Selective Regulation of Eosinophil Degranulation by Interleukin 1β (43355)

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Abstract. **Recent evidence confirms that cytokines such as IL-1, IL-4, IL-5, and GM-CSF may enhance or inhibit eosinophil function. Functions that are susceptible to modulation include eosinophil-mediated antibody-dependent damage of helminthic parasites, oxidative metabolism and degranulation. We have employed IgG and IgE-coated Sepharose beads to investigate selective modulation of IgG and IgE-mediated enzyme release by IL-1β. Both IgG and IgE-coated beads induced release of granular enzymes β-glucuronidase and arylsulfatase. Enzyme release from IgG-stimulated eosinophils was inhibited by preincubation with IL-1β (100 pg/ml, $P \leq 0.05$). In contrast, enzyme release by IgE-stimulated eosinophils was enhanced by IL-1β (100 pg/ml, $P \leq 0.05$). These studies support the hypothesis that IL-1β has specific selective actions on eosinophil function. Furthermore, these actions on particle-stimulated enzyme release suggest that IgG and IgE mediated processes in eosinophils are differentially regulated.**

[P.S.E.B.M. 1992, Vol 199]

Recent evidence has established that eosinophil function is susceptible to modulation. For instance, eosinophil-mediated, antibody-dependent cellular cytotoxicity for schistosomula of *Schistosoma mansoni* is enhanced by tumor necrosis factor, granulocyte-macrophage colony stimulating factor, and interleukin (IL) 5, and inhibited by IL-4 (1–3). There has been divergence in the reports of cytokine actions, in part depending on the assay selected and the particular cytokine employed. Degranulation indicated by release of granular enzymes from otherwise unstimulated eosinophils is modestly increased by IL-1, whereas IL-5 is effective in inducing release of granular contents as measured by release of eosinophil-derived neurotoxin (4, 5) when IgA-coated Sepharose beads are used as the stimulus. Using antibody-dependent cellular cytotoxicity as a measure of the action of eosinophils, γ-interferon has been shown to enhance receptor expression after 24 hr of incubation (6). Thus, there may be both immediate and delayed activators of eosinophil function. Eosinophils possess cell surface receptors for IgG and IgE, both of which may be involved in eosinophil-mediated cytotoxicity (7). Normal eosinophils appear to use IgG, specifically the Fcγ-rII receptor in eosinophil-mediated damage to schistosomula, whereas activated (or hypodense) eosinophils appear to use Fcε-rII in damaging parasites (8, 9). Modulation of such receptors is a possible mechanism by which cytokines can act.

The complexity and specificity of regulation of eosinophil function is increasingly evident. For instance, our recent report on IL-4 actions demonstrates selective regulation of the Fc IgG receptor by IL-4. In the current report, we have established that IL-1β has selective actions on IgG and IgE receptor functions as measured by degranulation and enzyme release from eosinophils stimulated by IgG- or IgE-coated Sepharose beads. These studies provide support for the thesis that eosinophil function is selectively and specifically regulated.

Materials and Methods

Cell Preparation. Human eosinophils were purified on discontinuous metrizamide gradients from normal, mildly hypereosinophilic (up to 5%) volunteers, as described previously (3). Briefly, heparinized (10 units/ml of preservative-free heparin; A.H. Robins Co., Richmond, VA) blood drawn from healthy normal volunteers was mixed with 4.5% dextran (T-500; Pharmacia Laboratories, Piscataway, NJ, or Dextran

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Received April 17, 1991. [P.S.E.B.M. 1992, Vol 199]
Accepted September 23, 1991.

0037-9727/92/1992-0249$3.00/0

150,000, Sigma Chemical Co., St. Louis, MO) in phosphate-buffered saline and was allowed to sediment for 30 min at 37°C. The leukocyte-rich plasma was removed and washed twice with Eagle's minimal essential medium ([MEM] Gibco, Grand Island, NY) containing 100 units/ml of penicillin, 100 μg/ml of streptomycin, 10% heat-inactivated fetal calf serum (FCS), and 2 n*M* l-glutamine ([MEM/FCS] Flow Laboratories, Rockville, MD). Unless otherwise stated, eosinophils were pooled from Layers 23, 24, and 25 of the metrizamide gradient. Routine preparations contained greater than 85% eosinophils; contaminating cells were exclusively neutrophils.

