1	0
Keratosis follicularis	1	-	2	-	1	-	2	1
Subcorneal pustulosis	-	2	-	-	1	1	2	-
Porphy. cut. tarda	0	1	-	-	-	2	1	1
Persistent light react.	-	-	2	-	-	1	-	1
Alopecia areata	4	1	-	-	2	1	3	-
Lichen planus	6	-	-	-	2	-	2	-
Ichthyosis	4	-	-	-	4	-	4	-
Uremia	5	-	-	-	-	-	5	-
UV-B erythema	6	-	-	-	6	-	6	-
Various	20	-	-	-	20	-	20	-
Healthy subjects	10	-	-	-	10	-	10	-

found to be 2-16.0  $\mu\text{g/l}$ . We therefore decreased our initial values by 70% in order to get comparable figures when presenting our results.

#### Skin histology

Punch biopsies (3 mm) were snap frozen and kept at  $-70^\circ\text{C}$  until sectioned for immunohistological examination. The immunoperoxidase technique (PAP) was used as described by Sternberger (5). The monoclonal antibody EG-2 was received from Pharmacia, Uppsala and used in a 1/100 dilution. In a small number of patients we also used the EG-1 antibody (dilution 1/100), which also recognizes non-activated eosinophils in the tissues. The number of cells per section was counted and the mean from three sections was calculated (objective 16; ocular 10). The following grading system for the number of eosinophils was used; occasional = 0-3 eosinophils, some increase 4-10, a clear increase 11-30 and a very marked increase >30 eosinophils per section. Controls without antibody were always performed. These controls were especially important in patients with increased granulocytes. If freshly prepared 0.3% hydrogen peroxide in phosphate buffered saline was not used, the

granulocytes could be non-specifically positive on account of endogenous peroxidase production.

#### Patients

The number of patients investigated and their diagnoses are given in the results and in Table I. Treatment with systemic corticosteroids was withheld one month before the specimens were taken. The following disorders need some special comments.

*Chronic urticaria.* These patients had had almost daily eruptions of wheals for  $\frac{1}{2}$ -10 years. The individual urticarial lesions disappeared within 24 h and also, the patient's sedimentation rate was normal. The biopsy was taken from the most recent lesion. Corticosteroids had been withheld for the last month and antihistamines for the last 3 days.

*Psoriasis.* Most patients had used an emollient or had had no treatment at all when investigated. Some of them had previously been treated with UV-B irradiation. The lesions were of the progressive plaque type, involving 20-50% of



Fig. 1. EG-2 positive eosinophils in chronic urticaria.

the body surface, except in two patients, one whom had pustular and the other generalized erythrodermic lesions. The biopsies were taken in the outer portion of the lesion.

**Papular erythematous eruptions.** This diagnosis is not a recognized entity and the patients are mostly described with other terms in the literature. The clinical signs in these patients were a rather sudden onset (2–8 days) of papules 2–4 mm in diameter and involving 20–50% of the body surface, with severe itching. The papules faded after some weeks, leaving a more diffuse erythema which could persist for several months. Recurrences after 1–3 years were common. An initial diagnosis of non-specific eczema was often made and a history of non-atopic eczema was often found. A conventional formalin-fixed biopsy specimen showed non-specific dermatitis with spongiosis and a mainly lymphocytic infiltrate with an increased number of eosinophils, especially around blood vessels. Follicular involvement was rare. There were no acantholytic features indicating Grover's disease. The general investigation revealed an increased proportion of circulating eosinophils (5–20%) and a slight increase in the total number of white cells (7000–12000/mm<sup>3</sup>). The patients' condition was good despite the fact that the itching interfered with sleep and that antihistamine was needed for sedation at night.

**Wells' syndrome.** The diagnostic criteria for this syndrome were episodes of eosinophilia (20–50%) and infiltrated, sharply demarcated urticaria-like annular lesions in otherwise unaffected patients (6). The biopsy findings were in accordance with those usually described for this condition.

## RESULTS

### *EG-2 and EG-1 monoclonal antibody recognition of eosinophil up-take compared with eosin staining*

When continuous sections from the same patient were stained with eosin, EG-1 and EG-2, the eosinophils were readily revealed by EG-1 and EG-2 in contrast to the findings with eosin. EG-1 not infrequently also showed diffuse staining of the collagen and rarely revealed more eosinophils than EG-2. With EG-2 the eosinophils often appeared elongated and sometimes also as dendritic cells, which with eosin hardly would be recognized as an eosinophil (Fig. 1). Free eosinophil granules around degranulated eosinophils were also observed. The good contrast against other cells probably also accounted for the fact that more eosinophils were detected with EG-2 than with eosin. This was especially evident in patients in whom no or a few eosinophils were found in sections stained with eosin. When there was a marked increase in eosinophils, this was easily detected by both EG-1 and EG-2. The results for EG-2 are shown in Table I, which also gives the serum ECP concentrations and the numbers of circulating eosinophils. Since in some cases both ECP determination and biopsy were not performed, the total numbers of patients examined are not always the same.

