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This information is current as
of September 24, 2021.

J Immunol 1998; 161:1510-1515; ;
<http://www.jimmunol.org/content/161/3/1510>

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Secretory IgA Induces Degranulation of IL-3-Primed Basophils¹

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We examined whether secretory IgA (sIgA), known to mediate eosinophil stimulation, has an effect on basophil functions. An immobilized preparation of sIgA, but not of monomeric IgA, induced histamine release (approximately 15% of total histamine contents) from human basophils in vitro. sIgA-induced basophil histamine release was totally dependent on pretreatment with IL-3. IL-5 and granulocyte-macrophage CSF also primed basophils for sIgA-mediated release. Exogenous divalent ions, i.e., Ca²⁺ and Mg²⁺, were essential for sIgA-mediated basophil degranulation, and the degranulation was completed within 45 min. A newly synthesized lipid mediator, leukotriene C₄, was also liberated from IL-3-primed, sIgA-stimulated basophils. Enzyme digestion experiments revealed that the (Fc)₂-secretory component portion of sIgA is important for sIgA-mediated basophil activation, but the functional binding sites of sIgA on basophils were surmised to be different from FcαR. These observations reveal the novel finding that sIgA is able to stimulate basophils as well as eosinophils. Since sIgA is the most abundant Ig isotype in the secretions from mucosal tissues, and basophils are active participants in allergic late-phase reactions, sIgA-mediated basophil mediator release is potentially involved in exacerbation of the inflammation associated with allergic disorders. *The Journal of Immunology*, 1998, 161: 1510–1515.

Since the first description by Paul Ehrlich, basophils have been recognized to be unique white blood cells that stain metachromatically with a variety of chemical dyes. They express high affinity receptors for IgE (FcεRI) on their surface and contain potent vasoactive amines in their granules. When the surface-bound IgE is cross-linked by specific multivalent Ags, basophils release granule-associated mediators as well as newly synthesized mediators such as leukotriene (LT)³ C₄ (1). Through the release of these proinflammatory mediators, basophils play an active role in allergic reactions in concert with mast cells. Although basophils and mast cells show remarkable similarities, such as the possession of FcεRI and granule histamine, and their histochemical properties, these two cell types belong to distinct cell lineage. Substantial evidence has indicated that the cells most closely related to basophils are eosinophils (reviewed in Ref. 2). Tissue eosinophilia is a fundamental trait of allergic diseases in which infiltrated eosinophils appear to play a key role (3). Similarly, an increasing body of evidence indicates that basophils represent another type of proinflammatory cells involved in the pathogenesis of allergic disorders. The role of basophils in allergic reactions has

become more apparent with the recognition and understanding of allergic late-phase reactions (LPRs) (1, 2). Basophils and basophil-derived mediators have been identified in a number of LPRs induced by experimental Ag challenge in patients with nasal (4, 5) or bronchial hypersensitivity (6, 7).

Pivotal roles of IgE in the activation of basophils have been well established, but secretory IgA (sIgA) is the most abundant Ig isotype in the mucosal tissues, in which a variety of allergic inflammatory cells such as eosinophils and basophils exert their effector functions. The dimeric IgA and J chain are locally synthesized by plasma cells located in the lamina propria of the mucous membranes. After binding to a secretory component (SC), which is produced by epithelial cells, sIgA is transported into the epithelial lining fluid. In vitro studies demonstrate that sIgA stimulates eosinophils to undergo degranulation (8), indicating that sIgA plays an important triggering role in eosinophil activation. On the other hand, to date there has been no information regarding any possible role for sIgA in basophil activation. Given the potential importance of sIgA and basophils in allergic inflammation, we decided to conduct analyses designed to detect sIgA-induced basophil mediator release. In the present study, we demonstrate that immobilized sIgA, but not IgA, induces mediator release from IL-3-primed basophils.

Materials and Methods

Reagents

Human rIL-3, IL-5, and granulocyte-macrophage CSF (GM-CSF) were kindly donated by Kirin Brewery (Tokyo, Japan), Suntory Institute of Biomedical Research (Osaka, Japan), and Sumitomo Pharmaceutical (Tokyo, Japan), respectively. Purified human IgA preparations, i.e., serum IgA, myeloma IgA, and sIgA, were obtained from Cappel-Organon Teknika (West Chester, PA). Goat anti-human IgE was purchased from Medical Biologic Laboratory (Nagoya, Japan). sIgA was divided into Fab and (Fc)₂-SC fragments Fab-sIgA and (Fc)₂-SC-sIgA, respectively) by enzyme digestion using IgA protease from *Clostridium ramosum* (9). Human free SC was purified from pooled colostrum by heparin Sepharose column chromatography, followed by gel filtration, as described elsewhere (10).

