LETTERS

Eosinophil cationic protein is not only a distinctive eosinophil protein

We read with interest the article by Qiu et al (Thorax 2007;62:475–82). In this paper, neutrophils and eosinophils were identified using mouse anti-human neutrophil elastase and anti-eosinophil cationic protein (ECP), both monoclonal antibodies (mAbs). mAbs against ECP have been used to detect total eosinophils, but immunostaining techniques evidenced that the number of ECP+ cells was higher than the number of eosinophils.1 Recent studies show that ECP is not only a distinctive eosinophil protein, but has been found in neutrophils.2,3

In this regard, it has been reported that these cells can take up ECP from the bloodstream after phagocytosis and store it, but not synthesize it.4 In agreement with these reports, our recently published study5 indicated that in resting (unstimulated) neutrophils, no ECP mRNA was detected, and only a small amount of intracellular or released protein was found. However, after cellular stimulation, ECP was synthesised. This was verified by several lines of evidence: (1) after neutrophil stimulation, bands of PCR product corresponding to ECP mRNA were detected; (2) de novo biosynthesis protein was detected by S35 radiolabelling; (3) an increase in intracellular ECP protein was observed by flow cytometry, fluorescence microscopy and western blotting; and (4) accompanying ECP release was detected by ELISA.

Our results are exclusively due to neutrophils and cannot be ascribed to possible contamination by eosinophils for several reasons: (1) both cells types had a different course of ECP release. Eosinophils released ECP after 30 min of cell stimulation; neutrophils only released protein after 3–18 h; (2) PAF failed to induce ECP release by eosinophils whereas it induced ECP release by neutrophils; (3) lipopolysaccharide induced ECP release from eosinophils but not from neutrophils; (4) allergen, anti-IgE, anti-FceRI and anti-galectin-3 dependent ECP production was observed in neutrophils but not in eosinophils; (5) CD66b is a specific marker for neutrophils and we found that these cells (CD66b+) do in fact highly express ECP; (6) Charcot Leyden Crystal protein is a marker of eosinophils and basophils but not of neutrophils and we did not find its transcript in our neutrophil preparation.

Collectively, these results suggest that caution should be exercised in the interpretation of immunohistochemistry when antibodies to ECP are used as specific markers for eosinophils, especially in endobronchial biopsy specimens from patients with severe exacerbation of asthma in which an accumulation of activated cells exist.

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REFERENCES


Authors’ reply

We thank Monteserin and Vega for their comments concerning the specificity of the clone EG2 (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden), mouse anti-human eosinophil cationic protein (ECP) monoclonal antibody used by us (Thorax 2007;62:475–82). We read with interest their results of isolated cells, which lead the authors to suggest caution in our interpretation of paraffin wax embedded tissues. Thus despite what manufacturers may indicate their results of isolated cells, it is not surprising that we published in Thorax 2008;63:185. doi:10.1136/thx.2007.088807.

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