### *The urticarias*

All the 20 patients with chronic urticaria had normal eosinophil blood counts, whereas the serum ECP value was increased in 9 and normal in 11 patients (Table I, Fig. 2). All the 9 patients with increased ECP had had daily eruptions for a long time. One of these 9 patients also had a healed IgA nephritis and three also had an increased sensitivity to pressure, although no typical pressure urticaria could be established with various pressure tests. Otherwise no clinical differences were noted between those with high and low ECP values. An increase in EG-2-positive cells in the deep dermis was found in 7 patients with chronic urticaria and 3 with pressure urticaria, of whom 5 also had increased serum ECP. In the other types of urticaria investigated no increase in EG-2 or ECP was found (Table I).

### *Psoriasis*

In 50% of the patients with psoriasis serum ECP was increased, whereas the blood eosinophils were within normal limits (Fig. 2). None of the patients

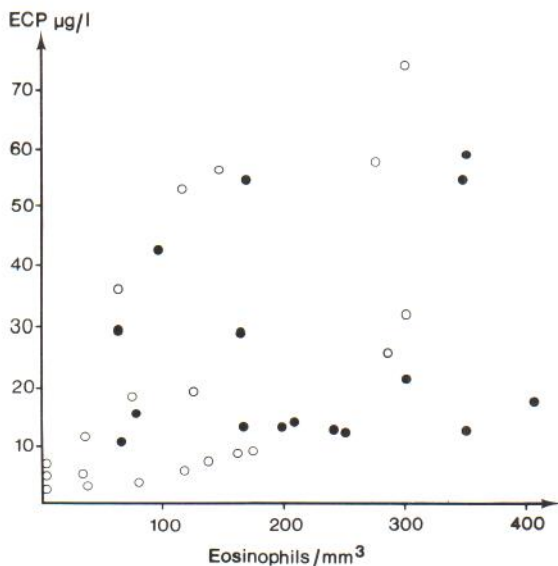


Fig. 2. Serum ECP and number of blood eosinophils in the blood in patients with psoriasis (●) and chronic urticaria (○).

had any signs of atopic disease. In the tissues no or only occasional eosinophils were seen, except in two patients with eosinophils in the dermis and also a few in the epidermis. The biopsies here were taken during an acute flare-up of the disease, with papulopustules. The flare-up in one patient occurred in association with severe infection which was treated with penicillin and in the other a reaction to diltiazem was suspected. Both had a temporary increase in ECP. When their lesions were examined on later occasions, no eosinophils were seen. No eosinophils were found in the skin of a patient with erythrodermic psoriasis.

#### Other disorders

An increase in serum ECP and in the number of eosinophils in the blood and tissue was common in patients with atopic dermatitis, prurigo nodularis, papular erythematous eruption, light dermatosis, vasculitis, toxicodermic reactions to drugs, and Wells' syndrome. Supranormal values of ECP without any increase in eosinophils were found in a few patients with acute conglobate acne, severe aphthae, subcorneal pustulosis (Sneddin-Wilkinson's disease), and erythralgia with swelling of the hands and feet. Normal serum ECP values and eosinophil counts were found in patients with leg ulcer, folliculitis, various non-specific eczemas, Sweet's syndrome, myelodysplastic syndrome, teleangiectasia

macularis persistans, Sjögren's syndrome and ichthyosis.

#### Relationship between circulating human eosinophils and ECP

In some patients with eosinophilia the serum ECP was markedly increased, but in others it was within normal limits. Two patients who repeatedly showed no circulating eosinophils had an elevated serum level of ECP. In both patients an increased number of eosinophils was detected in the bone marrow and in the skin lesions. The relationship between ECP and eosinophils in patients with urticaria and psoriasis is illustrated in Fig. 2 and in those with atopic dermatitis, prurigo nodularis and papular erythematous eruptions in Fig. 3. In these patients those with the more severe and widespread lesions tended to have the highest ECP and the most marked eosinophilia.