Preparation of Human IgG-Coated Beads. Beads were prepared with cyanogen bromide-activated Sepharose 4B particles (Sigma), as described previously (10). Particles were swollen in 1 m*M* HCl and washed with coupling buffer 0.2 *M* NaHCO3 (pH 8.7) containing 0.5 *M* NaCl. Aliquots (1 ml) of gel were incubated with 7.5 mg of human IgG (Sigma) and dissolved in coupling buffer for 2 hr at room temperature with continuous mixing, followed by overnight incubation at 4°C. Any remaining reactive groups on the Sepharose were blocked by incubating particles with 1.0 *M* ethanolamine (pH 8) for 2 hr at room temperature with frequent mixing. The particles were washed alternately with coupling buffer and acetate buffer (0.1 *M* sodium acetate (pH 4.0) containing 0.5 *M* NaCl) to remove any non-covalently bound protein. This resulted in preparations containing 5.0–5.5 mg of IgG per 1×10^6 beads.

Preparation of Human IgE-Coated Sepharose 4B Beads. Purified human IgE was kindly provided by Dr. Kimshige Ishizaka (The Good Samaritan Hospital, Baltimore, MD). Human IgE was coated onto cyanogen bromide-activated Sepharose 4B particles, as described above. Equivalent amounts of IgG and IgE were bound to the Sepharose beads as determined by calculation of the amount bound.

Enzyme Assays. β-Glucuronidase (specific granule marker) activity was assayed using 4-methylumbelliferyl β-D-glucuronide (Sigma), as described previously (4). An appropriate aliquot of supernatant was incubated with 10.0 m*M* 4-methylumbelliferyl glucuronide in 0.1 *M* acetate buffer (pH 4.8) for 15 min at 37°C (final vol 0.25 ml). The reaction was terminated by the addition of glycine carbonate buffer (pH 10.0; final vol 2.0 ml). The amount of 4-methylumbelliferone was determined by changes in fluorescence (excitation 365, emission 450), and comparison with a standard curve was prepared by the addition of 4-methylumbelliferone. Results are expressed as the percentage of total enzyme release and represent the means of duplicate reactions. Percentage of release was defined as:

$$\frac{\text{supernatant activity}}{\text{total activity}} \times 100 \qquad \text{[Eq. 1]}$$

Total activity was determined using an appropriate aliquot of cells for each experiment. The cell suspension was freeze-thawed three times, the debris was removed by centrifugation at 2500 rpm for 5 min at 4°C, and an aliquot was removed for enzyme assay. Arylsulfatase B (small granule marker) activity was measured using 10.0 m*M* 4-methylumbelliferyl sulfate (Sigma) and 0.2 *M* sodium acetate buffer (pH 5.7). The percentage of release was determined as described above. Under these conditions, eosinophils contained 8- to 10-fold more arylsulfatase on a per cell basis. Thus, the contribution from the 0–15% contaminating neutrophils was not significant and was less than 1–7% of the calculated total activity. Our previous studies have shown no detectable lactate dehydrogenase activity under these conditions (4, 10). Statistical significance was determined using the Student's *t* test.

Secretion of Granular Contents. Release of the granular enzymes β-glucuronidase and arylsulfatase was studied under resting or stimulated conditions (IgG- or IgE-coated beads), according to our standard method (10). Briefly, 2×10^5 eosinophils were incubated in the presence or absence of stimulus for 60 min in Hanks' balanced salt solution in 12 × 75 mm polypropylene tubes (Falcon). Where indicated, endotoxin-free human recombinant IL-1β (a gift from Dr. Charles Dinarello) was added one-half hour prior to the addition of particles. After incubation, intact cells were removed by centrifugation for 10 min at 1000 *g*. The supernatant was decanted and stored at −20°C until assayed for enzyme activity.

