short report
Haematologica 1995; 80:50-53

BENIGN IDIOPATHIC HYPEREOSINOPHILIA: A FEEBLE MASQUERADER OR A SMOLDERING FORM OF THE HYPEREOSINOPHILIC SYNDROME?

Marco Confalonieri, Fiorenzo Tacconi*, Mara De Amici°, Rita Maccario°, Camillo Porta#, Luciana Gioglio@, Enrico Bobbio-Pallavicini*

*Divisione di Pneumologia e *Divisione di Medicina Generale, Ospedale Maggiore, Crema; °Istituto di Clinica Pediatrica e #Istituto di Terapia Medica, IRCCS Policlinico San Matteo, Università degli Studi di Pavia; @Istituto di Anatomia Umana Normale, Università degli Studi di Pavia, Italy*

ABSTRACT

In a patient with long-standing idiopathic hypereosinophilia with no apparent organ damage we measured serum eosinophil cationic protein (ECP) and eosinophil protein X (EPX) titers, activated circulating eosinophil rates (by means of monoclonal antibodies EG1 and EG2), and the release of ECP and EPX *in vitro* by leukocytes at different cultures stages in order to detect possible functional abnormalities associated with hypereosinophilia. Our patient had elevated serum levels of both ECP and EPX, together with a high EG2 count, which would suggest eosinophil activation. However, serum levels of ECP and EPX were not significantly high in relation to the total number of eosinophil cells, although they were more numerous than in healthy controls. Moreover, the release of intracytoplasmic basic proteins by the patient's eosinophils was poor even after *in vitro* stimulation. Since hypereosinophilic syndrome (HES) with organ damage can appear as long as 8-9 years after the presence of a hypereosinophilic state, the absolutely benign nature of our patient's condition still cannot be defined. Thus, there is the possibility it could be slow-onset or smoldering HES.

Key words: benign idiopathic hypereosinophilia, eosinophil cationic protein, eosinophil protein X, hypereosinophilic syndrome, eosinophils

The most common primary type of eosinophilia is the hypereosinophilic syndrome (HES), which often presents clinically with organ damage and has a potentially poor prognosis.[1,2] The so-called *benign* idiopathic hypereosinophilia – including the familial and the constitutional forms – is much rarer. Its *benignness* depends on the fact that an increased eosinophil count is the only abnormal finding. No evidence has been offered yet to support the hypothesis that non-activated or innoxious eosinophils are present in benign hypereosinophilia.[3]

Serum measurement of eosinophil cationic proteins (ECP) has recently been suggested as a marker of eosinophil activity,[4] especially for monitoring clinical conditions characterized by allergic inflammation. Moreover, the monoclonal antibodies EG1 (anti-ECP) and EG2 (anti-ECP and anti-eosinophil protein X, EPX) have been reported to distinguish between activated and non-activated eosinophils.[5] In order to detect possible functional anomalies associated with hypereosinophilia, we measured a) serum ECP and EPX, b) EG1 and/or EG2-positive circulating eosinophil rates, c) *in vitro* ECP and EPX release by leukocytes at different culture stages, in a patient with long-standing idiopathic hypereosinophilia with no apparent organ damage.

Case report

C.G., a 77-year-old woman, was hospitalized in 1990 for persistent major eosinophilia of unknown origin. The patient had been aware of the eosinophilia for at least 10 years, but the first positive medical records dated to 1988: 1.722×10^9/L total eosinophils and 21% of 8.2×10^9/L WBC. Tests performed on an outpatient basis in 1989 and 1990 confirmed the per-

Correspondence: Dr. Marco Confalonieri, via Crotti 24, I-29100 Piacenza, Italy.
Received July 29, 1994; accepted October 3, 1994.

sistent hypereosinophilia (more than 1.5×10^9/L cells). The patient, who had always been a housewife, was asymptomatic; at that time she was receiving calcium antagonists for moderate essential arterial hypertension. Her remote case history was unremarkable. Physical examination revealed no unusual findings. Several investigations and laboratory tests were performed (and in some cases repeated) with normal or negative results. Bone marrow biopsy demonstrated a normal relationship between myeloid and erythroid series, both normally maturing, and megakaryocytes, and there were no major alterations. The eosinophil component of the myeloid population was markedly hyperplastic in all maturation phases, with no abnormal features. Electron microscopy morphometry showed no findings referable to an activation process, e.g. increased membrane surface or larger, more numerous mitochondria. Cytochemical study showed a negative CAE, while cytogenetic characteristics were not assessed. Blood tests of the 48-year-old patient's daughter demonstrated a normal eosinophil count in the peripheral blood.

The patient was followed up on a regular outpatient basis and hypereosinophilia (steady > 1.5×10^9/L) with no associated symptoms was always confirmed. Even during hospitalization three years later, in 1993, no other clinical or laboratory anomalies were found, and the leukocyte count demonstrated 34% eosinophils out of a total of 6.1×10^9/L WBC.

