Interaction and complex-formation between the eosinophil cationic protein and α_2 -macroglobulin

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The interaction between the highly basic and cytotoxic eosinophil cationic protein (ECP) and human plasma proteins is described. The major plasma protein responsible for complex-formation with ECP was shown to be the 'fast' form of α_2 -macroglobulin (α_2 M). Large amounts of complexes were observed in a serum obtained from a patient with hypereosinophilic syndrome. The amount of complexes that could be generated *in vitro* in normal fresh serum was rather low and was even less in fresh citrated plasma. Complex-formation between the non-proteolytic ECP and α_2 M was augmented in the presence of methylamine. Binding of ECP to α_2 M was also induced by the proteinases cathepsin G and thrombin, and the binding was competitive with cathepsin G. Methylamine and the proteinases seem to share a common mechanism in inducing binding of ECP. The nature of the ECP- α_2 M interaction is non-covalent, but withstands high salt concentrations. The interaction with α_2 M may reflect a mechanism by which the organism protects itself against the deleterious effects of the highly cytotoxic protein ECP.

INTRODUCTION

Eosinophil granulocytes contain in their granules several highly cationic proteins [1,2]. One of these is the eosinophil cationic protein (ECP). ECP is a highly basic zinc-containing single-chain protein with a molecular mass of about 21 kDa. The ECP content of human eosinophils from normal subjects is about 26 $\mu\text{g}/10^6$ eosinophils and accounts for approx. 30% of the granule protein content [3,4]. A sensitive radioimmunoassay has been developed for ECP, and in serum the normal ECP concentrations are in the range 5–55 $\mu\text{g}/\text{l}$ [5]. A number of biological functions have been reported for ECP. For example, ECP has been shown to be toxic to various mammalian cells [2] as well as to parasites [2]. Early observations were the effects of ECP on coagulation, fibrinolysis and kallikrein formation [2]. In individuals with high serum levels of ECP due to hypereosinophilia [6] or to activation of the eosinophils [7,8], coagulation abnormalities are frequently found [9]. High ECP levels have been shown in serum obtained from patients with various inflammatory conditions, which indicates that the eosinophil releases considerable amounts of ECP into the circulation upon stimulation [10]. However, in some cases, such as in asthmatic patients, serum ECP levels may be low in spite of blood eosinophilia [11], and if such a patient was challenged with allergen, a profound decrease in the ECP level was seen [8]. It was suggested that these phenomena were due to the interaction of ECP with products formed during the asthmatic process. These putative agents, however, have remained unidentified. In the present study we describe the interaction between a plasma protein, α_2 -macroglobulin (α_2 M), and ECP, which may be a clue to these early findings, since the interaction takes place only under certain conditions.

MATERIALS AND METHODS

Materials were obtained as follows: Sephadex G-100 (fine grade), Sephadex G-75 (superfine grade), Sephadex G-25 (fine grade), Superose 12 (prep grade), chelating Sepharose 6B and Sephadex G-25 (ultrafine grade) (Pharmacia Fine Chemicals, Uppsala, Sweden); Bio-Rex 70 (Bio-Rad Laboratories, Richmond, CA, U.S.A.); agarose indubiose A37 (L'Industrie Biologique, France); thrombin [1300 NIH (National Institutes of Health) units/mg of protein] and soybean trypsin inhibitor (SBTI) (Sigma, St. Louis, MO, U.S.A.); antisera to human serum, α_2 M, IgG, albumin, α_1 -proteinase inhibitor, α_1 -antichymotrypsin, inter- α -trypsin inhibitor and complement components C3 and C4 (Behringwerke Co., Marburg, Germany); Na^{125}I (The Radiochemical Centre, Amersham, Bucks., U.K.); X-Omatic G X-ray film (Kodak, Rochester, NY, U.S.A.). All other materials were of reagent grade.

ECP was isolated from human myeloid leukaemic cells as described previously [3] or from normal human granulocytes. Briefly, ECP was purified to homogeneity by using buffy-coat cells from normal blood donors as starting material. Isolated granulocyte granules were extracted, and the extracted proteins were gel-filtered on a Sephadex G-75 column. ECP-containing fractions were pooled and further purified by ion-exchange chromatography on a Bio-Rex 70 column. Eluted ECP was sometimes contaminated with neutrophil elastase, which was removed by chromatography on a zinc-Sepharose column. Homogeneity was checked by means of SDS/PAGE as described by Laemmli [12], together with Western immunoblotting [13], single radial immunodiffusion by the method of Ouchterlony [14] with antibodies against EPX [15], ECP [4], neutrophil elastase

Abbreviations used: α_2 M, α_2 -macroglobulin; ECP, eosinophil cationic protein; EPX, eosinophil protein X; SBTI, soybean trypsin inhibitor; PBS, phosphate-buffered saline (Dulbecco's); PAGE, polyacrylamide-gel electrophoresis.

