

Antigen-Specific IgG and IgA, but Not IgE, Activate the Effector Functions of Eosinophils in the Presence of Antigen

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Key Words

Eosinophils · Dansyl · Immunoglobulins · Superoxides · Eosinophil granule proteins · Eosinophil-derived neurotoxin

Abstract

Background: Activated eosinophils are thought to play an important role in allergic inflammation. Prior reports suggest that eosinophils have receptors recognizing IgA, IgG and IgE; however, little is known regarding the direct effects of antigens and antigen-specific immunoglobulins on the functions of eosinophils. **Methods:** To investigate eosinophil activation by antigens mediated by the various antigen-specific immunoglobulins, we used dansyl-conjugated bovine serum albumin (DNS-BSA) and recombinant dansyl-specific antibodies (human IgG 1–4, IgA and IgE). Eosinophils from healthy donors were incubated in the wells coated with dansyl-specific immunoglobulins with or without DNS-BSA. Eosinophil activation was monitored by superoxide production and eosinophil-derived neurotoxin (EDN) release. **Results:** Superoxide production and EDN release by eosinophils were induced by the dansyl-specific reaction via all IgG subclasses (IgG 1–4) and IgA in the presence of DNS-BSA; the responses were not observed in the absence of antigen, DNS-BSA. The immune complexes (ICs) of DNS-BSA and dansyl-specific IgE did not induce these responses. Furthermore,

IgE ICs did not enhance eosinophil activation stimulated with various immunoglobulins, IL-5 or platelet-activating factor. **Conclusion:** These data suggest that ICs of antigens and antigen-specific IgGs and IgA, but not IgE, in inflamed tissues may activate eosinophils and play an important role in allergic inflammation.

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Introduction

Eosinophils express a wide array of cell-surface molecules, including cytokine receptors, chemokine receptors, FcγRII (CD32), FcαRI (CD89), complement receptors, adhesion molecules, CD9 and CD69 [1]. They can be activated by various classes of secretagogues, such as cytokines (IL-5 and GM-CSF), lipid mediators (platelet-activating factor, PAF), immunoglobulins and complement fragments (C5a) [2, 3]. These activated eosinophils may play an important role in allergic inflammation and the pathological remodeling in allergic diseases [3, 4].

Ligation of cell surface IgE on mast cells by antigen (that is allergen) induces release of histamine, arachidonic acid derivatives and other mediators. These mast cell-derived mediators play central roles in the acute-phase allergic response [5]. In the late-phase response after antigen exposure in patients with allergic asthma, eosino-

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phils infiltrate predominantly into the airway tissues and are involved in airway inflammation [5]. Increased levels of eosinophil peroxidase are detected after stimulation of purified eosinophils from hypereosinophilic patients by cross-linking with anti-FcεRI monoclonal antibody [6]. IgE-dependent cytotoxicity of eosinophils against parasites is inhibited by FcεRI antibody [6]. Kayaba et al. [7] detected unoccupied FcεRI on the surface of eosinophils from normal donors, approximately 4,500 antibodies binding sites per cell, while 50,000 antibodies binding sites per cell are detected intracellularly. However, other reports have not demonstrated expression of functional FcεRI by eosinophils [8, 9], and the expression of FcεRI on eosinophils may be minimal. Thus, the roles of IgE and FcεRI in eosinophilic inflammation are not totally clear.

In airway secretions, IgA (60–70%) and IgG (30–40%) account for 99% of the total immunoglobulins [10]. Not only allergen-specific IgE, but also allergen-specific IgA and IgG, have been detected in the nasal secretions of patients with intermittent allergic rhinitis [11]. Furthermore, levels of specific IgA and IgG antibodies to antigen (*D. farinae*) are elevated in induced sputum from patients with atopic asthma, who are sensitized to *D. farinae*, and sputum eosinophil cationic protein levels correlate significantly with levels of IgA and IgG to *D. farinae* [12]. These results suggest that antigen-specific IgG and IgA may be involved in eosinophilic inflammation.

In a previous study, Kaneko et al. [13] showed that immune complexes (ICs) obtained from short ragweed pollen and sera from short ragweed pollen-sensitive patients induced superoxide production and eosinophil-derived neurotoxin (EDN) release. They also showed that plate-coated myeloma IgG1 and IgG3, and less consistently IgG2, induce eosinophil degranulation and that these reactions are abolished by anti-FcγRII antibodies. FcαR have been established on a variety of cell types, including eosinophils [14]. IgA also induced superoxide production and degranulation by eosinophils [15].

To the best of our knowledge, there have been few previous reports that systematically examined the direct effects of antigen and antibody ICs on eosinophil activation. Therefore, by taking the advantage of recombinant immunoglobulins, we used dansyl (5-dimethylaminonaphthalene-1-sulfonyl radical)-conjugated bovine serum albumin (DNS-BSA) as an antigen and recombinant dansyl-specific antibodies (human IgG 1–4, IgA and IgE) and investigated systematically the effects of various Ig ICs on eosinophil activation.

Materials and Methods

Materials

Reagents were obtained as follows: IL-5 is a gift from Schering-Plough Research Institute (Kenilworth, N.J., USA), PAF is from Biomol (Plymouth, Pa., USA). DNS-BSA and recombinant human anti-dansyl IgG1, IgG2, IgG3, IgG4, IgA and IgE were generously provided by Dr. Sherie L. Morrison, University of California (Los Angeles, Calif., USA) [16, 17].

