

Eosinophils: from low- to high-affinity immunoglobulin E receptors

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Several experimental approaches have been used to identify immunoglobulin (IgE) binding molecules expressed by human eosinophils. After the description that Fcε RII/CD23 identified on eosinophils could participate in IgE binding and IgE-mediated cytotoxicity, Mac2/ε binding proteins belonging to the S-type lectin family were also detected on human eosinophils. Anti-Mac2 monoclonal antibodies inhibited eosinophil-dependent cytotoxicity towards parasitic targets. More recently, Fcε RI was demonstrated on human eosinophils from hypereosinophilic patients. The 3 components of Fcε RI, α, β and γ chains, were detected in eosinophils. The α chain of Fcε RI was shown to be involved in IgE binding to eosinophils and in the selective release of eosinophil peroxidase. The participation of Fcε RI-bearing eosinophils in a protective immune response against a parasitic infection indicates a so far unsuspected function of Fcε RI. The interactions between the different types of IgE binding molecules are discussed.

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Experimental evidence have suggested that eosinophils bearing receptors for (immunoglobulin E) IgE could participate in immune defense against parasites as well as in allergic manifestations (2, 9). The first type of IgE binding molecule to be identified on eosinophil membrane was the low-affinity IgE receptor Fcε RII/CD23 (2, 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, in parallel to high amounts of the corresponding mRNA (10). Inhibition of IgE-binding and IgE-mediated cytotoxicity towards parasites by anti-Mac2 antibodies indicated the functional role of such molecules in IgE-associated diseases. A role for Fcε RI was first neglected because this receptor was believed to be solely expressed on mast cells and basophils (7). The demonstration that Fcε RI is present on Langerhans cells in the skin (11) suggested that it might be expressed by other immune effector cells.

The present report summarizes recent data show-

ing that Fcε RI is expressed on human eosinophils from hypereosinophilic patients (8), is involved in IgE binding to eosinophils and participates in the selective release of one granule mediator, the eosinophil peroxidase (EPO). The participation of Fcε RI-bearing eosinophils in a protective immune response against a parasitic infection associated with IgE indicates the *in vivo* relevance of our findings in relation to the dual function of IgE.

Expression of Fcε RII/CD23 and Mac2/εBP by eosinophils

In order to identify the molecules that bind IgE on human eosinophils, our first strategy was based on the described antigenic similarities between eosinophil IgE receptor and the B-cell Fcε RII/CD23 (2). This approach allowed us to demonstrate the variable membrane expression of several epitopes of CD23 by eosinophils from patients. Moreover, CD23 was shown to participate in IgE-binding and IgE-mediated cytotoxicity of human eosinophils (3). However, the low amounts of mRNA hybridizing with the CD23 cDNA probe led to the hypothesis

that human eosinophils might express another type of IgE-binding molecule. A family of molecules has particularly drawn our attention: Mac-2, a murine macrophage cell surface protein shown to be highly homologous to the carbohydrate binding protein 35 (CPB35) and to the rat IgE-binding protein (ϵ BP). These molecules, belonging to the S-type lectins (thiol-dependent) with a specific β -galactoside recognition domain, are detected on the cell surface, in the cytoplasm, and in the nucleus. They are expressed by various cell types, specifically after activation. The precise functions of these lectins are still unknown; however, ϵ BP has recently been proposed as a cell adhesion protein.

We have investigated whether human eosinophils can express molecules of this lectin family, using binding of antibodies directed against Mac-2 or against h ϵ BP and Northern blot with the corresponding cDNAs. In addition, we have evaluated the involvement of these molecules in the IgE-dependent function of eosinophils (10). The expression of Mac-2 molecules on the eosinophil surface was first analyzed by flow cytometry and compared with the expression of IgE receptors. Independently of eosinophil origin and density, surface Mac-2 molecules were detected in about half of patients with eosinophilia. Northern blot analysis performed with eosinophil RNA and probed with hMac-2 or h ϵ BP cDNA revealed the same RNA band at 1.2 kb in all eosinophil preparations, whether expressing membrane Mac-2 or not. Our results suggest that the mechanism of surface expression of Mac-2/ ϵ BP can be differentially regulated according to the patients. Both anti-Mac-2 and anti- ϵ BP detected a molecule at 29 kDa in eosinophil extracts by Western blot and immunoprecipitation. However, two lower-molecular-weight molecules could be also detected at 20 kDa and 15 kDa (10).

The involvement of Mac-2/ ϵ BP in the IgE-dependent functions of eosinophils was also investigated, since Mac-2/ ϵ BP has been shown to bind IgE through its carbohydrate moiety. Anti-Mac-2 monoclonal antibody (mAb) significantly inhibited, in a dose-dependent manner, the binding of ^{125} I IgE, in contrast to control mAb. Moreover, anti-Mac-2 mAb strongly inhibited IgE-dependent cytotoxicity of hypodense eosinophils against schistosome larvae compared with control antibodies (10). These results indicate the participation of Mac-2/ ϵ BP in IgE binding and IgE-dependent cytotoxicity mediated by eosinophils. The involvement of the two families of IgE-binding molecules (CD23 and Mac-2/ ϵ BP) in the cytotoxic function of eosinophils suggests an important role in the recognition of IgE by CRD domains of C- and S-type lectins. In addition to the role of Mac-2/ ϵ BP in IgE binding to eosinophils, the inhibition by anti-Mac-2 mAb of eosinophil adhe-

sion to parasite targets also suggests the function of such molecules as cell adhesion proteins and should be explored in other adhesive properties of eosinophils. Taken together, these results indicate that not only transmembrane receptors such as Fc ϵ RII/CD23 but also soluble molecules expressed on the surface of eosinophils might participate in the IgE-dependent effector function.