[16], chymotrypsin-like cationic protein (cathepsin G; 'CCP') [3] and lysozyme (Dakopatts, Hägersten, Sweden). The absence of proteinase activity was checked by means of a solid-phase proteinase assay [17] with ^{125}I -fibrin as substrate. Quantification of purified ECP was done by assuming an absorption coefficient ($A_{280}^{1\%, 1\text{ cm}}$) of 15.45. Radioimmunoassay of ECP in fractionated plasma and serum was performed as described by Venge *et al.* [5]. The samples were incubated with Sephadex-bound anti-ECP for 2 h before the addition of ^{125}I -ECP, and the incubation was continued for a further 20 h. Radioactivity retained on particles was assessed in a γ -radiation counter and the amount was correlated with serial dilutions of known amounts of ECP.

Cathepsin G was purified to homogeneity as described by Olsson & Venge [3].

$\alpha_2\text{M}$ was purified from fresh pooled citrated human plasma by precipitation with poly(ethylene glycol) [18], gel chromatography on Superose 12 prep grade and zinc-chelate chromatography [19]. The purity of the preparation was checked by means of single radial immunodiffusion by the method of Ouchterlony [14] and SDS/PAGE as described by Laemmli [12]. No detectable amounts of the following plasma proteins were present: IgG, albumin, inter- α -trypsin inhibitor, α_1 -proteinase inhibitor, α_1 -antichymotrypsin, C3 and C4; however, trace amounts of IgM were present. The final $\alpha_2\text{M}$ preparation contained only the electrophoretically 'slow' form which, upon treatment with proteinases, converted to the 'fast' form [18]. The concentration of purified $\alpha_2\text{M}$ was determined by spectrophotometry at 280 nm, an $A_{280}^{1\text{ cm}, 1\%}$ value of 9.1 being assumed [20].

Labelling of ECP was performed by the ICI method of McFarlane [21]. The following reagents were mixed and incubated for 5 min: 50 μl of ECP (0.6 g/litre) in 50 mM-acetic acid, 250 μl of 0.9 M-glycine, 0.225 M-NaCl, 0.1 M-NaOH, pH 9.3, 10 μl of ICI solution (4 mM) in dilute HCl and 10 μl of Na^{125}I (1 mCi). Labelled protein was separated from free $^{125}\text{I}^-$ on a Sephadex G-25 (fine grade) column by using labelling buffer as elution buffer. Labelled ECP (sp. radioactivity about 1×10^7 c.p.m./ μg of ECP) stored in elution buffer at 4 °C for no longer than 1 month did not show any significant loss of bound radioactivity or disintegration of ECP.

Assays for the detection of ECP interactions in serum and plasma were as follows: ^{125}I -ECP was incubated in plastic tubes together with serum or citrated plasma at a final concentration of about 0.5 $\mu\text{g}/\text{ml}$ or about 1×10^7 c.p.m./ml. The incubation time and the temperature were as indicated. The pH in plasma and serum during experiments was always maintained at the physiological value by adding HCl or NaOH. Interaction between $\alpha_2\text{M}$, cathepsin G, thrombin and ECP was analysed as follows: $\alpha_2\text{M}$ (0.14 nmol) in PBS was incubated with cathepsin G, thrombin, labelled and/or unlabelled ECP as indicated. Stock solutions of cathepsin G and ECP were made in 50 mM-acetic acid. Stock solution of thrombin was made in 0.05 M-sodium citrate/0.15 M-NaCl, pH 6.5.