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Received for publication January 26, 1998. Accepted for publication April 6, 1998.

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¹ This work was partially supported by a grant from Manabe Medical Foundation and a grant-in-aid from Ministry of Education, Science, Sports, and Culture of Japan.

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³ Abbreviations used in this paper: LT, leukotriene; EDN, eosinophil-derived neurotoxin; GM-CSF, granulocyte-macrophage CSF; LPR, late-phase reaction; SC, secretory component; sIgA, secretory Ig A.

Buffer

PIPES A buffer contained 25 mM PIPES (Sigma, St. Louis, MO), 119 mM NaCl, 5 mM KCl, and 0.03% human serum albumin, and it was adjusted to pH 7.4. For stimulation of basophils and eosinophils, PIPES A containing 2 mM Ca^{2+} and 0.5 mM Mg^{2+} (PIPES ACM) was used, unless otherwise indicated.

Preparation of protein-conjugated Sepharose beads

Protein-conjugated Sepharose beads were prepared, as described elsewhere (11). In brief, cyanogen bromide-activated Sepharose 4B beads (Pharmacia, Uppsala, Sweden) were swollen in 1 mM HCl and washed with borate buffer (0.2 M H_3BO_3 , 0.5 M NaCl, and 0.02 M NaOH, pH 8.6). Proteins, dissolved in borate buffer, were added to the beads at 10 mg/ml of packed beads, and this was stirred overnight at 4°C. On the next day, the beads were washed at least three times in borate buffer and blocked with 0.1 M lysine monohydrochloride, pH 8.6, in borate buffer for 2 h at room temperature. Finally, the beads were washed alternately in borate buffer and 0.1 M acetate buffer (pH 4) and stored at 4°C in borate buffer until use. Greater than 85% of the total protein was bound to the beads, as determined by OD measurement of the supernatants and washes.

Cell preparation

Human basophils and eosinophils were isolated from venous blood obtained from consenting volunteers with no history of atopic diseases. For most of our experiments, semipurified basophils were prepared. In brief, anticoagulated blood was overlaid on isotonic Percoll solutions having different densities (1.079 and 1.070 g/ml). After centrifugation at $700 \times g$ for 15 min at room temperature, cell bands between the two Percoll solutions were collected. For some experiments, the basophils were purified further by an additional isolation step of negative panning selections using anti-CD2, anti-CD14, anti-CD16, and anti-CD19 Abs, as reported previously (12). The purity of semipurified and highly purified basophil preparations was 16% (range, 8-30%) and 72% (range, 60-83%), respectively. Both basophil preparations contained virtually no eosinophils. Their viability measured by the trypan blue exclusion test was consistently >95%.

Eosinophils were purified by Percoll density centrifugation, followed by negative selections using anti-CD16-bound micromagnetic beads and a magnetic activated cell sorter column, as previously described (13). The purity of eosinophils was more than 99%.

Mediator release from basophils

Histamine and LTC_4 release by soluble reagents was performed as previously described (14). Mediator release by protein-conjugated Sepharose beads was performed as follows. Based on the density of basophils, assessed by Alcian blue stain, the number of added beads was calculated to adjust the designated ratio of beads to basophils (shown as bead:cell ratio in the figures) in each sample. To increase the opportunity of direct contact between the cells and beads during basophil stimulation, cells suspended with beads (300 μl total) were immediately centrifuged at $50 \times g$ for 8 min at 4°C, transferred to a water bath, and incubated without agitation at 37°C for 45 min, unless otherwise stated. At the end of the incubation, the samples were chilled on ice, and 200 μl of ice-cold PIPES A was added to each sample. After centrifugation, the supernatants were collected and stored at 4°C. The concentration of histamine in supernatants was measured using an automated fluorometric technique. LTC_4 was quantified using an ELISA kit (Bühlmann Laboratories AG, Schönenbuch, Switzerland), according to the manufacturer's instruction.