#### DISCUSSION

Serum ECP values were increased in about 50 per cent of the patients with chronic urticaria and psoriasis despite normal levels of circulating eosinophils. This indicates that the eosinophils may play a role in the inflammatory mechanism in these disorders. Among patients with chronic urticaria the highest ECP levels were seen in those who had had almost daily eruptions for years, with a history of increased sensitivity to pressure in many cases. Some of these patients and patients with pressure urticaria also showed an increase in ECP in the urticarial lesions (EG-2-positive cells). Such activated, EG-2-positive cells have previously been observed in 12 of 14 patients with severe chronic urticaria (7). Russell Jones et al. studied these 14 patients further on the basis of paraffin-embedded sections and divided them into 3 groups. Group 1 had leukocytoclastic vasculitis, group 2 had a dense perivascular infiltrate, and group 3 had a sparse infiltrate and fulfilled the criteria of urticaria (8). The most marked increase in EG-2-positive cells was seen in the seven patients of group 2, with 4 to 43 such cells per field. Of the four patients with urticaria without vasculitis two had no EG-2-positive cells and the other two patients had 4 and 6 per field, respectively. There was a good correlation between the EG-2-positive cells and the number of eosinophils stained by eosin. Their studies support the concept that urticaria and urticarial vasculitis represent a spectrum of disease

activity rather than separate entities (9). Although our patients had no clinical signs of vasculitis such as an increased sedimentation rate or wheals persisting for more than 24 h, it is possible that those with an increased number of activated eosinophils in the dermis might have had increased vascular damage. Our finding that all the patients diagnosed as vasculitis and purpura showed an increased number of EG-2-positive eosinophils in the dermis would support such a possibility. Eosinophil granule major basic protein (MBP) has also been found in lesions of 12 out of 28 patients with chronic urticaria, in most biopsy specimens from 10 patients with pressure urticaria and in 4 patients with solar urticaria (10, 11, 12). The granular MBP material was mainly dispersed in the dermis and staining was seen on connective tissue fibers. It was not clear whether the MBP material derived from tissue eosinophils or from circulating MBP, which has been found to be elevated in 38% of patients with chronic urticaria (13). Thus the location of MBP in tissues differs from that of ECP, which in urticarial lesions is almost exclusively found in the eosinophils. Taken together, all findings suggest that activated eosinophils often are involved in the pathogenesis of chronic urticaria, pressure urticaria and solar urticaria. We found no increase in EG-2-positive cells or serum ECP in patients with immediate dermatographism, cholinergic urticaria or cold-induced urticaria. In both cholinergic and cold urticaria the release of an eosinophil chemotactic factor (ECF-A) into the circulation has been reported (14 for ref.). ECF-A does not, however, seem to have been able to induce the occurrence of eosinophils in the lesions.

Among patients with psoriasis, we have not identified any special clinical signs in those with increased serum ECP values. They all had progressive plaque-type psoriasis. In two patients with pustular and one with erythrodermic psoriasis the serum ECP was also increased. Our finding of increased ECP in psoriasis confirms an earlier report that serum ECP was above the normal range in five of 35 psoriatic patients (15).

Eosinophils are rarely seen in routine stained sections from lesions of psoriasis. Steigleder & Inderwisch (16) found that eosinophils were rare in the lesions of 35 psoriatic patients (0.04–0.07% of infiltrating cells). Only in two lesions produced by positive patch tests did they find eosinophils (1 and 8%, respectively). In cantharidin-induced blisters raised

on affected and unaffected skin in 44 patients with psoriasis, 0–2% eosinophils were found in 37 patients and 4–7% eosinophils in the other seven, in blisters on both normal and affected skin (17). In the blood the eosinophils were increased in three of the patients. The common migration of polymorphonuclear leukocytes in psoriatic lesions has recently been reviewed by van de Kerkhof & Chang (18). Here eosinophils are not mentioned. With eosin staining Lundin et al. (15) also found just a few eosinophils per section.

With a polyclonal rabbit ECP antibody, using the PAP procedure on paraffin sections, Lundin et al. (15) observed epidermal and dermal deposits of ECP in all 11 patients examined. The most intense deposition of ECP immunoreactivity was found in the upper part of the epidermis in association with granulocytes in lesions from patients with progressive psoriasis. In order to avoid unspecific staining in psoriasis it can, with this method, also be of importance to use hydrogen peroxide dissolved in buffered saline solution to block the endogenous production of peroxidase. With the EG-2 antibody and the APAAP procedure on paraffin sections they observed immunoreactivity in seven of 11 biopsies from lesional skin. In biopsies with no EG-2 reactivity less polymorphonuclear cells were seen. In the present investigation we found no EG-2-positive cells in the lesions except in two patients with a flare-up of the disease. In one of them no activated cells were found either before or after the flare-ups. The EG-2 immunoreactivity demonstrated previously (15) might be due to that these patients had a more active progression of their disease.