Immunofluorescence Flow Cytometry. Purified human eosinophils were suspended at 1×10^7 cells/ml and 100-μl aliquots (1×10^6 cells) were incubated with 100 pg of IL-1β for one-half hour at 37°C. The cells were washed with phosphate-buffered saline and incubated with either IgG or IgE antibodies at 4°C for 1 hr. Cells were washed thoroughly with phosphate-buffered saline and further incubated with goat antihuman IgG/IgE fluorescein isothiocyanate. After incubation, the cells were washed three times and fixed as described (11). Fixed eosinophils were analyzed in EPICS 541 FACS (Coulter Diagnostics, Hialeah, FL).

Results

IgG- and IgE-Coated Beads Stimulate Release of Granular Enzymes. IgG- and IgE-coated Sepharose beads were used as noningestible particles. We first asked whether these IgG- or IgE-coated particles would stimulate the release of the granular enzymes β-glucuronidase and arylsulfatase. Eosinophils were incubated with IgG- or IgE-coated beads at ratios of 2:1 or 20:1 for 1 hr at 37°C (Table I). Both IgG- and IgE-coated beads induced release of granular enzymes, whereas uncoated beads did not induce enzyme release above background. The maximum release of arylsulfatase and

β-glucuronidase was observed at an eosinophil to bead ratio of 2:1 (Table I). IgG-coated beads were more effective than IgE-coated beads in stimulating β-glucuronidase release at the ratios of eosinophils to particles tested ($P \leq 0.05$). The differences between IgG- and IgE-stimulated release of arylsulfatase were smaller; however, more arylsulfatase was released by IgG-coated beads at a 2:1 ratio (eosinophil to beads) ($P \leq 0.05$).

Differential Effects of IL-1β on Enzyme Release. IL-1β selectively enhances enzyme release from unstimulated eosinophils (4). Thus, we asked whether IL-1β altered IgG- and/or IgE-mediated enzyme release (Fig. 1). Our previous studies established that 100 pg of IL-1β were optimal for eliciting release from unstimulated eosinophils (4, 12). Purified eosinophils were preincubated with IL-1β (100 pg) for 30 min. Either IgG- or IgE-coated beads were gently mixed with the eosinophils and enzyme release was determined after 1 hr. IL-1β (100 pg) enhanced enzyme release from resting eosinophils, as noted previously (Fig. 1). Both β-glucuronidase (Fig. 1, top panel) and arylsulfatase (Fig. 1, bottom panel) release from IgG-stimulated eosinophils were significantly decreased by preincubation with IL-1β (Fig. 1). By contrast, IgE-stimulated enzyme release was significantly enhanced by preincubation with IL-1β. Because of the limited cell numbers and the small amount of IgE available, it was not possible to perform extensive dose-response studies.

Effect of IL-1β on Eosinophil Subpopulations. Our previous work established that normal eosinophils of differing density have differential responses to IL-1β (4). Thus, we asked whether IL-1β had similar differential effects on IgG- or IgE-mediated release from eosinophil subpopulations. Normal eosinophils were separated into three subpopulations according to their density on metrizamide gradients. Layer 23 (low density normal eosinophils) was harvested from the 22/23 interface, Layer 24 (intermediate density normal eosinophils) from the 23/24 interface, and Layer 25 (heavy normal eosinophils) from the 24/25 interface. Eosinophil purity was more than 90% in each layer. These eosinophil subpopulations were then employed in our standard assay of enzyme release. IL-1β (100 pg) was added 30 min prior to the addition of IgG- and IgE-coated beads. Both IgG- and IgE-coated beads stimulated release of β-glucuronidase (Fig. 2, top panel) and arylsulfatase (Fig. 2, bottom panel) from low density and intermediate density normal eosinophils (Fig. 2). Data from a representative experiment are displayed. The inhibitory effect of IL-1β on IgG-induced release was most evident in low density eosinophils from Layer 23. The enhancing effect of IL-1β on IgE-stimulated release of arylsulfatase and β-glucuronidase release was most prominent in cells of intermediate density (Layer 24). The more dense eosinophils (Layer 25) were relatively refractory to the effects of IL-1β on both IgG- and IgE-mediated degranulation. There were no differences in total enzyme content between normal eosinophils of differing density.

