Raised circulating levels of the eosinophil cationic protein in ankylosing spondylitis: relation with the inflammatory activity and the influence of sulphasalazine treatment

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SUMMARY The possibility of eosinophil involvement in ankylosing spondylitis (AS) was investigated by measuring serum levels of eosinophil cationic protein (ECP), a specific granule constituent of eosinophils. In a group of 48 patients with AS we found a threefold increase of the mean serum levels of ECP compared with a reference group ($p < 0.001$). The blood eosinophil counts were similar in patients and controls. A correlation was found between ECP and inflammatory activity defined by erythrocyte sedimentation rate (ESR) and serum haptoglobin. Fifteen patients were studied before and after three months' treatment with sulphasalazine (2–3 g/day). The ECP levels decreased in 13/15 and this paralleled reduction of the acute phase reaction and improvement of clinical parameters. The results point to eosinophil activation as part of the inflammatory process in AS. The signs of reduced eosinophil activation during sulphasalazine treatment suggest either a drug mediated, direct effect on eosinophils or an effect on the inflammatory mechanism stimulating eosinophils.

Key words: acute phase plasma proteins.

Activated neutrophil granulocytes have been suggested as one explanation of the increased disease susceptibility of HLA-B27 positive individuals. A role of the eosinophil has so far not been considered. On the whole the importance of eosinophils in inflammation has been an enigma, but the development of sensitive and specific assays for eosinophil granular constituents has facilitated investigations into the activity and turnover of eosinophils in disease.¹ Recently we directed our attention to the possible pathophysiological role of eosinophils in inflammatory arthritides and reported increased levels of eosinophil cationic protein (ECP), a specific granule protein of the eosinophil, in the circulation and in inflamed joint fluid in rheumatoid arthritis.^{2,3} This study deals with the possible involvement of eosinophils in ankylosing spondylitis (AS) in order to elucidate whether or not signs of eosinophil activation are specific for

rheumatoid arthritis or shared by other chronic inflammatory diseases. As high circulating ECP levels occur in patients with AS we extended the study to include a group of AS patients treated with sulphasalazine. Recently we reported beneficial effects, of 'disease modifying' pattern, in a trial with this drug in AS.⁴ Despite the wide use of sulphasalazine in inflammatory bowel disease and a growing experience in rheumatoid arthritis its mode of action is largely unknown. Therefore, in an attempt to elucidate a possible sulphasalazine influence on eosinophil activation we measured the ECP levels in a group of patients with AS before and after treatment with sulphasalazine.

Patients and methods

PATIENTS

Forty eight patients (13 female, 35 male) with ankylosing spondylitis were included in the study. All patients met the New York criteria of definite disease.⁵ The mean age was 38 (SD 10) years; the mean duration of disease was 11 (SD 7) years. The

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Table 1 Coefficients of correlation between serum ECP, blood eosinophils, inflammatory activity, and serum immunoglobulins in patients with ankylosing spondylitis

	Eosinophils	ESR	Haptoglobin	IgA	IgG
ECP ($\mu\text{g/l}$)	0.52***	0.31*	0.55***	0.12	0.18
Eosinophils $\times 10^6/\text{l}$	—	0.17	0.09	0.01	0.11
ESR (mm/h)	—	—	0.58***	0.54***	0.51***
Haptoglobin (g/l)	—	—	—	0.50***	0.51***
IgA (g/l)	—	—	—	—	0.28
IgG (g/l)	—	—	—	—	—

Statistical significance of the coefficients of correlation: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

control group consisted of apparently healthy volunteers (48 women and 45 men), with a mean age of 37 years. None of the patients or controls had signs or symptoms suggesting parasitic infection. Individuals with symptoms of clinical atopy were excluded from the study.

Fifteen of the patients were studied before and after three months' treatment with sulphasalazine (2–3 g/day). Improvement or worsening of clinical parameters was estimated by measuring duration of morning stiffness, stiffness (visual analogue scale), pain (visual analogue scale), sleep disturbance, thoracic expansion, lumbar motion range (Schober's test), and sacroiliac pain. The patient was considered better if four or more of the parameters measured were improved.

