

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.^{1–3}

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.³ In agreement with these reports, our recently published results⁴ indicate 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-FcεRI 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; (7) 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.

J Monteseirín, A Vega

Servicio Regional de Inmunología y Alergia, Hospital Universitario Virgen Macarena, Sevilla, Spain

Correspondence to: Dr J Monteseirín, Servicio Regional de Inmunología y Alergia, Hospital Universitario Virgen Macarena, c/Asunción 27, 3^o Izda E-41011, Sevilla, Spain; fmonteseirin@meditex.es

Funding: This work was supported by grants from the Junta de Andalucía (Ayudas Grupos de Investigación), Fundación de la Sociedad Española de Alergia e Inmunología Clínica, Fundación Sanitaria Virgen Macarena and Fundación Alergol, Spain. AV was supported by fellowships from the Ministerio de Ciencia y Tecnología, and Fundación Alergol. JM is under the Programa de Intensificación de la Actividad Investigadora del Sistema Nacional de Salud.

Competing interests: None.

Thorax 2008;**63**:185. doi:10.1136/thx.2007.088807

REFERENCES

1. **Ying S**, Meng Q, Smith SJ, *et al*. Methods for identifying human eosinophils in blood and tissue. *ACI Int* 2002;**14**:64–71.
2. **Sur S**, Glitz DG, Kita H, *et al*. Localization of eosinophil-derived neurotoxin and eosinophil cationic protein in neutrophilic leukocytes. *J Leukoc Biol* 1998;**63**:715–22.
3. **Byström J**, Garcia RC, Hakansson L, *et al*. Eosinophil cationic protein is stored in, but not produced by, peripheral blood neutrophils. *Clin Exp Allergy* 2002;**32**:1082–91.
4. **Monteseirín J**, Vega A, Chacón P, *et al*. Neutrophils as a novel source of eosinophil cationic protein in IgE-mediated processes. *J Immunol* 2007;**179**:2634–41.

Authors' reply

We thank Monteseirín 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,¹ which lead the authors to suggest caution in our interpretation of paraffin wax embedded bronchial biopsies from patients experiencing severe exacerbations of asthma. We respond respectfully and as follows.

Firstly, in our experience and those of the originator (Per Venge; recent personal communication), the cells invariably stained with EG2 have the characteristic morphology of eosinophils (ie, bi-lobed granular cells) whereas neutrophils stain rarely and too weakly to be included in our counts of formalin fixed paraffin wax embedded tissue. Secondly, while it is recognised that monoclonal antibodies are highly specific for the epitopes against which they have been raised, it is also widely appreciated that any particular epitope may be present on more than one cell type, or become expressed at different stages of cell maturation or activation. For example, dependent on the manufacturer and clone applied, the anti-CD56 monoclonal anti-human antibody (used by many to detect natural killer cells) may also detect a subset of CD4⁺ or CD8⁺ cells and

even neurons, astrocytes and Schwann cells. Thus despite what manufacturers may claim, we have always been careful to conduct pilot studies in which we make comparisons against non-immunostains, in this case, of eosinophils (eg, chromotrope 2R), first titrating the antibodies that we plan to use and for each particular fixation and embedding protocol. Moreover, in order to reduce further non-specific staining due to any endogenous peroxidase present in large quantities in neutrophils and macrophages, we routinely apply the alkaline phosphatase anti-alkaline phosphatase technique² rather than use a peroxidase based method.

Finally, in our study, we compared EG2 positive and neutrophil elastase (Ne) positive cells in bronchial biopsies between patients with stable asthma and asthmatics experiencing severe exacerbations. We found, in the latter, that while there was a significant increase in Ne positive cells, the numbers of EG2 positive cells did not increase significantly. If the concerns expressed were to be realised, it would be expected that the increase in EG2 positive cells would have followed closely that of Ne positive cells, which was not the case.

In conclusion, while we appreciate that other markers (eg, anti-MBP or anti-EPO) may also be used to mark eosinophils, for formalin fixed paraffin wax embedded tissue, the continued application of EG2 allows us to compare our findings with those previously done by both ourselves and by others. We consider that application of EG2 antibody to detect eosinophils remains a robust and interpretable technique, particularly by those appropriately trained and experienced in quantitative histopathology. The novel findings kindly communicated by Monteseirín and Vega are interesting and important, but caution must also be exercised in extrapolating from the results of such studies on isolated cells to those of tissues fixed and embedded, a process known to alter protein tertiary structure and hence the expression/accessibility of the epitopes of interest. We trust that these findings and discussion are helpful in immunohistological studies of inflammatory conditions, both in the lung and in general.

Y Qiu, P K Jeffery

Lung Pathology, Department of Gene Therapy, Imperial College London, Royal Brompton Hospital, London, UK

Correspondence to: Dr P K Jeffery, Lung Pathology, Department of Gene Therapy, Imperial College London, Royal Brompton Hospital, Sydney Street, London SW3 6NP, UK; p.jeffery@imperial.ac.uk

Competing interests: None.

REFERENCES

1. **Monteseirín J**, Vega A, Chacón P, *et al*. Neutrophils as a novel source of eosinophil cationic protein in IgE-mediated processes. *J Immunol* 2007;**179**:2634–41.
2. **Mason DY**, Sammons R. Alkaline phosphatase and peroxidase for double immunoenzymatic labelling of cellular constituents. *J Clin Pathol* 1978;**31**:454–60.