Analytical agarose electrophoresis of ^{125}I -ECP-containing samples was performed with purified agarose as described [3,22]. The electrophoresis buffer was 0.075 M-sodium barbital, pH 8.6. Crossed immuno-electrophoresis was performed as described by Laurell [23] with the electrophoresis buffer mentioned above. Single radial

Fig. 1. Gel filtration of 1 ml of human serum on a Sephadex G-75 column (1.5 cm $\times$ 90 cm)

The serum, containing 2.56 mg of ECP/litre, was obtained from a patient with hypereosinophilic syndrome. Proteins in fractions (1.8 ml) eluted with 0.05 M-Tris/HCl/0.5 M-NaCl, pH 8.0, at a flow rate of 15 ml/h, were determined by means of their absorbance at 280 nm ($\blacktriangle$) and a specific radioimmunoassay for ECP ($\bullet$). V_0 is the void volume and V_t the total volume.

immunodiffusion with labelled material was performed by the method of Ouchterlony [14]. Autoradiography of agarose plates containing ^{125}I -ECP was done by exposing the fixed and stained plates to X-ray film for 1–7 days at -20°C before development. Gel-filtration chromatography of serum, citrated plasma and incubation solution containing purified $\alpha_2\text{M}$ was performed on a Sephadex G-75 (superfine grade) column (1.5 cm $\times$ 90 cm) or on a Sephadex G-100 (fine grade) column (1.5 cm $\times$ 90 cm). Proteins were eluted with 0.05 M-Tris/HCl/0.3 M-NaCl, pH 8.0. Proteins in the eluted fractions were measured by their A_{280} and by the γ -radiation they emitted. ECP was sometimes quantified in the eluted fractions by the radioimmunoassay described above. SDS/PAGE of labelled material was performed by the method of Laemmli [12], with samples treated with 2% SDS for 15 min at 60°C . Autoradiography of the PAGE plates was done as described above.

RESULTS

To study the interaction between ECP and serum proteins *in vivo*, sera from patients which were known to contain high amounts of ECP were chromatographed on Sephadex G-75. The elution profile of a serum from one such patient with hypereosinophilia is shown in Fig. 1. ECP was eluted in two main peaks. The first ECP-containing peak corresponds to proteins eluted at the void volume. The second peak corresponds to the elution volume of pure and unbound ECP. Sera from patients with rheumatoid arthritis were also chromatographed; the elution profile of ECP was similar, but the relations between the heights of the first and second peak were quite variable (not shown).

In experiments *in vitro*, labelled ECP was incubated together with serum or citrated plasma for 2 h at 37°C and then subjected to chromatography on Sephadex G-75 or Sephadex G-100 and to agarose-gel electrophoresis.

Fig. 2. Gel-filtration chromatography on Sephadex G-100 of plasma samples (0.5 ml) incubated with ^{125}I -labelled ECP for 2 h at 37°C

(a) and (b) correspond to 10-year-old citrated plasma obtained from a patient with serine-proteinase-FXII deficiency and a fresh normal citrated plasma respectively. The column (1.5 cm $\times$ 90 cm) was equilibrated with 0.05 M-Tris/HCl/0.3 M-NaCl, pH 8.0. Fractions (2.35 ml) were collected at a flow rate of 20 ml/h. Proteins were determined by their absorbance at 280 nm (results not shown) and labelled ECP was determined by its radioactivity ($\bullet$). V_0 and V_t are defined in Fig. 1. The insert shows autoradiographs (A–C) of the electrophoretic pattern of the samples above: A, serine-proteinase-FXII-deficient plasma; B, normal plasma; C, labelled ECP alone; D, Coomassie Blue-stained electrophoretic pattern of a normal plasma (for comparison). The electrophoresis was run in purified agarose as described. The cathode is at the top. The arrow indicates the starting position.

In experiments with citrated plasma which had been stored for 10–15 years at -70°C , about 35% of total radioactivity was recovered in the high-molecular-mass peak (Fig. 2a). When fresh normal citrated plasma or serum were used in the experiments, very small amounts, i.e. less than 5–10% of the total radioactivity, were bound to high-molecular-mass proteins (Fig. 2b).

In one experiment the peak fraction from the lower-molecular-mass peak in Fig. 2(b) was re-incubated with citrated plasma known to have a high complex-forming capacity, and thereafter chromatographed on a Sephadex G-100 column. A significant amount of labelled ECP was recovered in the high-molecular-mass peak (results not shown), which means that the complex-formation observed with fresh serum/plasma is not limited by the availability of certain forms of ECP. When labelled ECP was chromatographed together with human serum albumin, only one radioactive peak, corresponding to free labelled ECP, was eluted from the column. This experiment excluded the presence of high-molecular-mass aggregates of ^{125}I -ECP.