Eosinophil Isolation

Eosinophils were purified from peripheral blood obtained from healthy volunteers using the negative selection method, as described previously [18, 19]. Briefly, venous blood anti-coagulated with heparin was diluted with PIPES (with NaOH, glucose, KCl, and NaCl) at a 1:1 ratio. Diluted blood was overlaid on Percoll (density = 1.085 g/ml) and centrifuged at 900 *g* to remove mononuclear cells and plasma. Erythrocytes were lysed by two cycles of hypotonic shock, and neutrophils were removed from the eosinophils by separation using anti-CD16-conjugated magnetic beads (Miltenyi Biotec, Auburn, Calif., USA) and a magnetic cell separation system (Miltenyi Biotec). Eosinophil purity exceeded 95% as determined by Randolph's stain. Purified eosinophils were washed with medium (Hanks' balanced salt solution; Life Technologies, Grand Island, N.Y., USA) with 0.01% human serum albumin (HSA; Sigma, St. Louis, Mo., USA) and 10 mM HEPES (Celox Laboratories, St. Paul, Minn., USA), pH 7.4, and resuspended to 5×10^5 cells/ml in cytochrome c solution (2.4 mg/ml cytochrome c in medium). The study protocol was approved by the Institutional Review Board at Mayo Clinic (Rochester, Minn., USA), and informed consent was obtained from all donors.

Superoxide Production Assay

Ninety-six-well tissue culture plates were coated with 50 μl of DNS-BSA (50 μg/ml in phosphate-buffered saline, PBS) or PBS at 4°C overnight. Wells were washed with PBS and blocked with 50 μl of 2.5% HSA for 2 h at 37°C. Blocked wells were incubated with 50 μl of dansyl-specific IgG subclasses (IgG1, IgG2, IgG3 or IgG4; 0.03–3 μg/ml in PBS each), IgA (0.03–30 μg/ml in PBS), IgE (0.03–30 μg/ml in PBS) or PBS alone 2 h at 37°C and washed with PBS. One hundred microliters of freshly isolated eosinophils (5×10^4 cells/well) and 100 μl of medium were placed in the wells and incubated for 4 h at 37°C. Immediately after eosinophils were placed, the absorbance at 550 nm was analyzed in a Thermomax plate reader (Molecular Devices, Sunnyvale, Calif., USA) followed by readings every 15–30 min. Conversion of absorbance to nanomoles of cytochrome c reduced per 1×10^6 eosinophils used the following equation: $19.1 \times (\text{absorbance} - \text{absorbance}_{\text{time 0}})/0.05 = \text{nmol cytochrome c reduced}/10^6 \text{ cells}$ [20, 21].

In separate experiments, to examine the combined effects of IgG subclasses and IgA and IgE, the wells coated with DNS-BSA and dansyl-specific IgG subclasses (IgG1, IgG2, IgG3 or IgG4; 1 μg/ml in PBS each), dansyl-specific IgA (1 μg/ml in PBS) or PBS were further coated with 50 μl of dansyl-specific IgE (10 μg/ml in PBS) for 2 h at 37°C. To examine the effects of IL-5 and PAF on IgE-mediated eosinophil responses, 100 μl of freshly isolated eosinophils and 100 μl of IL-5 (at final concentration of 0.1–10 ng/ml) or PAF (at final concentration of 0.001–1 μM) were incubated in the wells coated with DNS-BSA and dansyl-specific IgE (10 μg/ml in PBS).

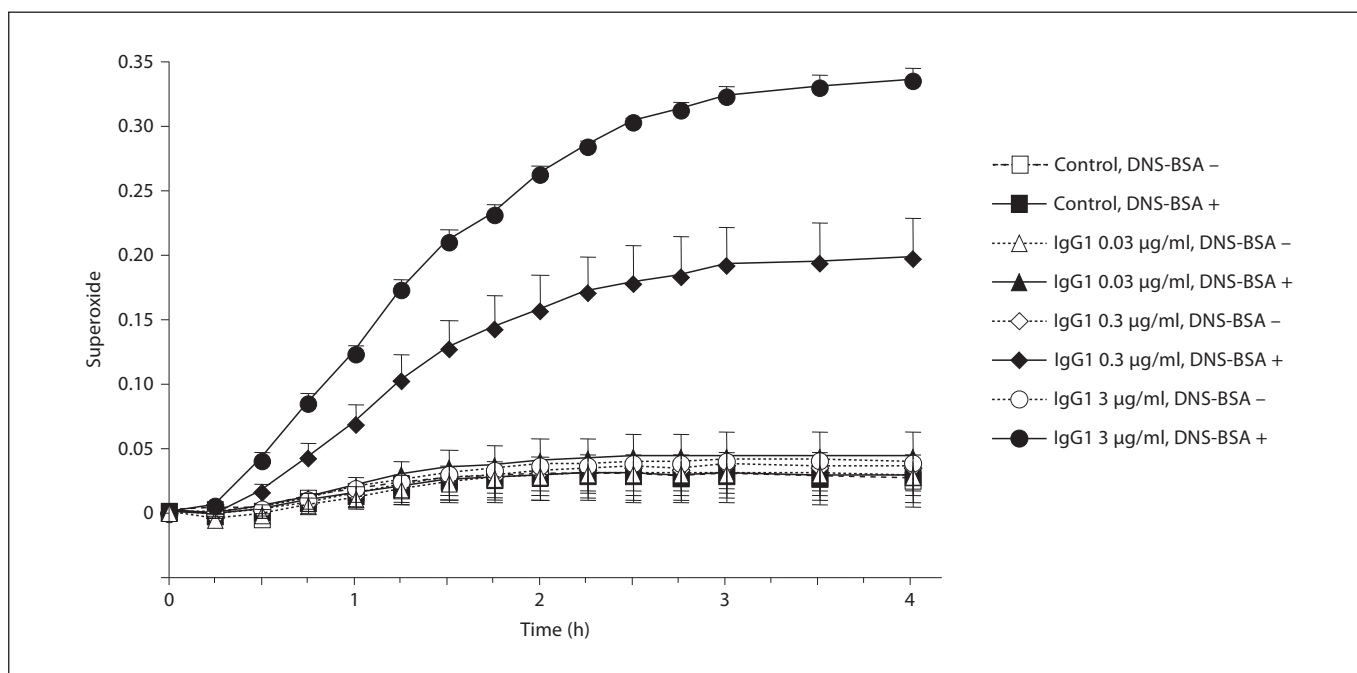


Fig. 1. Superoxide production by eosinophils via dansyl-specific IgG1 in the presence or absence of DNS-BSA. Isolated human eosinophils were added to the wells that had been coated with or without DNS-BSA and preincubated with dansyl-specific IgG1 at the concentrations shown. To detect superoxide production, the absorbance at 550 nm was monitored in a Thermomax plate reader every 15–30 min for 4 h at 37°C. (n = 6, each).