Flow Cytometry Analysis Does Not Reveal Changes in IgE and IgG Receptors. One possible explanation for the observed IgG- and IgE-mediated enzyme release is alteration in the number of receptors. Purified eosinophils were incubated at 37°C for 30 min with 100 pg of IL-1β and the number of IgG and IgE receptors was determined by FACS analysis. No changes in the percentage of IgG and IgE of receptor positive cells was observed after treatment with IL-1β (data not shown). Thus, modulation of receptors does not appear to explain the observed effect.

Discussion

Although the biologic role of the eosinophil is incompletely understood, numerous studies support the concept that eosinophils are important in allergic hypersensitivity diseases and in host response to helminthic parasitic infections. IL-1β is an important cytokine in host defense because of its diverse biologic actions. Our previous studies have demonstrated that IL-1β selectively enhances enzyme release from eosinophils (4, 12). Though IL-1β may have relatively modest effects on otherwise unstimulated eosinophils, the

Table 1. Release of Enzymes Stimulated by IgG- and IgE-Coated Sepharose Beads[a]

	IgG beads		IgE beads	
	β-Glucuronidase	Arylsulfatase	β-Glucuronidase	Arylsulfatase
Resting	6.6 ± 0.9	3.5 ± 0.6	4.8 ± 1.8	6.0 ± 0.6
Eos:bead 54.6 ± 2.5	21.1 ± 1.2	34.8 ± 0.9	12.3 ± 2.0	
Eos:bead 20:1	20.1 ± 1.4	5.6 ± 1.5	11.0 ± 0.7	8.1 ± 0.2

[a] Eosinophils were incubated for 1 hr with either IgG- or IgE-coated beads at a ratio of 2:1 or 20:1 (eosinophils to bead). Enzyme release was measured after 1 hr. Results are expressed as the mean ± SE of the percentage of release of the total enzyme content for two experiments performed in duplicate for each immunoglobulin. IgG-mediated release of both β-glucuronidase and arylsulfatase was significantly greater than IgE-mediated release at eosinophil to bead (Eos:bead) ratio of 2:1 ($P \leq 0.05$).

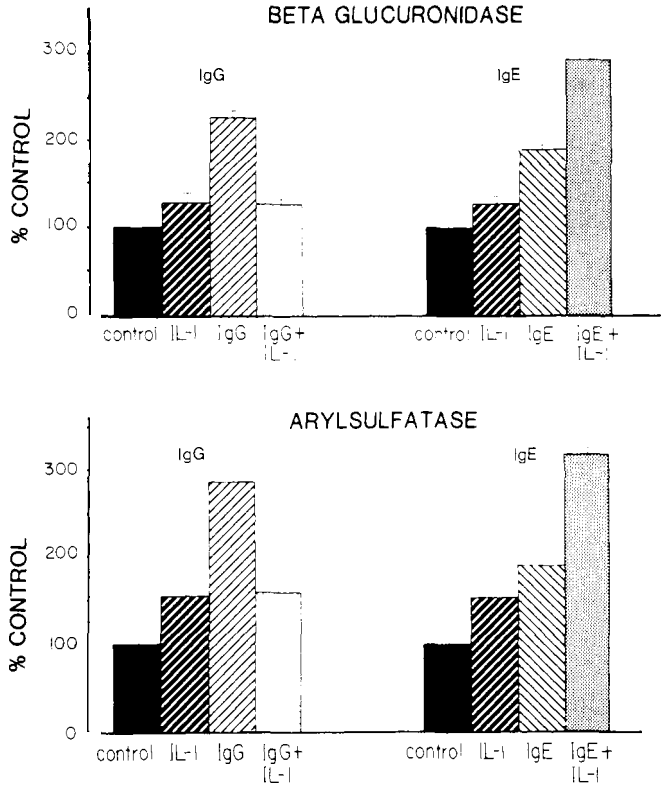

Figure 1. Eosinophil degranulation induced by IgG- and IgE-coated beads with and without rhIL-1β. Eosinophils were preincubated for one-half hour with rhIL-1 (100 pg) prior to the addition of particles, as described in Materials and Methods. After an additional incubation of 1 hr at 37°C, release into the supernatant of granular enzymes β-glucuronidase (top panel) and arylsulfatase (bottom panel) was determined. Results are expressed as percentage of unstimulated control and represent the mean ± SE of two experiments for each immunoglobulin performed in duplicate. The unstimulated release for β-glucuronidase was 8.9 ± 0.7% total enzyme, and for arylsulfatase, 6.0 ± 0.5% total enzyme. The significance of the difference between IgG beads with and without rhIL-1β was $P \leq 0.005$ for both β-glucuronidase and arylsulfatase release, and for IgE beads with and without rhIL-1β was $P \leq 0.01$ for both β-glucuronidase and arylsulfatase release.