Fig. 2 also gives the agarose-gel-electrophoretic patterns and the corresponding autoradiographs for these experiments. On agarose-gel electrophoresis the ^{125}I -ECP–protein complex migrated towards the anode to a position corresponding to $\alpha_2\text{M}$, whereas free ^{125}I -ECP migrated towards the cathode. These experiments suggested that $\alpha_2\text{M}$ might be one of the proteins responsible for ECP binding. This was confirmed by crossed immunoelectrophoresis with antibodies against whole serum and $\alpha_2\text{M}$ (Fig. 3). With antibodies against whole serum, only one precipitation peak containing

radioactivity was seen. The peak corresponded exactly to the radioactive peak with antibodies against $\alpha_2\text{M}$. Binding to $\alpha_2\text{M}$ was also shown by double immunodiffusion, where identity of radioactive precipitation lines

Fig. 3. Autoradiography of crossed immunoelectrophoresis

The sample, citrated plasma from a patient with serine-proteinase-FXII deficiency (see Fig. 2a), was incubated with labelled ECP for 15 h at 37°C , followed by agarose-gel electrophoresis and crossed immunoelectrophoresis, with antibodies to $\alpha_2\text{M}$ (a) and human serum (b) included in the gels. The corresponding agarose-gel electrophoretic patterns are shown at the bottom, with the anode at the left.

Fig. 4. Gel-filtration chromatography on Sephadex G-100 of fresh normal citrated plasma treated with methylamine and incubated with labelled ECP

The column (1.5 cm $\times$ 90 cm) was equilibrated with 0.05 M-Tris/HCl/0.3 M-NaCl, pH 8.0. The sample volume was 0.5 ml and the fractions (2.35 ml) were collected at a flow rate of 20 ml/h. The elution of labelled ECP was monitored by its radioactivity ($\bullet$). V_0 and V_t are defined in Fig. 1.

was obtained between antibodies to human serum and those to α_2 M (results not shown). These experiments indicate that ECP binding to α_2 M is specific and exclusive among plasma proteins.

To study the nature of the interaction between ECP and α_2 M, the following experiment was performed. Fresh citrated normal plasma was preincubated with 50 mM-methylamine at pH 8 for 1 h, followed by incubation with 125 I-ECP for 2 h. Methylamine is known to cause complete inhibition of complex-formation between proteinases and α_2 M [28]. However, in this experiment the result was completely opposite, with nearly 100% binding of ECP to α_2 M, compared with 5–10% in the absence of methylamine (Fig. 4). A number of reports [27,28] have described the similarities in the action of methylamine and proteinases on α_2 M. We were therefore interested to see if proteinases would affect the binding of ECP to α_2 M. To exclude interaction between proteinases and other plasma proteinase inhibitors, purified α_2 M was used in these experiments instead of serum/plasma. Experiments were performed with two serine proteinases, thrombin and cathepsin G. α_2 M is the major plasma inhibitor for the latter [24]. When fixed amounts of α_2 M and labelled ECP were incubated at a 10:1 molar ratio with different amounts of proteinases, the binding of labelled ECP was increased (Fig. 5). The binding of ECP to α_2 M in the

Fig. 5. Effects of various amounts of cathepsin G (O) or thrombin (Δ) on the binding of 125 I-ECP (1.5×10^{-11} mol) to α_2 M (1.4×10^{-10} mol)

Mixtures with thrombin were incubated for 1.5 h at 37 °C, and mixtures with cathepsin G were incubated for 5 min at 22 °C, followed by addition of SBTI (10^{-8} mol) and further incubation for 1.5 h at 37 °C. The amount of ECP bound to α_2 M was calculated from the gel-filtration chromatogram of the incubation mixtures.

presence of cathepsin G was optimal at a 1:1 molar ratio of cathepsin G and α_2 M. At higher doses of cathepsin G, decreased binding was observed, which could indicate either that ECP is digested by free cathepsin G or that ECP and cathepsin G compete for the same binding site. The incubation time before addition of SBTI could be decreased to 1 min without any effects on the binding of ECP (results not shown). In these experiments, ECP, cathepsin G and α_2 M were mixed simultaneously. When α_2 M and cathepsin G were preincubated for 5 min at 22 °C, followed by addition of labelled ECP, a 55% decrease of ECP binding was observed (results not shown). These results suggest that ECP binds to α_2 M very quickly. Fig. 5 also shows the results of ECP binding in the presence of thrombin. Thrombin is less efficient than cathepsin G in promoting ECP binding, and the binding is slightly enhanced above equimolar amounts of thrombin and α_2 M, which could mean that ECP is not digested by thrombin or that thrombin does not compete with ECP for the binding site. As thrombin binds to α_2 M at a 1:1 molar ratio, the remaining binding site could be available for ECP binding.