EDN Assay

To quantitate eosinophil degranulation, supernatants were collected after the 4 h eosinophil incubation in the superoxide assay and stored at –20°C. Concentrations of EDN in sample supernatants were measured by radioimmunoassay. The radioimmunoassay is a double-antibody competition assay in which radioiodinated EDN, rabbit anti-EDN antibody, and burro anti-rabbit IgG are used as described previously [18, 21]. All assays were carried out in duplicate.

Histamine Assay

In separate experiments, histamine release by basophils was measured, using the same methods as eosinophil degranulation assay. Ninety-six-well plates were coated with DNS-BSA or PBS overnight at 4°C and were blocked with 2.5% HSA for 2 h at 37°C. After washing with PBS, wells were then coated with 50 µl of dansyl-specific IgE (0.03–30 µg/ml in PBS, each) or PBS for 2 h at 37°C.

The mononuclear cell layer, including basophils, was isolated from peripheral blood obtained from healthy volunteers by density centrifugation with Histopaque-1077 (Sigma). The cells were suspended in Hanks' balanced salt solution with 0.01% HSA and 10 mM HEPES, placed in each well, and incubated 1 h at 37°C. Supernatants were collected and stored at –20°C. Histamine concentrations in supernatants were measured by an enzyme immunoassay kit (Immunotech, Marseille, France). The histamine levels in supernatants were normalized to histamine levels per 1,000 basophils.

Statistical Analysis

Data are summarized as mean ± SE or box plots. Statistical differences were assessed with the paired nonparametric Wilcoxon signed rank test and were significant with a p value <0.05.

Results

Superoxide Production by Eosinophils via Dansyl-Specific IgG Subclasses, IgA, and IgE

Eosinophil superoxide production stimulated with ICs of DNS-BSA and dansyl-specific IgG subclasses (0.03–3 µg/ml), IgA (0.03–30 µg/ml) or IgE (0.03–30 µg/ml), or by each immunoglobulin class or subclass alone without DNS-BSA was investigated. Figure 1 shows the kinetics of eosinophil superoxide production induced by ICs of DNS-BSA and IgG1. When eosinophils are incubated with dansyl-specific IgG1 with DNS-BSA, the levels of superoxide production increased in a time-dependent manner and reached a plateau at 3 h after incubation. No superoxide production was observed, when wells were not coated with DNS-BSA. Other IgG subclasses ICs or IgA ICs also induced superoxide production in similar time course (data not shown). The results from 6 eosino-