EOSINOPHIL COUNTS AND EOSINOPHIL CATIONIC PROTEIN

Eosinophils were counted according to the method of Forsham *et al.*⁶ The ECP was measured in serum stored at -70°C by the use of a solid phase radioimmunosorbent technique.⁷

SERUM IMMUNOGLOBULINS AND ACUTE PHASE PLASMA PROTEINS

Total IgE levels in serum were measured by a paper radioimmunoassay technique (Pharmacia Diagnostics, Uppsala, Sweden). The median total IgE concentration in 175 healthy controls of the county of Uppsala is 17.0 kU/l (actual range ≤ 5 –163).⁸ Serum levels of IgG and IgA were measured by nephelometry at the department of clinical chemistry, University Hospital, Uppsala; normal ranges are 7–18 and 0.8–4 g/l respectively. Erythrocyte sedimentation rate (ESR) was read after one hour by the Westergren method. The acute phase plasma protein haptoglobin was measured by nephelometry; normal range is 0.3–2.0 g/l.

STATISTICAL ANALYSIS

Student's *t* test was used for statistical analysis unless otherwise stated. The logarithmic values of ECP,

blood eosinophils, and IgE were used when calculating means, comparing groups, and performing correlative analyses. The logarithmic values of these variables and the arithmetic values of the other variables studied were normally distributed. The means are presented as the antilog values of the logarithmic means (SD) or as the arithmetic mean (SD).

Results

The geometric mean serum level of ECP in patients with ankylosing spondylitis was 46 (24–87) $\mu\text{g/l}$, which is a threefold increase compared with the reference group with a mean ECP concentration of 15 (6–42) $\mu\text{g/l}$ ($p<0.001$). The geometric mean value of blood eosinophils in the patients was 155 (82–294) $\times 10^6/\text{l}$. Similar eosinophil counts were seen in the controls (mean value 140 (72–271) $\times 10^6/\text{l}$). In the patients there was a positive correlation between the ECP values and the eosinophil counts ($r=0.52$, $p<0.001$). We noted a relationship also in the controls ($r=0.68$, $p<0.001$). No age and sex dependency of ECP concentrations or eosinophil counts was noted in patients or controls.

The mean (SD) serum levels of IgG and IgA in the patient group were 12.8 (2.8) and 3.3 (1.3) g/l, respectively. There was a positive correlation between serum IgG and serum IgA and the inflammatory activity defined by serum haptoglobin or ESR (Table 1). The total serum IgE level in the patient group was 27 kU/l (range 8–84). None of the immunoglobulins were related to the ECP levels.

The mean (SD) value for ESR was 28 (23) mm/h and for serum haptoglobin 3.1 (1.2) g/l. The ECP levels correlated with haptoglobin ($p<0.001$, Fig. 1) and with ESR ($p<0.05$, Table 1). The relation between ECP and serum haptoglobin remained when the patients were subgrouped into men ($r=0.47$, $p<0.01$) and women ($r=0.77$, $p<0.01$). The laboratory signs of acute phase reaction did not correlate with the eosinophil counts.

The fifteen patients studied before and after

Fig. 1 Serum ECP levels v serum haptoglobin in patients with ankylosing spondylitis. ○=female patients; ●=male patients.

treatment with sulphasalazine showed both clinical and laboratory improvement. As outlined in Fig. 2 the ECP levels decreased in 13/15 patients and on average from 46 µg/l before therapy to 29 µg/l after treatment ($p<0.01$, Wilcoxon's signed rank test). The mean difference (SD) was 28 (38) µg/l. The eosinophil counts did not change significantly. A

reduction of the acute phase reaction was noted in 12/15 patients (Fig. 2); the mean (SD) value for serum haptoglobin before institution of sulphasalazine was 3.0 (1.2) g/l and after three months' treatment with the drug 2.3 (1.2) g/l. An improvement in the clinical parameters was seen in nine patients. No statistical relationship was found between the clinical outcome and the degree of the reduction of ECP levels.