To examine the stoichiometry of the ECP- α_2 M interaction, various amounts of ECP, together with a fixed amount of labelled ECP, were incubated with fixed amounts of α_2 M, cathepsin G or thrombin. The extent of binding was calculated, as described above, from the two peaks obtained on Sephadex G-100 chromatography. As seen in Fig. 6, an increased binding of ECP was observed in the presence of cathepsin G and thrombin with increasing doses of ECP. Further, the binding was not saturable with the amounts of ECP used in the experiments, and complex-formation was higher in the presence of cathepsin G than in the presence of thrombin, which is in agreement with the results in Fig. 5. In agreement with the results with methylamine (Fig. 4), methylamine seems to be far more efficient in inducing binding of ECP to α_2 M. In spite of this, methylamine and proteinases may induce binding of ECP to α_2 M by similar mechanisms. This hypothesis was

Fig. 6. Stoichiometry of binding of ECP to α_2 M

The incubation mixtures contained fixed amounts of 125 I-ECP (1.5×10^{-11} mol), α_2 M (1.4×10^{-10} mol) and either cathepsin G (1.3×10^{-10} mol) (O) or thrombin (3.9×10^{-10} mol) (Δ) and increasing amounts of unlabelled ECP. Incubation times and calculations are given in the legend to Fig. 5.

Fig. 7. Effects of ECP on the activity of α_2 M-cathepsin G complexes

The incubation mixtures contained 0.2×10^{-10} mol of cathepsin G and 0.28×10^{-10} mol of α_2 M and increasing amounts of ECP. These mixtures were incubated for 10 min at $+22^\circ\text{C}$ before assessment of the proteolytic activity by means of the solid-phase fibrin method [17].

further strengthened by an experiment where methylamine was added to α_2 M which had been incubated with cathepsin G at a 1:2.6 molar ratio together with labelled ECP. The addition of methylamine in this situation caused no further binding of ECP to α_2 M (results not shown).

To examine the interaction between α_2 M, cathepsin G and ECP further, the proteolytic activity of unbound cathepsin G was measured by means of the solid-phase-fibrin method [17]. An increasing proteolytic activity was observed which was proportional to the amount of ECP present (Fig. 7). Control experiments with mixtures of ECP and cathepsin G revealed the same results as with cathepsin G alone. ECP alone had no proteolytic activity. The molar ratio of cathepsin G and α_2 M was slightly below 1, which means that the cathepsin G activity, in the absence of ECP, was completely inhibited by α_2 M. These results probably means that ECP inhibits cathepsin G- α_2 M complex-formation. The proteolytic activity of cathepsin G at the highest ECP concentration approached the activity of 100% free cathepsin G.

EDTA at a concentration of 4 mM had no effect on complex-formation (results not shown). Samples with a high content of ECP- α_2 M complexes were treated with 2% SDS and then subjected to SDS/PAGE as described by Laemmli [12], with 7.5% (w/v) polyacrylamide in separating gel. Almost all labelled ECP was separated from the complex, which indicated that the binding is of non-covalent character (results not shown). On the other hand the binding was stable enough to withstand 0.5 M-NaCl or the electrical forces encountered in agarose-gel electrophoresis.

DISCUSSION

The interaction and complex-formation between the ECP and a serum component is described. The identification of α_2 M as the major ECP-binding protein in serum and plasma is based on the identical precipitation lines obtained when testing with antibodies against serum and α_2 M. In experimental systems *in vitro* with normal serum and citrated plasma, very little complex-formation was observed compared with serum and plasma stored for several years at -70°C . Considerable complex-formation could also be generated in the presence of methylamine, which is a result quite opposite to its effect on proteinase- α_2 M complex-formation [18]. The phenomenon in the above experiments indicates that ECP binds to the 'fast' form of α_2 M, which is generated from the native 'slow' form by methylamine treatment or by cleavage by proteinases [26-28]. We observed in experiments with purified α_2 M and the proteinases cathepsin G and thrombin a proteinase-dependent binding of ECP to α_2 M. The binding of ECP was dose-dependent, in respect to both the proteinases and to ECP. Equimolar amounts of cathepsin G and α_2 M were optimal for binding of ECP, whereas a molar ratio of > 1 inhibited ECP binding. Cathepsin G is, as shown by Virca & Travis [29], bound to α_2 M preferentially at a 1:1 molar ratio at low concentrations of both proteins, and binding of a second molecule was shown to occur only at higher concentrations. They also showed that a second molecule does not need to have proteolytic capabilities to be entrapped. In the light of their results, the optimal binding of ECP that occurred at a 1:1 molar ratio of cathepsin G and α_2 M may reflect binding of ECP to the other available binding site of α_2 M. A molar ratio over 1:1 for cathepsin G and α_2 M may therefore lead to competition between ECP and cathepsin G for this second binding. In experiments with thrombin, no decline in binding of ECP was observed when over-saturable