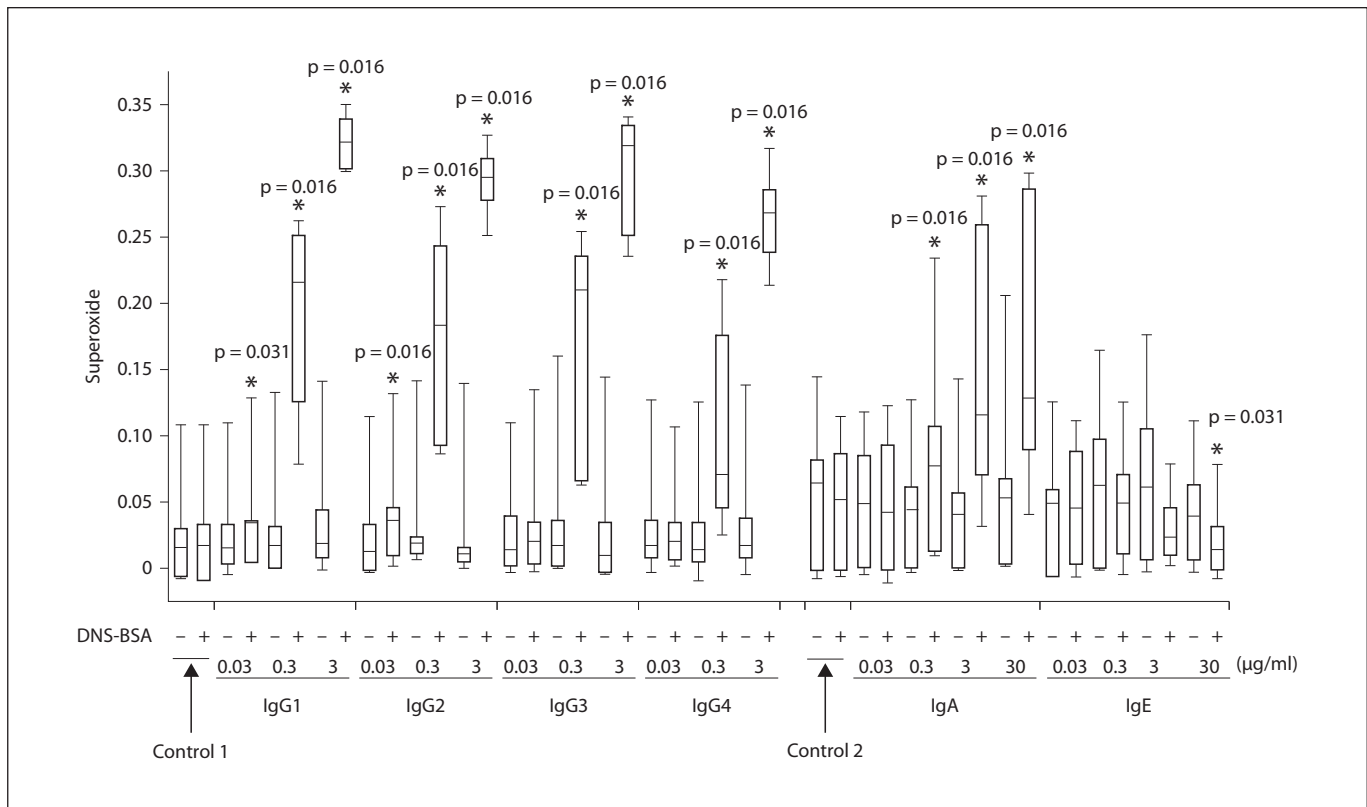


Fig. 2. Superoxide production by eosinophils via dansyl-specific IgG subclasses (IgG1–4), IgA and IgE in the presence or absence of DNS-BSA. Using the same conditions as described in figure 1, the levels of superoxide production (after 3 h of incubation) are shown for the IgG subclasses, IgA and IgE. Control 1 is the control for all IgG subclasses and control 2 is for IgA and IgE (n = 6, each). * p < 0.05 vs. control group with DNS-BSA.

phil donors are summarized in figure 2. ICs of DNS-BSA and all IgG subclasses or IgA dose-dependently induced eosinophil superoxide production at 3 h after incubation (p < 0.05, n = 6). Furthermore, all IgG subclasses and IgA did not induce superoxide production, when wells were not coated with DNS-BSA. In contrast, dansyl-specific IgE (30 µg/ml) did not induce superoxide production with or without DNS-BSA. Instead, IgE antibody suppressed basal superoxide production slightly (p < 0.05, n = 6).

EDN Release by Eosinophils via Dansyl-Specific IgG Subclasses, IgA and IgE

EDN release by eosinophils stimulated with ICs of DNS-BSA and dansyl-specific IgG subclasses (0.03–3 µg/ml), IgA (0.03–30 µg/ml) or IgE (0.03–30 µg/ml), or by each immunoglobulin class or subclass alone was investigated. ICs of DNS-BSA and all IgG subclasses or IgA

dose-dependently induced EDN release by eosinophils (fig. 3). On the other hand, similarly to superoxide production, ICs of DNS-BSA and IgE (3 and 30 µg/ml) did not induce degranulation but slightly suppressed basal EDN (p < 0.05, n = 6).

Superoxide Production and EDN Release by Eosinophils from Each Donor

We investigated the distribution of eosinophil superoxide production and EDN release from all 6 different donors. In figure 4, each symbol represents the response of each donor (concentration of each immunoglobulin: 3 µg/ml). The ICs from all IgG subclasses induced elevated levels of both superoxide production and degranulation in all donors. However, the ability of IgA ICs to induce superoxide production and degranulation was dependent on eosinophil donors and showed a different distribution.

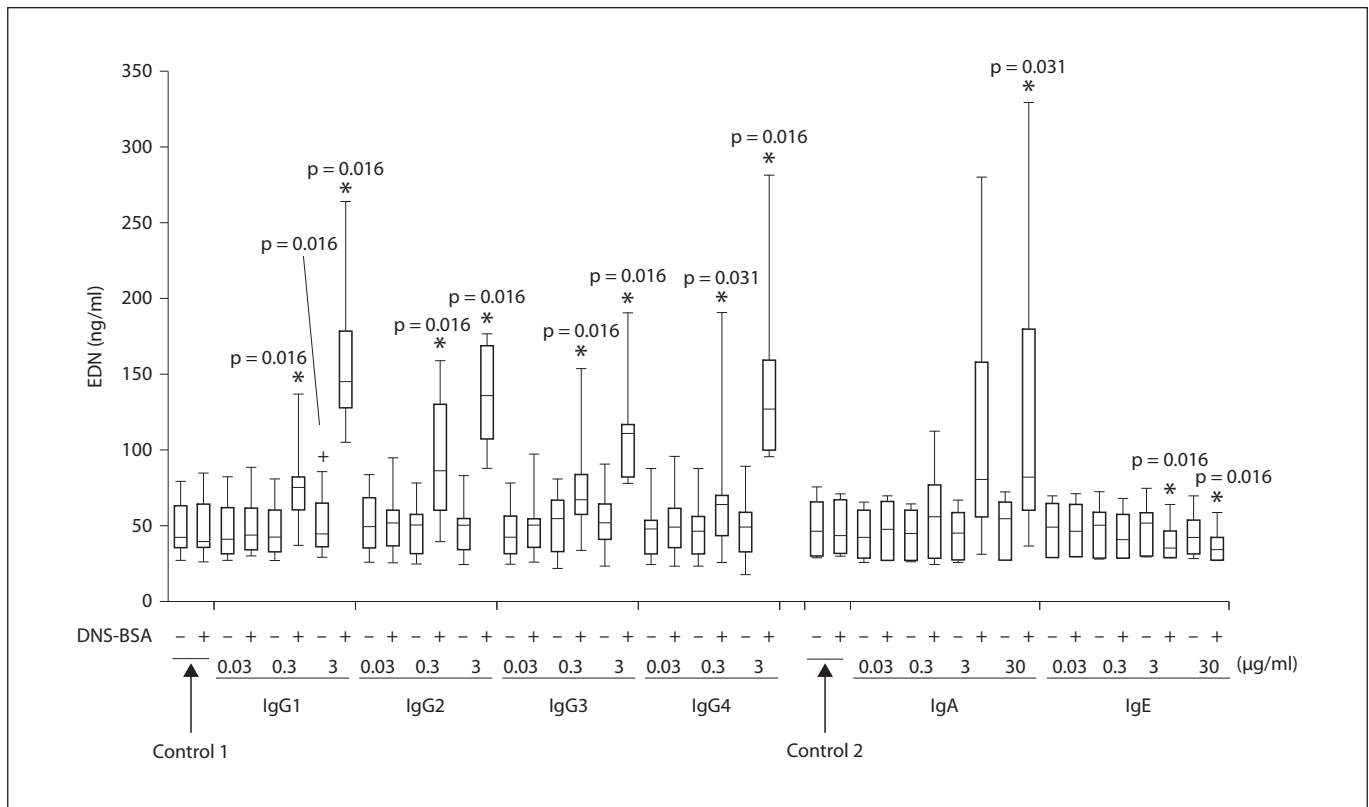


Fig. 3. EDN release by eosinophils via dansyl-specific IgG subclasses (IgG1–4), IgA and IgE in the presence or absence of DNS-BSA. To quantitate eosinophil degranulation after 4 h of incubation as described in figure 1, the concentration of EDN in cell supernatants was measured by radioimmunoassay. Control 1 is the control for all IgG subclasses and control 2 is for IgA and IgE (n = 6). * p < 0.05 vs. control group in the presence of DNS-BSA; † p < 0.05 vs. control group in the absence of DNS-BSA.