Discussion

The patients with AS investigated in this study had normal eosinophil counts. Despite this they had raised serum levels of ECP, a specific protein of eosinophils, which appears in the circulation when released from eosinophils.¹ The significance of the circulating numbers of eosinophils in relation to the rate of production and utilisation of the cells is obscure since the blood contains only a small part of the total eosinophil mass in the body. In most situations, however, it is reasonable to assume that the peripheral counts reflect the total eosinophil number.⁶ Normally a positive correlation is noted between serum ECP concentrations and blood eosinophil numbers,¹⁰ suggesting that the turnover of the eosinophil mass in health is mirrored by serum ECP levels. In our patients with AS this same relationship was observed, suggesting that the turnover of a normally sized eosinophil mass is increased in AS.

Inflammatory mediators may accelerate the

Fig. 2 Serum ECP values and serum haptoglobin levels in patients with ankylosing spondylitis ($n=15$) (A) before and (B) after treatment with sulphasalazine (2-3 g/day) for three months.

turnover of eosinophils or activate eosinophils by an enhanced degranulation.¹⁰ In support of the hypothesis that inflammatory processes may govern the abnormal eosinophil homeostasis in AS we found a strong positive correlation between the acute phase reaction and circulating ECP levels. This evidence of eosinophil activation in AS expands the range of granulocyte involvement in this disease, as previously the neutrophil has been shown to be hyper-reactive in AS and other HLA-B27 associated diseases.^{11 12}

We have suggested that the extraordinarily high concentrations of ECP in inflammatory synovial effusions may contribute to the increased circulating levels of ECP in patients with rheumatoid arthritis.³ Only two of our patients with AS, however, had signs of peripheral arthritis. Thus the hypothesis that circulating ECP levels reflect a flow of this protein from a specific target organ, like the joint, to the circulation is not as attractive as in rheumatoid arthritis. Yet one cannot exclude the possibility that eosinophils are involved in local inflammatory events in entheses, spine, and sacroiliac joints and that a local degranulation of eosinophils at these sites is reflected in the circulation.

There are apparent exceptions to the rule that circulating eosinophil numbers reflect the total eosinophil mass of the body.¹³ Thus raised circulating levels of ECP in AS in the absence of peripheral eosinophilia may reflect an increased total eosinophil mass located in tissues other than the blood. The intestine is one of the tissues most abundantly populated by eosinophils.¹⁴ Recently we have demonstrated the presence of ECP in intestinal perfusion fluid from healthy controls and especially high levels in patients with inflammatory bowel disease (to be published). It appears likely that ECP in the circulation to a substantial extent originates from eosinophils accumulated in the intestine, and our findings in this study are a reminder of possible connections between the gut and certain arthritic conditions.

A role for intestinal micro-organisms in HLA-B27 associated arthritides has been proposed by several authors.^{15 16} Subclinical colitis has been reported in patients with various forms of arthritis.¹⁷ Against this background it seemed appropriate to test sulphasalazine in the treatment of AS, especially as it has been widely used in inflammatory bowel disease¹⁸ and has been shown to have second line properties in rheumatoid arthritis.^{19 20} Recently we have reported beneficial effects of this treatment in a double blind trial in AS.⁴ The study presented here shows a reduction of serum ECP levels in patients with AS treated for three months with sulphasalazine. During this time no significant

lowering of the eosinophil counts was seen, but there was both a reduction of the acute phase reaction and clinical improvement. The most straightforward explanation of the effect on the ECP levels is that the drug primarily reduces the acute phase reaction and secondary to that the eosinophil activation is reduced. The other more speculative explanation might be a sulphasalazine effect via its influence on an inflammatory process in the intestine involving the eosinophils. To elucidate the mode of action of sulphasalazine and a possible role of eosinophils in AS further studies with intestinal perfusion, like those performed in inflammatory bowel diseases, are needed.

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