

The high-affinity IgE receptor on eosinophils: From allergy to parasites or from parasites to allergy?

Abdelillah Soussi Gounni, MSc,^a Bouchaïb Lamkhied, MSc,^a
Emmanuel Delaporte, MD,^b Ariane Dubost, MD,^{a, b} Jean-Pierre Kinet, MD,^c
André Capron, MD,^a and Monique Capron, PhD^a
Lille, France, and Rockville, Md.

Experimental evidence has suggested that eosinophils bearing receptors for IgE could participate in immune defense against parasites, as well as in allergic manifestations.^{1, 2} The first type of IgE-binding molecule to be identified on the eosinophil membrane was the low-affinity IgE receptor FcεRI/CD23.³ However, the low level of membrane expression and CD23 mRNA in eosinophils from patients led us to investigate the existence of other IgE-binding molecules. Recently, Mac2/ε-binding protein, a molecule belonging to the S-type lectin family, was detected on human eosinophils, parallel to high amounts of the corresponding mRNA.⁴ Inhibition of IgE-binding and IgE-mediated cytotoxicity toward parasites by anti-Mac2 antibodies indicated the functional role of such molecules in IgE-associated diseases. A role for FcεRI was first neglected because this receptor was believed to be solely expressed on mast cells and basophils.⁵ The demonstration that FcεRI is present on Langerhans cells in the skin⁶ suggested that it might be expressed by other immune effector cells.

In this report, we summarize recent data showing that FcεRI is expressed on human eosinophils from patients with hypereosinophilia,⁷ is involved in IgE binding to eosinophils, and participates in the selective release of one granule mediator, eosinophil peroxidase (EPO). The participation of FcεRI-bearing eosinophils in a protective im-

Abbreviations used

ECP: Eosinophil cationic protein
EPO: Eosinophil peroxidase
mAb: Monoclonal antibody

mune response against a parasitic infection and their detection in various skin disorders associated with IgE indicate the *in vivo* relevance of our findings to the dual function of IgE.

RESULTS AND DISCUSSION

Surface expression of FcεRI α chain on eosinophils

FcεRI is a tetrameric structure composed of an IgE-binding α chain, a β chain, and two disulfide-linked γ chains.⁵ Anti-FcεRI α chain monoclonal antibodies (mAbs) were raised in mice immunized with Chinese hamster ovary cells transfected with human FcεRI α γ complexes.⁶ One of these mAbs, the anti-FcεRI α chain mAb 15-1, was used to investigate the surface expression of FcεRI α chain on human eosinophils from patients with hypereosinophilic syndromes. By flow cytometry, positive staining was detected on 13% to 73% of eosinophils from individual donors. The mean percent of FcεRI-expressing eosinophils did not differ from the mean percent of eosinophils able to bind IgE. These results indicate that in contrast to mast cells, which constitutively express FcεRI, expression of FcεRI by blood eosinophils was variable among patients.

Immunostaining experiments with anti-FcεRI α chain mAb 15-1 were performed on cytocentrifuged preparations of blood eosinophils. The staining was revealed by the alkaline phosphatase-anti-alkaline phosphatase procedure.⁶ Positive staining was found in some eosinophils. In contrast, no staining was detectable with the isotypic control mAb.

From ^aCentre d'Immunologie et de Biologie Parasitaire, Institut Pasteur, Lille; ^bService de Dermatologie A, Centre Hospitalier Régional Universitaire, Lille; and ^cMolecular Allergy and Immunology Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville.

Reprint requests: Monique Capron, Centre d'Immunologie et de Biologie Parasitaire, Unité Mixte INSERM U167-CNRS624, Institut Pasteur, Lille, France.

J ALLERGY CLIN IMMUNOL 1994;94:1214-6.

1/0/59921

Inhibition of iodine 125-labeled IgE binding to eosinophils by anti- α chain mAb 15-1

The involvement of Fc ϵ RI α chain in the IgE-binding capacity of eosinophils was next investigated in a binding assay with radiolabeled IgE myeloma protein. Highly purified eosinophils from patients with hypereosinophilia were incubated with ¹²⁵I-labeled monomeric human IgE together with increasing concentrations of the anti-Fc ϵ RI α chain mAb 15-1 or a control mAb, and the cell-bound radioactivity was evaluated. The mAb 15-1, but not the isotypic control mAb, induced a dose-dependent inhibition of monomeric IgE binding. At the higher dose of mAb 15-1 (50 μ g/ml), the inhibition was complete and comparable to the inhibition seen with 1 mg/ml of unlabeled human IgE. Under the same conditions, 1 mg/ml of human IgG did not inhibit IgE binding, whereas mAb 15-1 was not able to inhibit the binding of ¹²⁵I-labeled human IgG, confirming the absence of binding of mAb 15-1 to the Fc γ receptor.