In each experiment, the total content and spontaneous release of histamine were analyzed. The histamine in the supernatants of basophil preparations incubated with OVA (Sigma)-coated beads was referred to spontaneous release (consistently <5%). Histamine release was expressed as a percentage of the total cellular histamine after subtracting the spontaneous release. Experiments were performed at least in duplicate.

Mediator release from eosinophils

Nonprimed eosinophils were stimulated similarly to basophils, except that the cells were stimulated for 4 h at 37°C, as previously described (11). Eosinophil-derived neurotoxin (EDN) in supernatants was measured using an RIA kit (Pharmacia). EDN release was calculated based on the same formula as histamine release. Experiments were performed at least in duplicate.

Statistical analysis

Data are presented as the mean \pm SEM. Statistical significance of the differences between various treatment groups was assessed by Student's *t* test (paired).

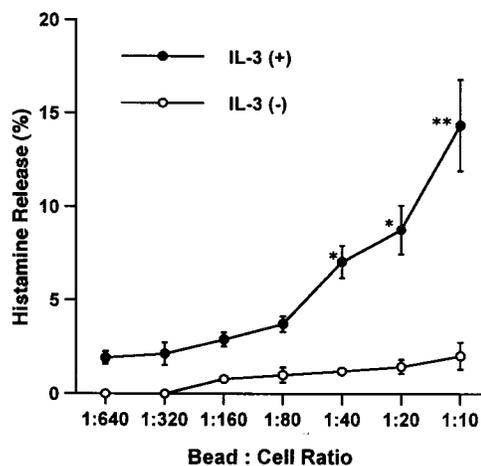


FIGURE 1. Induction of histamine release from basophils by sIgA. Semipurified basophils were preincubated with (closed circles) or without (open circles) IL-3 at 5 ng/ml for 30 min at 37°C, and then mixed with a buffer containing various amounts of sIgA-coated beads (the relative numbers of beads to basophils are indicated by the bead:cell ratio). After incubation for 45 min at 37°C, the supernatants were collected. Histamine release is expressed as a percentage of the total cellular histamine content after subtracting the spontaneous release in the presence of OVA-coated beads instead of sIgA-coated beads. Bars represent the SEM ($n = 5$). * $p < 0.05$, ** $p < 0.01$, vs OVA-induced release from corresponding cells.

Results

sIgA-induced histamine release from IL-3-primed basophils

We first tested the direct effect of soluble sIgA on basophil histamine release, but no significant release was initiated by sIgA over a wide range of concentrations. To further determine whether sIgA is capable of activating human basophils, we tested the effect of immobilized sIgA on basophils. Based on our previous findings that hemopoietic growth factors such as IL-3 prime basophils for increased releasability (15), semipurified basophils were pretreated with or without IL-3 (5 ng/ml) for 30 min and then incubated with sIgA-conjugated Sepharose beads for 45 min at various bead:cell ratios. As shown in Figure 1, nontreated basophils showed little or no histamine release at all tested bead:cell ratios. In IL-3-primed basophils, however, apparent histamine release was elicited by immobilized sIgA, depending on the bead:cell ratio. Approximately 15% of histamine release was obtained at a high ratio (1:10) of sIgA-coated beads to basophils. Five to ten percent of release occurred at a bead-to-cell ratio of 1:20-40.

Since the basophil preparations in the aforementioned experiments were separated by Percoll centrifugation, they contained appreciable numbers of contaminating leukocytes, such as lymphocytes, monocytes, and neutrophils. To exclude possible contributions from these contaminating cells in our experiments, we further purified basophils by means of negative selections and tested the effect of IL-3 and sIgA on these preparations. As shown in Figure 2, sIgA also induced histamine release from IL-3-primed, highly purified basophils. There was no significant difference in the percentage of sIgA-induced histamine release between the semipurified and highly purified basophil preparations. These results indicate that both IL-3 and sIgA act directly on basophils and provoke the histamine release. Treatment with IL-3 before stimulation with sIgA was essential for sIgA-induced basophil histamine release, since histamine release was mostly abrogated when IL-3 was added 30 min after incubation of the basophils with sIgA (Fig. 3).