Surface expression and molecular characterization of Fc ϵ RI in eosinophils

Fc ϵ RI is a tetrameric structure composed of an IgE-binding α chain, a β chain and two disulfide-linked γ chains (7). Anti-Fc ϵ RI α chain mAb were raised in mice immunized with CHO cells transfected with human Fc ϵ RI α , γ complexes (11). One of these mAbs, the anti-Fc ϵ RI α chain mAb 15-1, was used to investigate the surface expression of Fc ϵ RI α chain on human eosinophils from patients with hypereosinophilic syndromes. By flow cytometry, positive staining was detected on 13 to 73% eosinophils, according to individual donors (8).

These results indicate that, in contrast to mast cells that constitutively express Fc ϵ RI, expression of Fc ϵ RI by blood eosinophils was variable according to patients. Immunostaining experiments with anti Fc ϵ RI α chain mAb 15-1 were performed on cytocentrifuged preparations of blood eosinophils. The staining was revealed by the APAAP procedure (11). Positive staining was found in some eosinophils. In contrast, no staining was detectable with the isotopic control mAb.

The α chain of the high-affinity receptor is known to contain the binding site of IgE. To examine the expression of mRNA corresponding to the α chain of Fc ϵ RI receptor by eosinophils, Northern hybridization was performed using a cDNA probe coding for human α chain of Fc ϵ RI. RNA extracts from eosinophils of all patients tested gave a signal at 1.2 kb but with varying intensity according to individual patients (8).

As previously shown, transfection of cDNA for either the human α and rat γ subunits (1) or a chimeric human α subunit alone into heterologous mammalian cells resulted in cells capable of binding human IgE myeloma protein with high affinity (5). The question remained whether eosinophils could express β and γ chains. Owing to 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 Fc ϵ RI β chain and 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 3 components of the high-affinity IgE receptor Fcε RI (8).

Functional aspects of Fcε RI expressed by human eosinophils

The involvement of Fcε 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 hypereosinophilic patients were incubated with ¹²⁵I-labeled monomeric human IgE together with increasing concentrations of the anti-Fcε RI α chain mAb 15-1 or a control mAb, and the cell-bound radioactivity was evaluated. The monoclonal 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 μg/ml), the inhibition is complete and comparable to the inhibition seen with 1 mg/ml of unlabeled human IgE. Under the same conditions, 1 mg/ml 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 Fcγ receptor. 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 (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO) (4). 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 protein (9, 6). Experiments were then performed to determine whether cross-linking of Fcε RI could induce EPO release from eosinophils. Highly significant levels of EPO could be detected after stimulation of purified eosinophils with anti-Fcε RI α chain mAb 15-1, and anti-mouse IgG antibodies as 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 Fcε RI, and they also indicate that IgE bound to Fcε RI expressed by eosinophils could trigger their degranulation. The selectivity of EPO release through Fcε 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-Fcε RI α chain mAb 15-1 and further incubation

with antimouse 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 (6, 9). The reasons for differential exocytosis of eosinophil granule proteins are not clear. However, our findings are consistent with the hypothesis that engagement of Fcε RI drives the same signal inducing eosinophil degranulation as IgE immune complexes.

Finally, we analyzed whether Fcε RI is involved in the cytotoxicity of eosinophils against parasites. Purified eosinophils, *Schistosoma* targets and IgE antibody-containing immune sera were incubated in the presence of mAb 15-1 or an isotype control mAb. A dose-dependent inhibition of cytotoxicity is observed with mAb 15-1 but not with the control antibody, in the presence of one immune serum used as positive control. Moreover, when larger numbers of sera from *Schistosoma mansoni* infected patients were used, not only cytotoxicity levels significantly correlated with specific anti-*S. mansoni* IgE levels but were also significantly inhibited by mAb 15-1 at the optimum concentration. None of the 5 control isotype sera from allergic patients was able to induce target cell lysis.

This study provides the first evidence that, in addition to its role in mediating allergic responses (7), Fcε RI may participate in a physiologic protective immune response. At least *in vitro*, Fcε RI participates in eosinophil-mediated cytotoxicity against schistosomes. We propose that, *in vivo*, parasitic infestations not only up-regulate IgE and eosinophil production but also up-regulate eosinophil Fcε RI expression. Fcε RI-expressing eosinophils are then able to bind IgE and participate in IgE-dependent cytotoxicity directed at the parasite, resulting in a coordinated anti-parasitic immune response.

We postulate here that Fcε RI on eosinophil membrane could drive a transduction signal to the cells that, in turn, could lead to the release of specific mediators. Since we have previously reported that IgE-, IgG- or IgA-dependent activation of eosinophils induced a differential release of the granule proteins (6, 9), it would be interesting to compare the different transduction signals delivered by various membrane receptors for immunoglobulins. On the other hand, since only a subpopulation of eosinophils expressed detectable Fcε RI, it will be of interest to investigate the factors able to influence Fcε RI expression by eosinophils. In a kinetic study of eosinophil differentiation from cord blood cell precursors, it has recently been shown that Fcε RI expression was an early and transient event (Morita et al., in preparation).

In conclusion, the present results indicate a so far unsuspected function of Fcε RI in IgE-dependent antiparasite immune defense, in addition to its role in allergic manifestations. They lead therefore to suggest that not only IgE and 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 pathological allergic reactions. Although the precise interactions between the different types of IgE binding molecules remain to be elucidated, their expression on eosinophil membrane as well as on other proinflammatory cells reinforces the dual function of these cell populations both in immune defense and in disease.

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