The finding of an elevated level of ECP in the serum without an increase in circulating eosinophils or even without any circulating eosinophils at all, indicates that it comes from eosinophils outside the blood. The blood contains only a minor fraction of all eosinophils in the body. The elevated ECP levels may also be a consequence of the activation of circulating eosinophils by various cytokines. Little is known about the factors that increase the production of eosinophils and the release of ECP. Immunological reactions involving T lymphocytes and/or IgE are possible (19). To explain the increase of eosinophils in the skin, Stoughton hypothesized that there is a factor in the epidermis attracting eosinophils into the dermis (20). An increase in eosinophils in the skin and blood is common in several skin disorders. It would be interesting to know

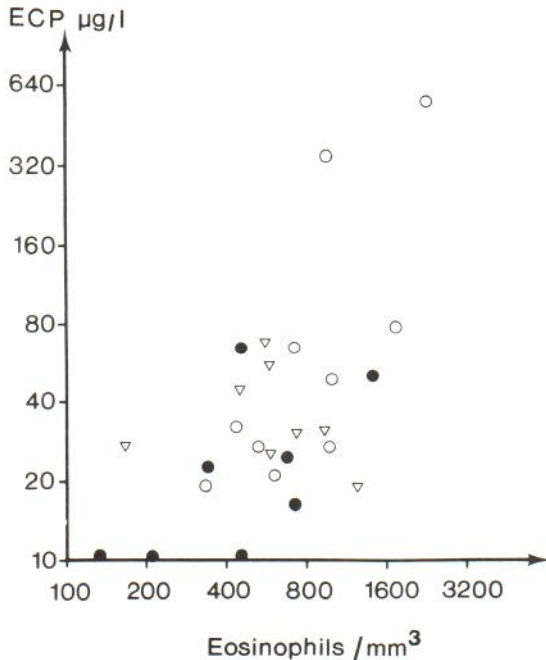


Fig. 3. Serum ECP and number of circulating eosinophils in patient with atopic dermatitis (●), prurigo nodularis (▽) and erythematous eruptions (○).

whether the diseased skin could be involved in the regulation of eosinophil production.

A correlation between the number of circulating eosinophils and the serum level of ECP is best seen in patients with eosinophilia (Fig. 3) and in individual patients when the values are followed week after week. The correlation is especially evident in patients treated with corticosteroids, where the parallel changes are dependent on the dose given.

Itching is a frequent feature in dermatology and is common in dermatoses with an increase in tissue eosinophils. Recent observations suggest the occurrence of a peripheral neuropathy caused by toxic agents derived from eosinophils (21). Since EG-2 recognized both EPX/EDN and ECP, the results from patients with very severe itching disorders, such as prurigo nodularis and papular erythematous eruptions, are of special interest. Here the serum ECP was always markedly increased, but not always the number of circulating eosinophils. In these disorders the EG-2-positive eosinophils in the skin are also increased. Both are normalized when the disorder has healed. As mentioned above under "Patients" the papular erythematous eruption is not a recognized entity. It has some features in common with the papuloerythroderma of Ofuji (22 for ref),

but can occur in women; it resolves within a few months, lacks lymphopenia, has no "deck chair signs" and has less solid flat papules. Atopic dermatitis, contact eczema, mycosis fungoides and eosinophilic folliculitis are other differential diagnoses. Since our patients did not fit into these diagnoses, we used the term papular erythematous eruption, but ECP is always increased in the blood and skin in these cases making it tempting to propose the term "ECP syndrome".

Itching can also be a problem in atopic dermatitis and in this disorder a certain correlation between the severity of the disease and the ECP concentration in the blood and tissue is found. In patients with atopic dermatitis an increased number of activated eosinophils was found in patch test reactions to inhalent allergens (23). In patients with certain itching dermatoses such as lichen planus and in uremia there were no EG-2-positive cells in the skin, although increased serum levels of ECP have been described in uremia (24). The patients with uremia were treated with UV-B irradiation, which might have influenced our results. It is also evident that there are disorders with an increased number of eosinophils in the skin but usually no itching, e.g. Kimura's disease, granuloma faciale, keratosis follicularis, vasculitis and toxicodermia. It therefore seems likely that in several conditions other factors and neuropeptides can be involved in eliciting pruritus.

The cationic proteins ECP and EPX in tissue infiltrations have been found to be related to tissue damage (25). The inflammatory mediators produced by the human eosinophil have recently been reviewed (26). In many internal diseases accompanied by tissue destruction there is an infiltration of eosinophils. Both MBP and ECP have been found to destroy epithelial cells in vitro. The involvement of eosinophils in several inflammatory dermatoses strengthen the concept that these cells have an important function in inflammation. In some of the dermatoses measurement of ECP in the serum and tissues can also be of diagnostic value.

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*Added in proof:* Increased levels of ECP in atopic dermatitis was recently reported (Kapp et al., *J Am Acad Dermatol* 1991; 24: 555-558 and Jakob et al., *Arch Dermatol Res* 1991; 283: 5-6). In the first study also several patients with psoriasis had an increased level of ECP but the increase of their mean value was not significant.