The time kinetics of sIgA-induced histamine release indicates that the reaction was a rapid process: apparent release (~5%) was

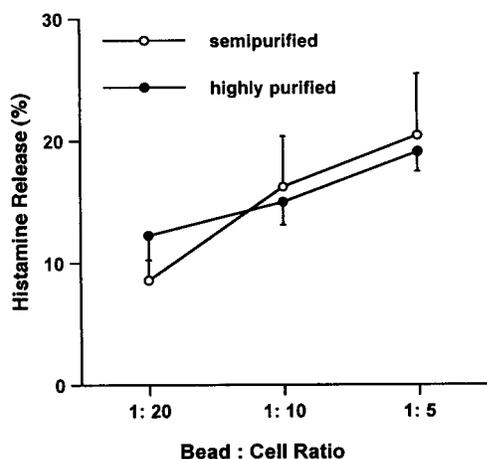


FIGURE 2. Comparison of sIgA-induced histamine release between semipurified and highly purified basophil preparations. Basophils were semipurified by Percoll density centrifugation (open circles), or further purified by means of negative panning selection (closed circles) from single donors. Basophils were pretreated with IL-3 (5 ng/ml for 30 min) and stimulated with sIgA-coated beads. Basophil purity was $16 \pm 8\%$ (semipurified), and $72 \pm 12\%$ (highly purified). Bars represent the SEM ($n = 4$).

observed after 5 min of stimulation with sIgA (Fig. 4). The release reached a plateau within 45 min of stimulation with sIgA.

We have reported previously that picomolar concentrations of IL-3 are sufficient to prime basophils for enhanced releasability (15). The same situation was also observed in sIgA-mediated basophil histamine release: IL-3 exhibited a significant priming effect on basophils at as low as 5 to 50 pg/ml (Fig. 5). IL-3 at 5000 pg/ml strongly primed basophils and induced 15 to 20% of histamine release following stimulation with sIgA. Pretreatment of basophils with two other basophil-active growth factors, i.e., GM-CSF and IL-5, also induced sIgA-mediated histamine release (Fig. 5).

sIgA-induced basophil histamine release requires exogenous Ca^{2+} and Mg^{2+}

To examine whether the degranulation evoked by sIgA is dependent on extracellular Ca^{2+} or Mg^{2+} , IL-3-treated basophils were stimulated with sIgA in PIPES A buffer (no Ca^{2+} , no Mg^{2+}),

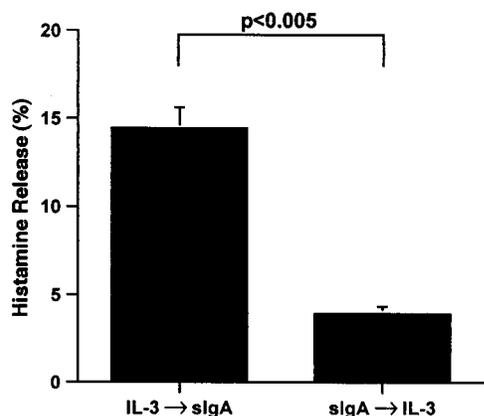


FIGURE 3. Requirement of prior incubation with IL-3 for sIgA-induced basophil histamine release. Basophils were treated with IL-3 for 30 min, followed by stimulation with sIgA-coated beads at a 1:10 bead:cell ratio for 45 min at 37°C (left column), or incubated with sIgA for 45 min, followed by treatment with IL-3 for 30 min at 37°C (right column). Bars represent the SEM ($n = 4$).

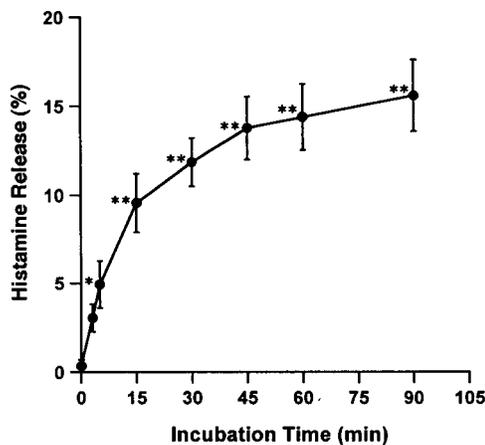


FIGURE 4. Time course of sIgA-induced histamine release. IL-3-primed basophils were stimulated with sIgA at a 1:10 bead:cell ratio for various time periods. Bars represent the SEM ($n = 5$). * $p < 0.05$, ** $p < 0.01$, vs percentage of histamine release at 0 min.