Materials ans Methods

Eosinophil characterization with cytofluorimetry techniques

After erythrolysis, peripheral blood was submitted to leukocyte membrane permeabilization according to the FOG method described by Hed et al.[4] Indirect immunofluorescence with EG1 and EG2 monoclonal antibodies (Pharmacia) and flow cytofluorimetry techniques were used to detect intracytoplasmic ECP.[5]

In vitro stimulation of polymorphonuclear leukocyte cultures

Polymorphonuclear leukocytes were separated and cultured in RPMI medium as described by Skoog and Beck.[6] Supernatants were collected at 2, 24, 48, 72, 96, 120 and 160 hours, and ECP and EPX assays were carried out on them.

Eosinophil cationic protein (ECP) and eosinophil protein X (EPX) assay in serum and in the supernatants of polymorphonuclear leukocyte cultures

A radioimmune method (Ria, Pharmacia) was used to assay serum ECP and EPX in our patient, in healthy controls and in the supernatants of polymorphonuclear leukocyte cultures. This technique has already been described elsewhere.[7]

Statistical analysis

Spearman's non-parametric correlation was used to evaluate release of the two proteins at different times.

Results

Serum ECP and EPX, EG1- and EG2-positive cell rates and eosinophil count in our patient and in healthy controls are reported in Table 1.

Our patient's serum levels were much higher than those in the paired control and in a previously tested group of healthy subjects (ECP, 20 subjects: 9±5 μg/L, EPX, 60 subjects: 20±10 μg/L).

Nevertheless, serum levels of both proteins were comparable in the patient and in the healthy control when expressed as pg/L/1,000 eosinophils (Table 1). As for $EG1^+$ and $EG2^+$ cells, our patient presented with a predominating number of $EG2^+$ cells, which would seem to suggest that most circulating eosinophils were activated and/or secreting.

Figures 1 a,b,c,d show the release of dosed ECP and EPX in the supernatants of polymorphonuclear cell cultures from both our patient and the control. The release of these mediators was observed to increase significantly over time in both patient (ECP: $r = 0.86$, $p<0.001$; EPX: $r = 0.98$, $p<0.001$, Figure 1a and 1b) and control (ECP: $r = 0.96$, $p<0.001$; EPX: $r = 0.90$, $p<0.001$, Figure 1c and 1d).

Peak values were recorded in the patient at 96 hours and maintained at 120 hours for both ECP (96h: 116.27 g/L, 120h: 123.79 g/L) and EPX (96h: 524.76 g/L, 120h: 541.17 g/L). As for

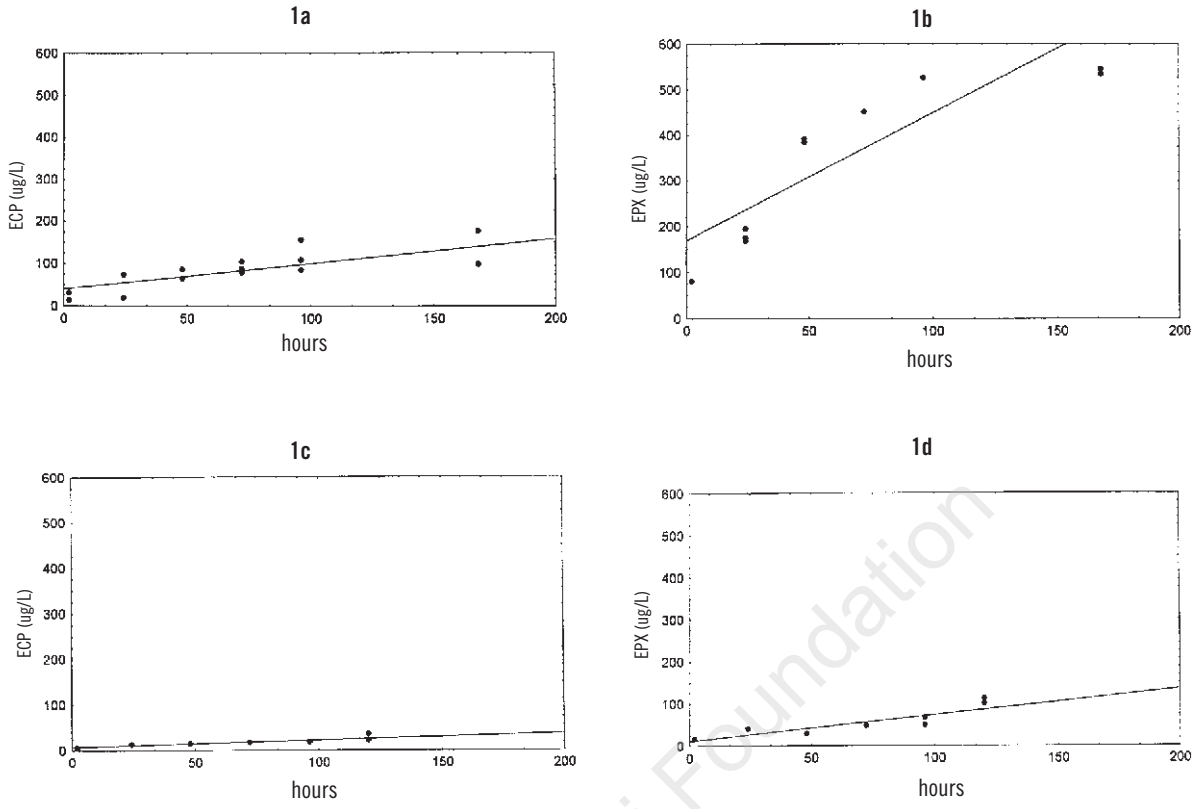

Figure 1. Dosage of ECP and EPX released in the supernatants of polymorphonuclear cell cultures from our patient and from a control.