dramatic effects on particle-stimulated enzyme release noted here suggest that IL-1β may have a greater than previously appreciated regulatory role in the interactions between eosinophils and noningestible parasites. The current studies suggest that the actions of IL-1β on eosinophils may, in part, be due to an alteration of the response to other components of host defense, such as the response to immunoglobulins.

Our reports demonstrating modulation of eosinophil degranulation are in contrast to those that failed to find effects of IL-1β (5). There are several possible reasons for this discrepancy, including the amount of IL-1β employed, the particular stimulus employed (IgE versus IgA), and the particular granular component selected for analysis (eosinophil-derived neurotoxin versus granular enzymes). Our results clearly establish that each cytokine and each action must be selectively analyzed, i.e., that positive or negative results cannot be extended from one system to another.

IgG- and IgE-mediated functions are of interest because of their roles in diseases associated with eosinophils. Both IgG and IgE participate in the release of contents from both specific granules (β-glucuronidase) and small granules (arylsulfatase) of eosinophils. Though IgG and IgE both induce release of granular enzymes, IgG is more effective at low cell to particle ratios. These results are consistent with previous observations demonstrating both IgE- and IgG-mediated enzyme release *in vitro*. Although both IgG and IgE may participate in antibody-dependent damage, different cellular mechanisms may be involved. Platelets demonstrate the differential selectivity of signals that accompany IgE and IgG binding. Upon IgG-depending activation, there is a significant release of serotonin, but no production of oxygen metabolites, while the reverse is observed after triggering by IgE (13). Similarly, in IgG-dependent reactions, eosinophils liberate significant amounts of cationic protein, whereas, upon IgE stimulation, eosinophil peroxidase and platelet-activating factor are preferentially released (14, 15). In our current study, the highly selective nature of the regulation of eosinophil function is further defined. Thus, IgE-stimulated release was enhanced by IL-1β, while IgG-stimulated release was inhibited. Such differential regulation by IL-1β raises the possibility that IgG- and IgE-mediated release may occur by different mechanisms. Given that FcγRII and FcϵRII belong to different receptor families, it is understandable that different regulatory mechanisms may exist. These observations suggest that *in vivo* highly specific and selective modulation of eosinophil function may occur and that IL-1 may act to influence host resistance by selective modification of granular release.

Our recent studies of the actions of IL-4 on eosinophil demonstrating inhibition of IgG-mediated function emphasize the highly selective, specific regulatory roles of cytokines in the effector actions of eosinophils (3). Furthermore, they provide a precedent for inhibition of eosinophil function, thus emphasizing that the current observations on the actions of IL-1β may be of importance. The mechanism of modulation of enzyme release by IL-1β is not addressed in the present study. Though receptor studies failed to show any changes with IL-1β treatment, we cannot exclude alterations in receptor affinity. Our previous studies demonstrating the complex nature of modulation of the actions of IL-1 on eosinophils provide alternative explanations for the actions of IL-1β (12). The observation concerning IL-1 actions on eosinophils is further complicated by recognition that eosinophils themselves may produce IL-1α (16). The current studies confirm that eosinophils from both normal and mildly hypereosinophilic donors constitute a heterogeneous population (17). Intermedi-