PIPES A supplemented with either Ca^{2+} (no Mg^{2+}) or Mg^{2+} (no Ca^{2+}), or PIPES ACM buffer (Fig. 6). In the absence of both Ca^{2+} and Mg^{2+} , sIgA caused nearly no histamine release. sIgA-induced histamine release was also very weak in buffer supplemented with either Ca^{2+} or Mg^{2+} alone ($\sim 35\%$ of the maximal release observed in the presence of Ca^{2+} and Mg^{2+}), suggesting that sIgA-induced histamine release is dependent on both Ca^{2+} and Mg^{2+} . On the other hand, basophil histamine release triggered by anti-IgE Ab was mostly dependent on exogenous Ca^{2+} , but not on Mg^{2+} , since it was not impaired in the absence of Mg^{2+} .

sIgA-induced LTC_4 release from basophils

In addition to preformed mediators, basophils can liberate newly synthesized mediators, such as LTC_4 , upon appropriate stimulation (16). As shown in Figure 7, immobilized sIgA also induced statistically significant release of LTC_4 from IL-3-primed basophils, and the amount of released LTC_4 was approximately one-half of

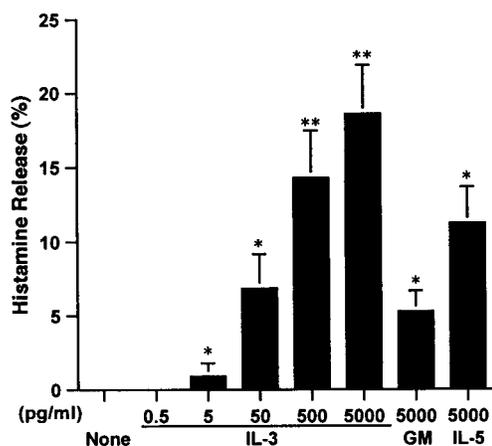


FIGURE 5. Comparison of priming effects of IL-3, IL-5, and GM-CSF on sIgA-induced basophil histamine release. Basophils were preincubated with IL-3, GM-CSF, or IL-5 at the indicated concentrations for 30 min at 37°C , and challenged for 45 min with sIgA-coated beads at a 1:10 bead:cell ratio. GM denotes GM-CSF. No difference was observed in spontaneous release among IL-3-, IL-5-, and GM-CSF-treated basophils (consistently $< 5\%$). Bars represent the SEM ($n = 5$). * $p < 0.05$, ** $p < 0.01$, vs percentage of histamine release from nonprimed, but sIgA-stimulated basophils (indicated as "none" in the figure).

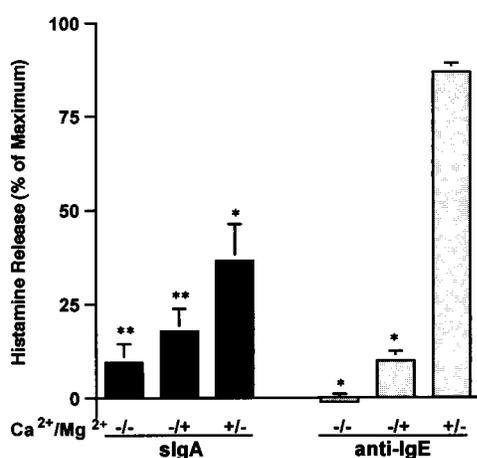


FIGURE 6. Basophil histamine release induced by sIgA-coated beads or anti-IgE in the presence or absence of Ca²⁺ or Mg²⁺. IL-3-primed basophils were stimulated with sIgA at a 1:10 bead:cell ratio in PIPES A buffer alone or PIPES A buffer supplemented with either Ca²⁺ at 2 mM, or Mg²⁺ at 0.5 mM (*left*). Basophils were stimulated with anti-IgE (1/3000 dilution) for 45 min without pretreatment with IL-3 (*right*). Data are expressed as the percentage of maximal histamine release observed in PIPES A buffer supplemented with both Ca²⁺ and Mg²⁺ (PIPES ACM). Bars represent the SEM ($n = 4$). * $p < 0.05$, ** $p < 0.01$, vs maximal histamine release in PIPES ACM.

that released from IL-3-primed, anti-IgE-stimulated basophils. Although LTC₄ release was observed in primed basophils from almost all donors, nonprimed cells from some donors (four of nine donors) released a comparable amount of LTC₄. This suggests that priming with IL-3 is not required for sIgA-induced LTC₄ generation in some, but not all, donors.

