Table 1 Specificities of monoclonal antibodies EG1 and EG2 for human eosinophil granule proteins

Purified granule protein	Monoclonal antibodies EG1	EG2
Eosinophil granule proteins	Optical density at 450 nm	
Eosinophil cationic protein, ECP	0.89*	1.02*
Eosinophil protein X, EP-X	0.16	0.95*
Major basic protein, MBP	0.09	0.06
Peroxidase, EPO	0.13	0.15
Neutrophil granule proteins		
Chymotrypsin-like cationic protein	0.08	0.06
Elastase	0.08	0.12
Lactoferrin	0.16	0.13
Myeloperoxidase	0.14	0.09

ECP was purified from blood granulocytes of a patient with chronic eosinophilic myeloid leukaemia[5]. EPO and EP-X were prepared from purified blood eosinophils of a patient with hypereosinophilia[6]. Their purity was assessed by agarose-gel electrophoresis, SDS–polyacrylamide gel electrophoresis (PAGE) of the reduced proteins, and double immunodiffusion in agar with specific polyclonal antibodies[3]. EPO was purified by Sephadex G-75 and CM-Sephadex column chromatography and identified by its enzyme activity. The purity of EPO was determined by comparison with an extinction value at 415 and 280 nm of 1.10, agarose-gel electrophoresis, SDS–PAGE after reduction, and by its failure to precipitate in double immunodiffusion gels using specific antibodies to the other eosinophil basic proteins. Purified neutrophil chymotrypsin-like cationic protein, elastase[25], lactoferrin[26] and myeloperoxidase[27] were also prepared. The binding of antibodies EG1 and EG2 to these purified granule proteins was assessed using microtitre plates (Dynatech) coated with 5 $\mu g\ ml^{-1}$ protein overnight at 4 °C. Unattached sites were blocked with 1% gelatin at 37 °C for 1 h. Endogenous peroxidase activity was removed using 0.3% H_2O_2 in methanol. The amount of antibody bound to these proteins was measured at 450 nm using peroxidase-conjugated rabbit anti-mouse IgG (Miles-Yeda) and H_2O_2 with *o*-phenylenediamine[28] (Sigma) and a Titertek multiskan reader (Flow).

* Significant binding above background levels.

titrating them against both cationic proteins (Fig. 1*a*). Antibody EG2 was studied further in a competitive inhibition assay with purified ECP and EP-X. Antibody EG2 was first incubated with EP-X, then residual binding for ECP was assessed. It was found that increasing amounts of EP-X caused a dose-dependent decrease in the binding of antibody EG2 to ECP (data not shown). In order to confirm the specificities of these two antibodies, western blots were made of eosinophil granule proteins, and the binding of antibodies EG1 and EG2 was assessed (Fig. 1*b*). These blots showed that antibody EG1 bound only to ECP, whereas antibody EG2 bound to ECP and EP-X.

The antibodies were then used for immunocytochemical staining of blood eosinophils from normal people and patients with the hypereosinophilic syndrome. In this disease, blood eosinophils may be degranulated and contain cytoplasmic vacuoles, which are thought to be areas of granule solubilization and secretion[22]. Antibody EG1 stained all the eosinophil granules in 25% of normal blood eosinophils (Fig. 2*a*), and all the granules in 70% of blood eosinophils from patients with the hypereosinophilic syndrome (Fig. 2*g*). Antibody EG2 did not stain normal blood eosinophils (Fig. 2*b*) but did stain them when: (1) They had phagocytosed zymosan–C3b particles. In some preparations, staining was found on the surface of adjacent cells, suggesting that the granule proteins had been secreted externally (Fig. 2*d*) (antibody EG1 also stained phagocytic vacuoles produced by zymosan-C3b, (Fig. 2*c*). (2) After incubation with eosinophil cytotoxicity enhancing factor[9] (Fig. 2*e*, *f*). Eosinophils which were incubated without this factor were not stained by antibody EG2 (Fig. 2*f*). (3) Blood eosinophils were obtained from patients with hypereosinophilic syndrome (Fig. 2*h*).

As EG2 recognizes antigens that are stable in formalin-fixed, paraffin-embedded tissues, we were able to examine the occurrence and distribution of activated and degranulating eosinophils in the skin of patients with chronic urticaria. Large numbers of activated cells were found in the skin biopsies from 12 out of 14 patients studied, and in some patients this was associated with areas of extracellular deposits of eosinophil secretion products (Fig. 3). Disease severity appeared to correlate with the presence of these activated cells.

The cross-reactivity of EG2 on ECP and on an epitope on EP-X suggested that there were structural similarities between these two proteins, at least on secretion. As these two cationic proteins are of similar molecular size, with closely related physicochemical and biological properties[6], it is possible that they are structurally related.

The observation of numerous activated eosinophils and their secretion products in the skin supports the view that eosinophils may contribute to tissue injury. It is now possible to extend these studies to other diseases in which eosinophil activation and degranulation in tissues have been thought to occur. The ability of monoclonal antibodies to detect antigenic differences between storage and secreted forms of granule proteins may have many applications in the study of activation and degranulation in secretory cells, in addition to their more obvious use in defining the tissues in which these processes occur.

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Erratum

Determination of the Si–O–Si bond angle distribution in vitreous silica by magic angle spinning NMR

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THE letter with the above title appeared in the 5 April issue (**308**, 523–525; 1984) with an incorrect initial for one of the authors—E. Dupree should have read R. Dupree.