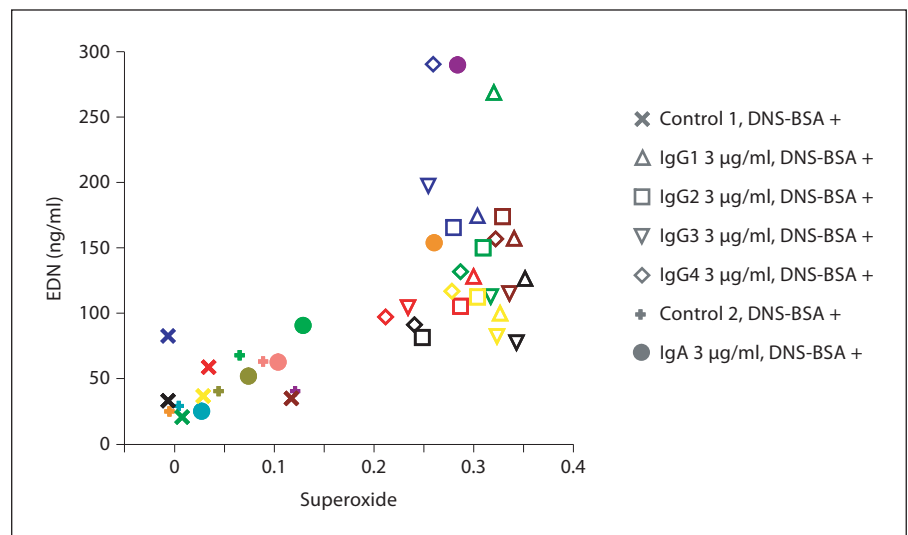


Fig. 4. Correlation of superoxide production and EDN release by eosinophils from each donor. This plot compares each donor's superoxide production (after 3 h of incubation) and EDN release in wells containing DNS-BSA and either an IgG subclass or IgA. Control 1 is the control for all IgG subclasses and control 2 is for IgA. Each individual donor was shown by each different color.

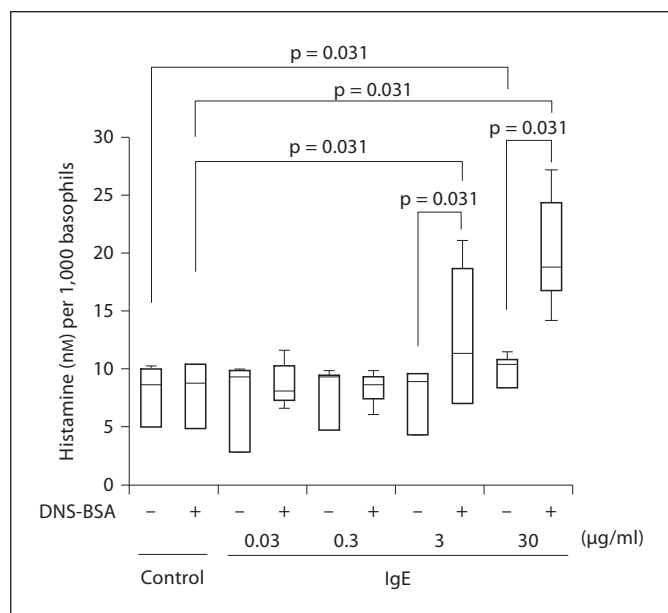


Fig. 5. Histamine release by basophils from healthy donors using IgE ICs. The monocyte layer, enriched in basophils, was incubated in wells that had been coated with or without DNS-BSA and preincubated with dansyl-specific IgE at the concentrations shown for 1 h at 37°C. Histamine levels released into the medium were measured. (n = 5).

Histamine Release from Basophils

As shown above, antigen-specific IgE did not induce superoxide production and degranulation by eosinophils. Therefore, to examine whether dansyl-specific IgE in the present study is functionally active, we investigated histamine release by basophils. IgE ICs using DNS-BSA and dansyl-specific IgE dose-dependently induced histamine release by basophils (fig. 5). No histamine release was observed, when wells were not coated with DNS-BSA.

Effect of IgE ICs on Enhanced Superoxide Production and EDN Release by Other Eosinophil Agonists

Because eosinophils are likely exposed to various inflammatory mediators together with IgE antibodies in the tissues, we examined whether priming of eosinophils with other agonists, such as IgG antibodies, IL-5 or PAF, could provoke the antigen-specific IgE responses in eosinophils.

First, the effect of IgE (10 µg/ml) ICs on eosinophil activation by IgG subclasses (1 µg/ml, each) ICs or IgA (1 µg/ml, each) ICs was investigated. Table 1 shows the superoxide production levels (after 3 h incubation) and EDN release. In both superoxide production and EDN

release assay, the IgE ICs did not affect the eosinophil activation stimulated by each immunoglobulin (IgG1, IgG2, IgG3, IgG4 and IgA) ICs'.