(Fc)₂:SC fragment of sIgA is important for basophil histamine release

To elucidate the mechanisms underlying sIgA-mediated basophil activation, we tested the effects of IgA, SC, Fab fragment, or (Fc)₂:SC fragment of sIgA on the degranulation of basophils as well as eosinophils. Consistent with the previous reports by others

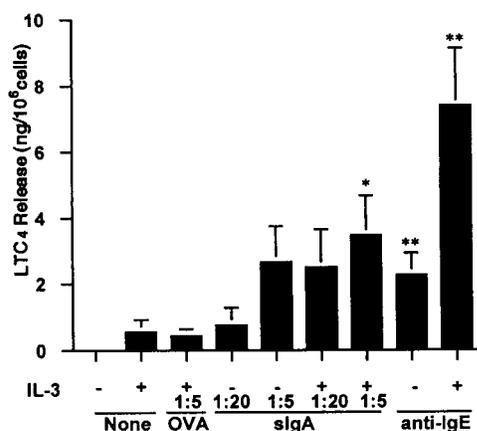


FIGURE 7. Induction of LTC₄ release from basophils by sIgA and comparison with release by anti-IgE. Semipurified basophils were pretreated with or without IL-3 (5 ng/ml) for 30 min. The cells were then incubated for 45 min in buffer alone (indicated as “none”), or either OVA- or sIgA-coated beads at the indicated bead:cell ratios, or anti-IgE Ab at 1/3000 dilution. Bars represent the SEM ($n = 9$). * $p < 0.05$, ** $p < 0.01$, vs LTC₄ release in the presence of OVA-coated beads (for sIgA-induced release) or in the buffer only (for anti-IgE-induced release).

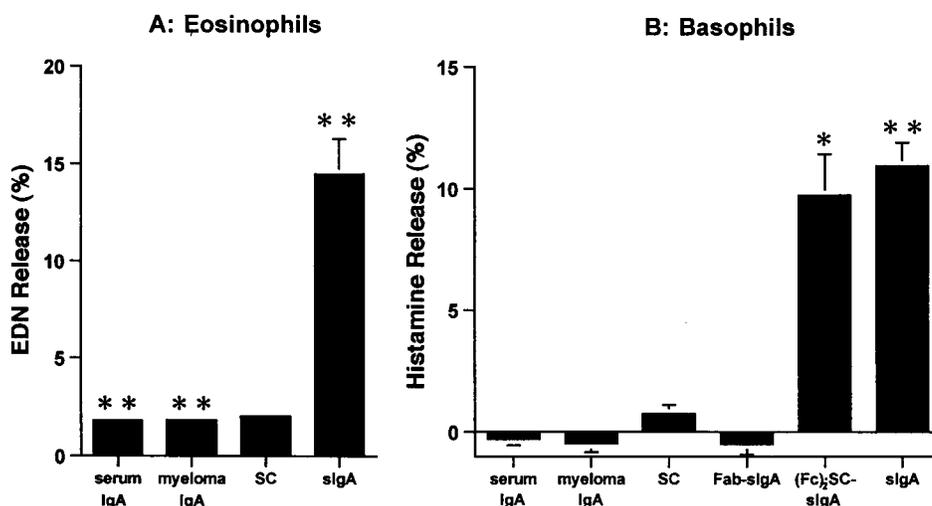
(8, 11), we observed that stimulation with immobilized preparations of serum IgA, myeloma IgA, or sIgA resulted in a significant release of EDN from human eosinophils (Fig. 8), and that the percentages of EDN release by the IgA preparations were similar to those in published reports (8, 11). It should be mentioned that sIgA induced statistically significantly more EDN release than serum IgA or myeloma IgA. On the other hand, in basophils, neither serum IgA nor myeloma IgA induced any histamine release. Although the Fab fragment of sIgA caused no basophil degranulation, apparent release was induced by the (Fc)₂:SC fragment of sIgA, suggesting that the (Fc)₂:SC portion is important for sIgA-mediated basophil activation. However, immobilized preparations of SC induced weak, but not statistically significant degranulation of IL-3-primed basophils as well as eosinophils.