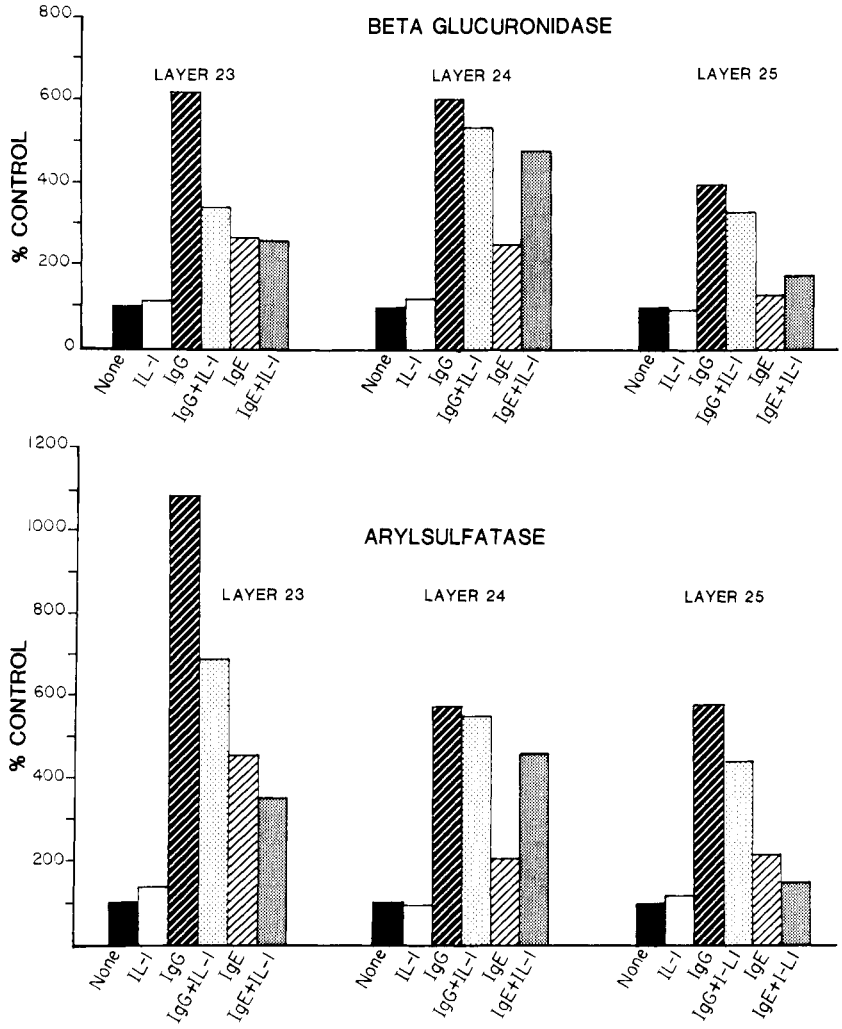

Figure 2. The effect of rhIL-1β on different populations of normal eosinophils. Gradients were prepared and layers of equivalent density were pooled as described in Materials and Methods. Eosinophils from Layers 23, 24, and 25 were incubated with rhIL-1β (100 pg) for one-half hour prior to the addition of IgG- or IgE-coated beads. Results represent one of three representative experiments performed in duplicate. Release is expressed as a percentage of control of unstimulated eosinophils for each layer. The unstimulated controls expressed as percentage of total enzyme release were 4.0% release (Layer 23), 5.9% release (Layer 24), and 4.1% release (Layer 25) for β-glucuronidase (top panel), and 2.4% release (Layer 23), 6.0% release (Layer 24), and 2.9% release (Layer 25) for arylsulfatase (bottom panel). IgG-mediated release of β-glucuronidase and arylsulfatase was reduced by the addition of IL-1β in Layer 23. In Layer 24, IgE-mediated release of both β-glucuronidase and arylsulfatase was prominently increased by the addition of IL-1β (100 pg).

ate density eosinophils are more responsive to modulation of IgG- and IgE-mediated secretion by IL-1β. The variability in sensitivity to IgG and IgE may perhaps be mediated through heterogeneity in either the number or affinity of IgE or IgG receptors in different density eosinophils. The concept that normal circulating eosinophils may represent a heterogenous population has been strengthened by recent observations of marked variability in the endogenous production of transforming growth factor-β (18). Markers other than density may become useful indicators of eosinophil heterogeneity.

Our studies further support the hypothesis that regulation of eosinophil function is highly specific and selective. The pathophysiology of eosinophil-associated diseases depends upon the specific interactions of stimuli, cytokines, and exogenous agents.

This work was supported by National Institutes of Health Grants AI16432 and AI123847.

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