Molecular characterization of Fc ϵ RI in eosinophils

The α chain of the high-affinity receptor is known to contain the binding site for IgE. To examine the expression of mRNA corresponding to the α chain of the Fc ϵ RI receptor by eosinophils, Northern blot hybridization was performed with a cDNA probe coding for the human α chain of Fc ϵ RI. RNA extracts from eosinophils of all patients tested produced a signal at 1.2 kb, but with varying intensity among individual patients.

As previously shown, transfection of cDNA for either the human α and rat γ subunits⁸ or a chimeric human α subunit alone into heterologous mammalian cells resulted in cells capable of binding human IgE myeloma protein with high affinity.⁹ The question of whether eosinophils could express β and γ chains remained. Because of the limited quantity of highly purified eosinophils, the analysis was done by polymerase chain reaction (PCR) of reverse-transcribed total RNA. The amplification products obtained from highly purified eosinophils revealed the predicted band for the Fc ϵ RI β chain and the γ chain. The specificity of the fragment was confirmed by restriction enzyme analysis and Southern blot with an internal oligonucleotide specific for each subunit. In summary, our studies provide the first evidence that eosinophils express the three components of the high-affinity IgE receptor Fc ϵ RI. These results are highlighted by the detection of

α , β , and γ chain mRNA in eosinophils but not in neutrophils and in lymphocytes, the contaminating cells, and the presence of α , β , and γ chains in HL60 cells differentiated toward the eosinophil lineage, as confirmed by their expression of IL-5R.¹⁰

Selective degranulation of eosinophils

Effector functions of eosinophils against parasites, as well as against mammalian cells, appear to be mainly mediated by the release of cationic proteins such as major basic protein, eosinophil cationic protein (ECP), eosinophil-derived neurotoxin, and EPO.¹¹ Moreover, it has been recently shown that EPO but not ECP could be released by purified eosinophils from patients with a parasitic infection or with allergic diseases after addition of the specific antigen or anti-IgE antibodies, suggesting that IgE-dependent activation could lead to a differential release of granule proteins.^{2, 12} Experiments were thus performed in order to investigate whether cross-linking of Fc ϵ RI could induce EPO release from eosinophils. Highly significant levels of EPO (index of release >15) could be detected after stimulation of purified eosinophils with anti-Fc ϵ RI α chain mAb 15-1, and anti-mouse IgG antibodies as the cross-linking reagent. In contrast, no significant EPO release was obtained in the presence of unrelated isotype control mAb or with the various controls. These results suggest that EPO could be released through engagement of Fc ϵ RI and indicate that IgE bound to Fc ϵ RI expressed by eosinophils could trigger their degranulation. The selectivity of EPO release through Fc ϵ RI was investigated by using a radioimmunoassay to evaluate the exocytosis of ECP in aliquots of the same supernatants in which EPO was detected. No significant release of ECP was detected after stimulation of eosinophils with anti-Fc ϵ RI α chain mAb 15-1 and further incubation with anti-mouse IgG antibodies. In contrast, elevated levels of ECP were obtained in supernatants of the same eosinophil preparations after activation with secretory IgA. These results confirm our previous reports showing EPO but not ECP release after IgE-dependent activation, whereas ECP was released after IgG- or IgA-dependent activation.^{2, 12} The reasons for differential exocytosis of eosinophil granule proteins are not clear. However, our findings are consistent with the hypothesis that engagement of Fc ϵ RI drives the same signal-inducing eosinophil degranulation as IgE immune complexes.

Role of FcεRI in eosinophil-mediated IgE-dependent cytotoxicity against schistosome targets

We attempted to determine whether FcεRI is involved in the cytotoxicity of eosinophils against parasites. Purified eosinophils, schistosoma targets, and IgE antibody-containing immune serum samples were incubated in the presence of mAb 15-1 or an isotype control mAb. A dose-dependent inhibition of cytotoxicity was observed with mAb 15-1 but not with the control antibody in the presence of one immune serum sample used as positive control. Moreover, when larger numbers of sera from patients infected with *Schistosoma mansoni* were used, not only were cytotoxicity levels significantly correlated with specific anti-*S. mansoni* IgE levels (Spearman's correlation test, $p < 0.05$) but they were also significantly inhibited by mAb 15-1 at the optimum concentration. None of the five control isotype serum samples from allergic patients was able to induce target cell lysis.