Discussion

Historically, sIgA has been recognized to play a protective role against invasion by microorganisms via the mucosal surfaces by virtue of toxin neutralization and prevention of bacterial adherence to the mucosal surfaces (17, 18). Individuals with IgA deficiency are apt to experience recurrent or unusual types of infections (17). Furthermore, recent studies have demonstrated that sIgA provides protection against HIV by neutralization (18), or some other mechanism, such as Ab-dependent cell-mediated cytotoxicity (19). In addition to the well-established protective roles of sIgA in mucosal immunity, several lines of evidence indicate potential involvement of sIgA in the inflammation associated with allergic disorders. Mucous secretions at the sites of allergic inflammation contain a large amount of IgA, and most of this mucus IgA consists of sIgA (20). IgA in the sputa of asthmatics was significantly higher than that in the normal controls (21). IgA in bronchoalveolar lavage fluids from allergic rhinitis or asthmatic patients was higher than that in nonallergic subjects (20). Furthermore, the levels of mite-specific IgA in sputa were significantly higher in mite-sensitive asthmatics than in mite-insensitive asthmatics (22). In vitro studies have demonstrated that both of sIgA and IgA have potent ability to cause degranulation of eosinophils (8, 11). In addition, studies of saline-induced sputa from asthmatic patients demonstrated that the increase in the level of eosinophil cationic protein correlated with an elevation of the IgA concentration (21, 23), suggesting a possible link between sIgA and cellular activation of eosinophils in vivo. In the present study, we have demonstrated for the first time that sIgA is capable of inducing mediator release from human basophils (Figs. 1 and 7). The in vitro mediator release by sIgA may indicate the existence of the in vivo mechanisms of basophil activation by this molecule. Basophils are identified at the sites of experimentally induced LPRs as well as in the airways of post-mortem cases of fatal asthma (1, 24). Basophil influx into the inflamed tissues is observed preceding LPRs, and the profile of proinflammatory chemical mediators in lavage samples indicates local basophil activation at the sites of LPRs. These notions along with the results shown herein collectively indicate that interaction of sIgA with basophils might play some role at mucosal surfaces in allergic disorders with a possible consequent contribution to the pathogenesis of LPRs. Although an Ag-sIgA immune complex might represent the physiologic form of immobilized sIgA in vivo, further studies analyzing how and to what extent sIgA acts on basophils in vivo will be of great importance.

One of the most striking findings in our study is that sIgA-induced degranulation of basophils was totally dependent on pretreatment with IL-3 (Fig. 1). Furthermore, sequence of addition of IL-3 and sIgA was crucial for sIgA-mediated histamine release (Fig. 3): IL-3 acts as a priming factor, and sIgA as a secretagogue

FIGURE 8. Degranulation of eosinophils and basophils by various IgA preparations or SC. **A.** Eosinophils were stimulated with Sepharose beads conjugated with various IgA preparations ($n = 4$) or SC ($n = 2$) at a 1:20 bead:cell ratio for 4 h at 37°C, and EDN in the supernatant was measured by RIA. **B.** IL-3-primed basophils ($n = 4$) were incubated with beads coated with whole IgA molecules (serum IgA, myeloma IgA, or sIgA) or sIgA-derived fragments (SC, F(ab')₂ fragment, or (Fc)₂SC fragment) at a 1:10 bead:cell ratio for 45 min at 37°C, and histamine in the supernatant was analyzed. Bars represent the SEM. * $p < 0.05$, ** $p < 0.01$, vs OVA-induced release from corresponding cells.