Second, the effect of antigen-specific IgE (10 µg/ml) ICs on eosinophil activation by IL-5 (0.1–10 ng/ml) or PAF (0.001–1 µM) was investigated. IgE ICs did not enhance eosinophil activation induced by IL-5 or PAF (table 1). Rather, IgE ICs slightly but significantly suppressed eosinophil superoxide production stimulated with 0.1 ng/ml and 1 ng/ml of IL-5 or with 0.01–0.1 µM of PAF (p < 0.05).

Discussion

In this study, we systematically examined the effects of various classes and subclasses of antigen-specific Igs complexed with antigen on eosinophil effector functions. ICs composed of DNS-BSA and anti-dansyl antibody have been used in other experimental models [22], and recombinant dansyl-specific immunoglobulins have been previously characterized by Morrison et al. [16].

Immobilized IgG, IgA, and secretory IgA (sIgA) induce eosinophil degranulation [13, 23], and various lipid mediators (such as PAF) and cytokines (such as IL-5) also activate eosinophils [21]. Furthermore, activated eosinophils release granule proteins (for example major basic protein and EDN), lipid mediators, reactive oxygen intermediates, cytokines and enzymes [2]. In the present study, ICs of all IgG subclasses induced eosinophil superoxide production and EDN release from 6 different cell donors. The responses to IgA ICs were dependent on eosinophil donors. However, IgE ICs did not induce superoxide production and degranulation, but slightly suppressed spontaneous basal response.

FcγRs on eosinophils have been extensively studied for their abilities to mediate eosinophil functions, including phagocytosis, antigen-dependent cytotoxicity, oxygen metabolism, leukotriene C4 production and degranulation [2]. Graziano et al. [24] showed that FcγR directly mediate eosinophil cytotoxicity. Flow-cytometric analyses of human cells revealed that freshly isolated eosinophils possess only FcγR II [25]. In addition, cytometric analyses and degranulation studies suggest that eosinophils also have IgA receptors, and Sepharose 4B beads coated with IgA or sIgA induce degranulation of eosinophils [26]. We found that ICs from all IgG subclasses induced similar eosinophil superoxide production and EDN release, but the eosinophils from different individuals reacted differently to IgA ICs (fig. 4). The concentra-

Table 1. Effect of IgE ICs on enhanced superoxide production and EDN release by IgG subclasses, IgA, IL-5 or PAF

	DNS-BSA	Superoxide (at 3 h after incubation)	EDN, ng/ml
Control 1	IgE–	0.026 ± 0.018 (p = 0.008)	31.10 ± 5.60
Control 1	IgE+	0.007 ± 0.010	28.89 ± 4.57
IgG1	IgE–	0.159 ± 0.042	56.33 ± 12.60
IgG1	IgE+	0.154 ± 0.038	56.87 ± 11.48
IgG2	IgE–	0.166 ± 0.037	60.96 ± 16.43
IgG2	IgE+	0.157 ± 0.037	59.71 ± 14.27
IgG3	IgE–	0.135 ± 0.039	46.89 ± 10.05
IgG3	IgE+	0.117 ± 0.042	43.78 ± 7.90
IgG4	IgE–	0.097 ± 0.038 (p < 0.1)	41.74 ± 9.17
IgG4	IgE+	0.079 ± 0.033	38.19 ± 7.12
IgA	IgE–	0.037 ± 0.021	30.67 ± 4.60
IgA	IgE+	0.034 ± 0.019	31.81 ± 5.42
Control 2	IgE–	0.054 ± 0.017 (p = 0.014)	51.06 ± 10.44 (p = 0.006)
Control 2	IgE+	0.029 ± 0.009	46.80 ± 8.83
IL-5, 0.1 ng/ml	IgE–	0.055 ± 0.038 (p = 0.031)	58.51 ± 23.86 (p = 0.031)
IL-5, 0.1 ng/ml	IgE+	0.022 ± 0.025	50.13 ± 17.48
IL-5, 1 ng/ml	IgE–	0.197 ± 0.035 (p = 0.020)	76.96 ± 16.35 (p < 0.1)
IL-5, 1 ng/ml	IgE+	0.181 ± 0.036	73.88 ± 15.27
IL-5, 10 ng/ml	IgE–	0.246 ± 0.022	92.75 ± 19.57 (p < 0.1)
IL-5, 10 ng/ml	IgE+	0.223 ± 0.030	88.62 ± 19.72
Control 3	IgE–	0.023 ± 0.018 (p < 0.1)	48.67 ± 16.51 (p = 0.016)
Control 3	IgE+	0.008 ± 0.011	44.60 ± 14.55
PAF, 0.001 μM	IgE–	0.111 ± 0.052 (p = 0.031)	75.05 ± 24.24 (p < 0.1)
PAF, 0.001 μM	IgE+	0.083 ± 0.050	66.61 ± 25.13
PAF, 0.01 μM	IgE–	0.166 ± 0.041 (p = 0.016)	73.47 ± 21.69 (p = 0.016)
PAF, 0.01 μM	IgE+	0.137 ± 0.050	64.84 ± 21.47
PAF, 0.1 μM	IgE–	0.175 ± 0.028 (p = 0.016)	81.85 ± 23.73
PAF, 0.1 μM	IgE+	0.150 ± 0.034	76.45 ± 25.20
PAF, 1 μM	IgE–	0.176 ± 0.021	105.05 ± 26.08
PAF, 1 μM	IgE+	0.179 ± 0.025	104.82 ± 28.39

Control 1, 2 and 3 are the control for immunoglobulins, IL-5 and PAF, each. p values obtained by comparison of IGE– with IgE+ DNS-BSA.

tions of immobilized immunoglobulins, which we used in the present study, were less than 0.03–0.01 of the concentrations used in other reports [23, 27–29]. Nevertheless, our results showed robust eosinophil activation (superoxide production and degranulation) similar to these earlier reports. We suspect that these lower concentrations of immunoglobulins were able to strongly stimulate eosinophils because we used an antigen-specific experimental system rather than patients' sera or polyclonal immunoglobulins.

sIgA is the main immunoglobulin in airway mucosa in allergic diseases and it may be the most important immunoglobulin to stimulate eosinophils [30, 31]. sIgA is

the most abundant class of antibodies at mucosal surfaces where eosinophils carry out many of their effector functions [32, 33]. Thus, future studies with recombinant, antigen-specific sIgA will provide more insights into the roles of sIgA in eosinophil activation in mucosal tissues.