Presence of FcεRI in tissue eosinophils from patients with IgE-associated skin disorders

Tissue sections with infiltrating eosinophils obtained after skin biopsies were performed on patients with atopic dermatitis or bullous pemphigoid. A combined immunostaining of tissue eosinophils by anti-FcεRI α chain mAb and antibodies raised against ECP, used as an eosinophil marker, confirmed that the labeled cells were eosinophils. This study provides the first evidence that in addition to its role in mediating allergic responses,⁵ FcεRI may participate in a physiologic protective immune response. At least in vitro, FcεRI participates in eosinophil-mediated cytotoxicity against schistosomes. We propose that in vivo, parasitic infestations not only upregulate IgE and eosinophil production but also upregulate eosinophil FcεRI expression. FcεRI-expressing eosinophils are then able to bind IgE and participate in IgE-dependent cytotoxicity directed at the parasite, resulting in a coordinated antiparasitic immune response.

We postulate here that FcεRI on eosinophil membranes could drive a transduction signal to the cells that in turn could lead to the release of specific mediators. Because we have previously reported that IgE-, IgG- or IgA-dependent activation of eosinophils induced a differential release of the granule proteins,^{2, 12} it would be interesting to compare the different transduction signals delivered by various membrane receptors for immunoglobulins. On the other hand, because

only a subpopulation of eosinophils expressed detectable FcεRI, it will be of interest to investigate the factors able to influence FcεRI expression by eosinophils.

In conclusion, the results of this study indicate a so far unsuspected function of FcεRI in IgE-dependent antiparasite immune defense in addition to its role in allergic manifestations. These results therefore suggest that not only IgE and the low-affinity IgE receptor, but also the high-affinity IgE receptor, have probably appeared during evolution to protect organisms against pathogens rather than to induce pathologic allergic reactions. Although the precise interactions between the different types of IgE-binding molecules remain to be elucidated, their expression on eosinophil membranes and on other proinflammatory cells reinforces the dual function of these cell populations in both immune defense and diseases.

REFERENCES

1. Capron M, Grangette C, Torpier G, Capron A. The second receptor for IgE in eosinophil effector function. *Chem Immunol* 1989;47:128-78.
2. Tomassini M, Tsicopoulos A, Tai PC, et al. Release of granule proteins by eosinophils from allergic and nonallergic patients with eosinophilia on immunoglobulin-dependent activation. *J ALLERGY CLIN IMMUNOL* 1991;88:365-75.
3. Capron M, Truong MJ, Aldebert D, et al. Heterogeneous expression of CD23 epitopes by eosinophils from patients. Relationships with IgE-mediated functions. *Eur J Immunol* 1991;21:2423-9.
4. Truong MJ, Gruart V, Liu FT, Prin L, Capron A, Capron M. Human eosinophils express functional IgE binding molecules (Mac 2/EBP) of the S-type lectin family. *Eur J Immunol* 1993;23:3230-5.
5. Ravetch JV, Kinet JP. Fc receptors. *Annu Rev Immunol* 1991;9:457-92.
6. Wang B, Rieger A, Kilgus O, et al. Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεRI. *J Exp Med* 1992;175:1353-65.
7. Soussi Gounni A, Lamkhioed B, Ochiai K, et al. High affinity IgE receptor on eosinophils is needed in defense against parasites. *Nature* 1994;367:183-6.
8. Blank U, Ra C, Kinet JP. Characterization of truncated alpha chain products from human, rat, and mouse high affinity receptor or immunoglobulin E. *J Biol Chem* 1991;266:2639-46.
9. Hakimi J, Seals, Kondas JA, Pettine L, Danho W, Kochan J. The alpha subunit of the human IgE receptor (FcεRI) is sufficient for high affinity IgE binding. *J Biol Chem* 1990;265:22079-81.
10. Plaetinck G, Van der Heyden J, Tavernier J, et al. Characterization of interleukin-5 receptors on eosinophilic sublines from human promyelocytic leukemia (HL-60) cells. *J Exp Med* 1990;172:683-91.
11. Gleich GJ, Adolphson CR. The eosinophilic leukocyte: structure and function. *Adv Immunol* 1986;39:177-253.
12. Khalife J, Capron M, Cesbron JY, et al. Role of specific IgE antibodies in peroxidase (EPO) release from human eosinophils. *J Immunol* 1986;137:1659-64.