that requires priming with IL-3. The priming effect of IL-3 on mature basophils was reported initially by us (12) and others (25): pretreatment (~15 min) of basophils with IL-3 results in enhancement of histamine release evoked by diverse stimuli including anti-IgE. In the present study, only a negligible response to sIgA was observed in IL-3 nontreated basophils, indicating that stimulation with sIgA is unable to transduce signals sufficient for degranulation, and IL-3 renders basophils susceptible to stimulation with sIgA. Similar phenomena have been reported for several secretagogues, such as C3a (25) and platelet-activating factor (26). Even picomolar concentrations of IL-3 were sufficient to induce a qualitative change in the response of basophils to sIgA (Fig. 5), indicating that the effect of IL-3 is exerted via high affinity IL-3R. Furthermore, IL-5 and GM-CSF also induced sIgA-mediated basophil degranulation (Fig. 5), indicating that intracellular signaling is mediated via the promiscuous β -chain of the receptors. IL-3, IL-5, and GM-CSF exert many other activities on basophils, including induction of migration (12), adherence to the endothelium (27), prolongation of survival (28), and augmentation of cytokine generation (29). These basophil-active cytokines are produced by Th2 lymphocytes, which are predominant cells at the sites of allergic inflammation. Therefore, the local production of these cytokines at sites of ongoing allergic reactions may contribute to the pathogenesis of local allergic responses through promotion of a wide array of basophil functions, possibly including sIgA-induced degranulation.

The results of our study suggest that human basophils possess functional binding sites for sIgA. At present, we have not directly identified the binding sites for sIgA on basophils, partly because of the difficulty in preparing a large number of pure basophils. Although the (Fc)₂SC fragment, but not the Fab fragment, of sIgA induced basophil degranulation (Fig. 8), the binding sites of sIgA are surmised to be distinct from Fc α R (CD89) for the following reasons. First, no significant histamine release was induced by immobilized monomeric IgA in the present study (Fig. 8). Second, flow-cytometric analysis did not detect CD89 on basophils (30). Finally, histamine release by immobilized sIgA was not antagonized in the presence of soluble serum IgA at up to 250 μ g/ml (data not shown). These results indicate that the binding sites of the (Fc)₂SC portion of sIgA differ from Fc α R. On the other hand, sIgA is also capable of causing degranulation of eosinophils, which express Fc α R (31). However, eosinophil degranulation by sIgA is not mediated merely via binding to Fc α R(s). Previous observations by

others (8, 11) and our present data (Fig. 8) showed that sIgA always induces a higher magnitude of EDN release than IgA. In addition, eosinophil superoxide production initiated by sIgA was only partially inhibited by treatment with anti-Fc α R Ab, whereas IgA-mediated superoxide production was completely abolished (32). These observations strongly indicate that sIgA transduces intracellular signals in eosinophils via two different pathways: Fc α R and an undetermined binding site(s) for sIgA. A substantial body of evidence indicates that basophils share a number of common surface structures with eosinophils (2). Presumably, sIgA-mediated activation of basophils is exerted through the undetermined binding site(s) for sIgA, identical with that on eosinophils. The existence of receptors/binding sites for SC has been reported in eosinophils (33). Although we failed to observe significant induction of degranulation of IL-3-primed basophils by immobilized SC, a binding site for SC could be a candidate for the functional binding site for sIgA on basophils. Because we cannot deny the possible involvement of a binding site on the Fc portion of sIgA other than CD89, which might transduce costimulatory signals in sIgA-mediated basophil degranulation, further functional and molecular biologic analyses of the binding sites of IgA/sIgA on eosinophils and basophils will be helpful to explain the mechanisms underlying sIgA-induced basophil degranulation.

In this study, we did not define the subclass of IgA preparations. Theoretically, all IgA preparations, including myeloma IgA, consist of mixtures of both IgA1 and IgA2. Serum IgA is enriched in IgA1, whereas sIgA is enriched in IgA2. However, we do not think that a difference in the subclass of IgA is the cause of the different responses of basophils to various IgA preparations for the following two reasons. First, an early report (8) demonstrated that there was no difference in EDN release from eosinophils in response to IgA1 and IgA2. Second, basophils were not stimulated by the beads coated with high concentrations of serum IgA, which contains about 10% IgA2.

In summary, we have demonstrated for the first time that sIgA induces mediator release from human basophils. sIgA-induced basophil degranulation requires priming with basophil-active cytokines, such as IL-3. Because sIgA is the most abundant Ig isotype in mucus secretions, and basophils at the sites of allergic inflammation are perceived to be activated by these cytokines, sIgA-mediated basophil mediator release may play an important role in the exacerbation of allergic inflammation of mucosal surfaces.

Acknowledgments

We thank Dr. Kaoru Kusama for providing us with reagents. Thanks are also extended to Ms. Yasuko Asada, Ms. Sayaka Jibiki, and Ms. Masako Imanishi for their excellent technical assistance.

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