Several studies have suggested that IgE stimulates the eosinophils' cytotoxic function and degranulation [34, 35], and that eosinophils express high-affinity receptors, FcεRI [7]. Also, PAF and LTB4 upregulated the expression of low-affinity receptors, FcεRII, on eosinophils in vitro [36]. In contrast, other studies reported that the numbers of FcεRI expressed on surfaces of eosinophils

from patients with allergic disease or airway eosinophilia were minimal or undetectable [8] and that ligation of FcεRI did not result in detectable eosinophil degranulation [9]. Our results showed that IgE ICs (even with high concentrations of IgE) did not induce or enhance eosinophil activation, but they seemed to slightly suppress the basal response. A direct comparison of antigen-specific IgG and IgE (fig. 2, 3) shows that antigen-specific IgE does not lead to eosinophil functional responses under the same experimental conditions that lead to the eosinophils' responses to antigen-specific IgG. In addition, eosinophils primed with IL-5 or PAF also failed to respond to IgE ICs. We also verified that ICs of DNS-BSA and dansyl-specific IgE are able to induce histamine release by basophils. Altogether, these findings suggest that IgE antibody may not contribute to the activation of eosinophils, unlike mast cells or basophils.

FcγRII (CD32) is the major FcγR expressed on mature human eosinophils with no or minimal expression of FcγRI (CD64) or FcγRIII (CD16) [25, 37]. However, various stimulants (for example PAF, IFN-γ [38,39] and non-specific IgG ICs [40]) did induce the enhanced expression of FcγRII and/or the expression of FcγRI or FcγRIII by eosinophils. Furthermore, the transient *in vivo* expression of FcγRIII is observed after aeroallergen challenge

of patients with asthma [40]. Eosinophils from allergic individuals also displayed an enhanced FcαR expression [14] and showed markedly increased IgA binding [29]. Thus, eosinophils in allergic inflammatory sites may be activated, and the expression of certain immunoglobulin receptors is enhanced in these eosinophils, resulting in increased antigen-specific eosinophil responses.

Eosinophil infiltration into airway tissue is observed after antigen challenge in patients with allergic asthma. In summary, our data show that antigen in the presence of antigen-specific antibodies (IgG1, IgG2, IgG3, IgG4 and IgA) induces activation and pro-inflammatory mediator release by human eosinophils. Eosinophils in the tissues of patients with allergic diseases may express increased levels of functional receptors for IgG and IgA. We propose that eosinophils, interacting with antigens via specific IgG and IgA antibodies and eosinophil receptors, may play roles in the pathophysiology of eosinophilic disorders, such as bronchial asthma.

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References

- Prussin C, Metcalfe DD: 4. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2003;111:S486–S494.
- Kita H, Adolphson CR, Gleich GJ: Biology of eosinophils; in Adkinson NFJr, Yunginger JW, Busse WW, Bochner BS, Holgate ST, Simons FER (eds): *Middleton's Allergy Principles and Practice*, ed 6. Philadelphia, Mosby, 2003, vol 1, pp 305–332.
- Trivedi SG, Lloyd CM: Eosinophils in the pathogenesis of allergic airways disease. *Cell Mol Life Sci*. 2007;64:1269–1289.
- Jacobsen EA, Ochkur SI, Lee NA, Lee JJ: Eosinophils and asthma. *Curr Allergy Asthma Rep*. 2007;7:18–26.
- Pearlman DS: Pathophysiology of the inflammatory response. *J Allergy Clin Immunol* 1999;104:S132–S137.
- Gounni AS, Lamkhieou B, Ochiai K, Tanaka Y, Delaporte E, Capron A, Kinet JP, Capron M: High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* 1994;367:183–186.
- Kayaba H, Dombrowicz D, Woerly G, Papin JP, Loiseau S, Capron M: Human eosinophils and human high affinity IgE receptor transgenic mouse eosinophils express low levels of high affinity IgE receptor, but release IL-10 upon receptor activation. *J Immunol* 2001; 167:995–1003.
- Seminario MC, Saini SS, MacGlashan DW Jr, Bochner BS: Intracellular expression and release of Fc epsilon RI alpha by human eosinophils. *J Immunol* 1999;162:6893–6900.
- Kita H, Kaneko M, Bartemes KR, Weiler DA, Schimming AW, Reed CE, Gleich GJ: Does IgE bind to and activate eosinophils from patients with allergy? *J Immunol* 1999;162: 6901–6911.
- Peebles RS Jr, Liu MC, Lichtenstein LM, Hamilton RG: IgA, IgG and IgM quantification in bronchoalveolar lavage fluids from allergic rhinitis, allergic asthmatics, and normal subjects by monoclonal antibody-based immunoenzymetric assays. *J Immunol Methods*. 1995;179:77–86.
- Benson M, Reinholdt J, Cardell LO: Allergen-reactive antibodies are found in nasal fluids from patients with birch pollen-induced intermittent allergic rhinitis, but not in healthy controls. *Allergy* 2003;58:386–392.
- Nahm DH, Kim HY, Park HS: Elevation of specific immunoglobulin A antibodies to both allergen and bacterial antigen in induced sputum from asthmatics. *Eur Respir J* 1998;12:540–545.
- Kaneko M, Swanson MC, Gleich GJ, Kita H: Allergen-specific IgG1 and IgG3 through Fc gamma RII induce eosinophil degranulation. *J Clin Invest* 1995;95:2813–2821.
- Monteiro RC, Hostoffer RW, Cooper MD, Bonner JR, Gartland GL, Kubagawa H: Definition of immunoglobulin A receptors on eosinophils and their enhanced expression in allergic individuals. *J Clin Invest* 1993;92: 1681–1685.
- Motegi Y, Kita H: Interaction with secretory component stimulates effector functions of human eosinophils but not of neutrophils. *J Immunol* 1998;161:4340–4346.