the control, peak values were observed at 120 hours for both ECP (28.54 g/L) and EPX (106.41 g/L). ECP and EPX values produced after *in vitro* culture and expressed as μg/L every 1,000 eosinophils are reported in Table 1, which shows that the eosinophils obtained from the control were more efficient in producing the two proteins than were the patient's.

Discussion

Benign idiopathic eosinophilia is an uncommon condition that has been widely discussed in hematology treatises but studied very little, probably because of its non-pathogenic nature. Since HES with accompanying organ damage can appear as late as 8-9 years after a hypereosinophilic state,[8] the absolutely benign nature

Table 1. Serum ECP and EPX, EG1-positive and EG2-positive cells, eosinophil percent and *in vitro* production of ECP and EPX.

	Serum ECP (g/L)	*Serum EPX (g/L)*	*Serum ECP (pg/L/1,000 eosinophils)*	*Serum EPX (pg/L/1,000 eosinophils)*	*EG1+ cells (%)*	*EG2+ cells (%)*	*Eosinophils (%WBC)*	*ECP produced (ng/L/1,000 eosinophils)*	*EPX produced (ng/L/1,000 eosinophils)*
Patient	94.13	290.43	45.38	140.03	4	39	34	0.36	1.59
Controls	9.32	27.22	49.70	145.56	3	2	2.5	1.14	4.25

of our patient's condition still cannot be defined. Many authors recommend a careful follow-up over time when no cause is found for the eosinophilia.[8,9] If biological criteria specific for HES could be established, this would allow early diagnosis and organ damage could be prevented in these subjects.

Our patient exhibited some controversial features. On the one hand, her serum levels of ECP and EPX were high, like the EG2 count, which would seem to suggest that most of the eosinophils are activated. On the other hand, the serum levels of ECP and EPX were not significantly high relative to the total number of eosinophils, although they were definitely higher than in the population with normal eosinophil counts. Moreover, the release of these intracytoplasmic basic proteins by the patient's eosinophils was poor even after *in vitro* culture. This would seem to confirm that eosinophils are hypofunctional in benign idiopathic hypereosinophilia, even though the total number of eosinophils increases for unknown reasons.[10] On the other hand, organ damage can occur even several years after the chance discovery of eosinophilia, thus there is also a possibility that this could be a case of slow-onset (smoldering) HES.

Further biological studies on benign idiopathic hypereosinophilia patients might not only define some of the features of this uncommon clinical condition, but they might also throw some light on why, when and how eosinophils become too active and harmful to the human body in much more severe clinical conditions.

References

1. Weller PF, Bubley GJ. The idiopathic hypereosinophilic syndrome. Blood 1994; 83:2759-79.
2. Cassi E, De Paoli A, Fava S, et al. Idiopathic hypereosinophilic syndrome and eosinophilic leukemia. Haematologica 1992; 77:430-2.
3. Lovisetto P, Manachino D, Olivetto L, Biarese V, Caliman L. Le grandi eosinofilie del sangue. Aspetti clinico-nosografici. Rec Progr Med 1988; 79:525-32.
4. Halldén G, Anderson U, Hed J, Johansson SGO. A new membrane permeabilization method for the detection of intracellular antigens by flow cytometry. J Immunol Methods 1989; 124:103-9.
5. Tai PC, Sry CJF, Peterson C, Venge P, Olsson I. Monoclonal antibody distinguishes between storage and secreted forms of eosinophil cationic protein. Nature 1984; 309:182-4.
6. Skoog WA, Beck WS. Studies on the fibrinogen, dextran and phytohemagglutinin methods of isolating leukocytes. Blood J Hematol 1956; 11:466-72.
7. Peterson CG, Enander I, Nystrand J, Anderson AS, Nilsson L, Venge P. Radioimmunoassay of human eosinophil cationic protein (ECP) by an improved method. Establishment of normal levels in serum and turnover *in vivo*. Clin Exp Allergy 1991; 21:561-5.
8. Lefebvre C, Bletry O, Degoulet P, et al. Facteurs prognostiques du syndrome hyperéosinophilique. Étude de 40 observations. Ann Med Interne 1989; 140:253-7.
9. Fauci AS, Harley JB, Roberts WC, Ferrans VJ, Gralnik HR, Bjornson BH. The idiopathic hypereosinophilic syndrome. Clinical, pathophysiological and therapeutic considerations. Ann Intern Med 1982; 97:78-92.
10. Spry CJF, Davies J, Tai PC. Clinical features of fifteen patients with the hypereosinophilic syndrome. Q J Med 1983; 205:1-22.