amounts of thrombin were used; instead, a slight increase of ECP binding was observed. This could indicate that there is no competition with regard to the second binding site between ECP and thrombin, a finding that is also in agreement with the fact that thrombin binds to α_2 M at a 1:1 molar ratio [30]. The binding of ECP to α_2 M seems to occur simultaneously with the action of the proteinases on α_2 M. A 55% decrease in ECP binding was observed when ECP was added after the binding of cathepsin G to α_2 M. The fact that methylamine and proteinases induce binding of ECP by the same mechanism was supported by an experiment where methylamine was added to a mixture consisting of α_2 M, labelled ECP and cathepsin G which had been preincubated for 5 min at 22 °C. Here no additional binding of ECP was observed when methylamine was added. The stoichiometry of binding of ECP to α_2 M was examined with increasing molar ratios of ECP to α_2 M in the presence of fixed amounts of proteinases. No saturation of binding was observed up to a molar ratio of 1.6 for ECP and α_2 M.

The relation between the ECP and cathepsin G binding to α_2 M was further examined by measuring the proteolytic activity of free cathepsin G. Increased proteolytic activity was observed proportional to the ECP concentration. This means that the binding of cathepsin G decreases when the ECP concentration is increased, and it appears that ECP decreases the binding capacity of α_2 M for cathepsin G. An example of the latter phenomenon was described by van der Veen *et al.* [31], who showed that myelin basic protein inhibited complex-formation between trypsin and α_2 M. They also showed that the stability of the α_2 M-trypsin complex was decreased, which may have a bearing on the results above. Incorporation of non-proteolytic proteins was also demonstrated by Sottrup-Jensen *et al.* [32], who described the incorporation of inactivated trypsin and insulin into α_2 M. This was only seen when α_2 M had been converted to a proteinase-activated 'nascent' form. Aspartate aminotransferase, another cationic non-proteolytic enzyme, was also shown to bind to α_2 M [33], and this cationic protein was cleared very rapidly from plasma of dogs after an intravenous injection. This latter finding is in agreement with our previous findings that ECP is cleared rapidly from circulation in asthma patients after challenge with allergen [8].

The nature of the binding between ECP and α_2 M was non-covalent, but withstood electrical and ionic forces. The concept that α_2 M encapsulates proteolytic enzymes may also apply to ECP. Complex-formation was shown to be limited, since not all ECP in serum or plasma was bound in complex. This behaviour, of limited complex-formation *in vitro*, could not be due to differences between unlabelled and labelled ECP, since the results of the radioimmunoassay and the use of 125 I-ECP in the experiments were similar.

ECP interactions with other serum and plasma components have been described previously. ECP enhances the formation of active kallikrein [34], interacts with coagulation factors, enhances serine-proteinase-FXII-dependent clot formation [34] and pre-activates plasminogen [35]. Whether any of these proteins are required for the development of complexes such as those described here remains speculative at this moment. A high degree of complex-formation was seen in sera with very high ECP levels obtained from, for example,

patients with hypereosinophilic syndrome. This indicates that complexes may be seen *in vivo*. However, normally this is not seen, probably owing to fast clearance. The ECP molecule is cytotoxic at very low concentrations to a number of cell types [2,6] and could therefore be hazardous to human cells. It has been suggested that ECP and other eosinophil secretory products are responsible for the damage to the endomyocardium seen in hypereosinophilic syndrome [36,37] and possibly causes epithelial desquamation in bronchi of asthmatic patients [38,39]. Under normal conditions ECP seems to be under control by some protective mechanism, and the interaction between ECP and α_2 M may be of importance in that respect.

The technical assistance of Mrs. Lena Björkman and Mrs. Kerstin Lindblad is greatly appreciated. This work was supported by the Swedish Medical Research Council and the Medical Faculty of the University of Uppsala.

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Received 5 January 1987; accepted 23 April 1987