- 16 Morrison SL, Porter SB, Trinh KR, Wims LA, Denham J, Oi VT: Variable region domain exchange influences the functional properties of IgG. *J Immunol* 1998;160:2802–2898.
- 17 Gala FA, Morrison SL: V region carbohydrate and antibody expression. *J Immunol* 2004;172:5489–5494.
- 18 Horie S, Gleich GJ, Kita H: Cytokines directly induce degranulation and superoxide production from human eosinophils. *J Allergy Clin Immunol* 1996;98:371–381.
- 19 Bankers-Fulbright JL, Bartemes KR, Kephart GM, Kita H, O'Grady SM: β 2-Integrin-mediated adhesion and intracellular Ca^{2+} release in human eosinophils. *J Membr Biol* 2009;228:99–109.
- 20 Bankers-Fulbright JL, Kephart GM, Loegering DA, Bradford AL, Okada S, Kita H, Gleich GJ: Sulfonyleureas inhibit cytokine-induced eosinophil survival and activation. *J Immunol* 1998;160:5546–5553.
- 21 Plager DA, Henke SA, Matsuwaki Y, Madaan A, Squillace DL, Dierkhising RA, Kita H: Pimecrolimus reduces eosinophil activation associated with calcium mobilization. *Int Arch Allergy Immunol* 2009;149:119–126.
- 22 Chen XM, Tanaka T, Kobayashi Y, Shigematsu H, Okumura K: Experimental glomerulonephritis induced by immune complexes of monoclonal antibodies produced by immunoglobulin class-switch variants. *Lab Invest* 1987;57:665–672.
- 23 Abu-Ghazaleh RI, Fujisawa T, Mestecky J, Kyle RA, Gleich GJ: IgA-induced eosinophil degranulation. *J Immunol* 1989;142:2393–2400.
- 24 Graziano RF, Looney RJ, Shen L, Fanger MW: Fc γ R-mediated killing by eosinophils. *J Immunol* 1989;142:230–235.
- 25 Hartnell A, Moqbel R, Walsh GM, Bradley B, Kay AB: Fc γ and CD11/CD18 receptor expression on normal density and low density human eosinophils. *Immunology* 1990;69:264–270.
- 26 Weiler CR, Kita H, Hukee M, Gleich GJ: Eosinophil viability during immunoglobulin-induced degranulation. *J Leukoc Biol* 1996;60:493–501.
- 27 Shin MH, Kita H, Park HY, Seoh JY: Cysteine protease secreted by *Paragonimus westermani* attenuates effector functions of human eosinophils stimulated with immunoglobulin G. *Infect Immun* 2001;69:1599–1604.
- 28 Bartemes KR, McKinney S, Gleich GJ, Kita H: Endogenous platelet-activating factor is critically involved in effector functions of eosinophils stimulated with IL-5 or IgG. *J Immunol* 1999;162:2982–1989.
- 29 Bracke M, van de Graaf E, Lammers JW, Coffer PJ, Koenderman L: In vivo priming of Fc α R functioning on eosinophils of allergic asthmatics. *J Leukoc Biol* 2000;68:655–661.
- 30 Wines BD, Hogarth PM: IgA receptors in health and disease. *Tissue Antigens* 2006;68:103–114.
- 31 Bartemes KR, Cooper KM, Drain KL, Kita H: Secretory IgA induces antigen-independent eosinophil survival and cytokine production without inducing effector functions. *J Allergy Clin Immunol* 2005;116:827–835.
- 32 Decot V, Woerly G, Loyens M, Loiseau S, Quatannens B, Capron M, Dombrowicz D: Heterogeneity of expression of IgA receptors by human, mouse, and rat eosinophils. *J Immunol* 2005;174:628–635.
- 33 Pleass RJ, Lang ML, Kerr MA, Woof JM: IgA is a more potent inducer of NADPH oxidase activation and degranulation in blood eosinophils than IgE. *Mol Immunol* 2007;44:1401–1408.
- 34 Truong MJ, Gruart V, Liu FT, Prin L, Capron A, Capron M: IgE-binding molecules (Mac-2/e BP) expressed by human eosinophils. Implication in IgE-dependent eosinophil cytotoxicity. *Eur J Immunol* 1993;23:3230–3235.
- 35 Dehlink E, Fiebiger E: The role of the high-affinity IgE receptor, Fc ϵ RI, in eosinophilic gastrointestinal diseases. *Immunol Allergy Clin North Am* 2009;29:159–170.
- 36 Walsh GM, Moqbel R, Nagakura T, Iikura Y, Kay AB: Enhancement of the expression of eosinophil IgE receptor (Fc ϵ R2) and its function by platelet-activating factor. *J Lipid Mediat* 1990;2:S177–S186.
- 37 Ebisawa M, Schleimer RP, Bickel C, Bochner S: Phenotyping of purified human peripheral blood eosinophils using blind panel mAb; in Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, Ritz J, Shaw S, Silberstein R, Springer T, Tedder TF, Todd RF (ed): *Leukocyte Typing V*. New York, Oxford University Press, 1995, p 1036.
- 38 Valerius T, Repp R, Kalden JR, Platzer E: Effects of IFN on human eosinophils in comparison with other cytokines. A novel class of eosinophil activators with delayed onset of action. *J Immunol* 1990;145:2950–2958.
- 39 Hartnell A, Kay AB, Wardlaw AJ: IFN- γ induces expression of Fc γ RIII (CD16) on human eosinophils. *J Immunol* 1992;148:1471–1478.
- 40 Davoine F, Labonte I, Ferland C, Mazer B, Chakir J, Laviolette M: Role and modulation of CD16 expression on eosinophils by cytokines and immune complexes. *Int Arch Allergy Immunol* 2004;134:165–172.