The effect of cationic charge on release of eosinophil mediators

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Background: In patients with atopic diseases, cationic-charged eosinophil proteins are deposited in inflamed tissues. Although the role of cytokines in cell activation is well established, the presence of cationic-charged tissue can also be an important factor in inflammatory cell function.
Objectives: We sought to determine whether increased cationic charge seen in an atopic microenvironment plays a role in the activation of eosinophils.
Methods: Human eosinophils were incubated with Sepharose beads coated with cationic or anionic compounds in the presence and absence of a cytokine cocktail (IL-3, IL-5, and GM-CSF) to simulate the milieu of inflammation. Eosinophil peroxidase and eosinophil-derived neurotoxin (EDN) release were compared with eosinophil morphology and expression of CD18, as determined by means of confocal microscopy.
Results: Cytokines with positively charged beads caused greater eosinophil peroxidase release (lysine coated, 44.2 nmol/L; compound 48/80, 40.0 nmol/L; or EDN coated, 49.1 nmol/L) than cytokines alone (14.9 nmol/L). Beads coated with heparin, dextran sulfate, and aspartic acid did not show this effect. EDN release was also induced by lysine-coated beads with cytokines (67.1 ng/100 μL) and blocked by heparin. Eosinophil incubation with wortmannin, genistein, and the src kinase inhibitor PP1 blocked cationic signaling. Eosinophils adherent to cationic-charged beads but not anionic-charged beads show polarization of CD18 expression toward the bead's surface.
Conclusion: Cationic-charged surfaces induce increased eosinophil mediator release by increasing the density of CD18 expression available at the target surface. (J Allergy Clin Immunol 2008;122:383-90.)

Key words: *Eosinophil, cationic charge, heparin, eosinophil peroxidase, eosinophil-derived neurotoxin, CD18*

Abbreviations used

CNBr:	Cyanogen bromide
DB-LysBD:	Dextran sulfate–blocked lysine-coated CNBr bead
DIC:	Differential interference contrast
EDN:	Eosinophil-derived neurotoxin
EPO:	Eosinophil peroxidase
HB-LysBD:	Heparin-blocked lysine-coated CNBr beads
HSA:	Human serum albumin
LysBD:	Lysine-coated CNBr bead
MBP:	Major basic protein
OPD:	*o*-Phenylenediamine
PI3:	Phosphatidylinositol-3
ROI:	Region of interest
RT:	Room temperature
sIgA:	Secretory IgA
TMB:	3,3′,5,5′-Tetramethyl-benzidine

Eosinophils are associated with a range of human pathologies in many organ systems. The eosinophil contains 5 cationic-charged proteins: major basic proteins (MBPs) 1 and 2, eosinophil peroxidase (EPO), eosinophil-derived neurotoxin (EDN), and eosinophil cationic protein.[1,2] In human subjects and animals inhalation of cationic substances induces airway hyperreactivity.[3,4] It has been surmised that the presence of extracellular eosinophil MBP seen in the tissue of patients with atopic disease[5-7] and around parasitic worms[8] plays a role in inflammatory cell activation. MBP induces mediator release from neutrophils by using phosphatidylinositol-3 (PI3) kinase– and tyrosine kinase–dependent pathways.[9] MBP can also induce eosinophil mediator release, although the mechanism remains unclear.[10]

As eosinophils migrate through tissue, they interact with other cells and structural proteins, which induce a state of activation.[11] Furthermore, the milieu of cytokines present in inflamed tissue plays an important role in eosinophil activation and survival.[12] Expression of cell-surface CD18 is upregulated in tissue-dwelling eosinophils,[13] and signaling through CD18 appears essential for eosinophil degranulation *in vitro*.[14,15]

In this study we attempted to reproduce the atopic tissue microenvironment and investigate the role of cationic charge within the context of eosinophil-activating cytokines (IL-3, IL-5, and GM-CSF) likely present in inflamed tissues. Our results indicate that exposure of eosinophils to cytokines and to a positively charged surface strikingly increases eosinophil degranulation. The mechanism of this sensitivity is through polarization of CD18 toward cationic surfaces, increasing the density of CD18 at the target.

METHODS

Please see the Methods section of the Online Repository at www.jacionline.org for more complete methodology.

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Eosinophil isolation

Human peripheral blood eosinophils were isolated as previously described (>99% purity).[16,17]

Measurement of EPO release

Eppendorf tubes were precoated with 2.5% human serum albumin (HSA) to block nonspecific cell activation.[18] The release of EPO was measured by using a standardized assay with purified human EPO[19] and peroxidase substrate solution containing *o*-phenylenediamine (OPD), as previously described.[17]

Measurement of EDN release

EDN release was measured by using an immunoluminometric assay with 2 mAbs. Briefly, microtiter plates were coated with anti-EDN capture antibody. After blocking and washing, standards, samples, and controls were added. Acridinium-labeled anti-EDN secondary antibody was added. After incubations, the plate was read in an LmaxII luminometer (Molecular Designs, Sunnyvale, Calif).

Cationic-charged bead preparation and eosinophil stimulation

Cyanogen bromide (CNBr)–coupled Sepharose 4 Fast Flow beads (CNBr beads; Amersham Biosciences, Uppsala, Sweden) are typically used in secretory IgA (sIgA) stimulation protocols with eosinophils. To create a cationic surface, we adapted the protocol of Abu-Ghazaleh et al,[20] using various cationic suspensions in place of sIgA (0.1 mol/L lysine monohydrochloride, compound 48/80 [5 μg/mL], or EDN [purified from human eosinophils, 2.5 mmol/L]). These cationic compounds were also studied directly in solution without the beads (lysine, 0.003 mol/L; compound 48/80, 1 μg/mL; EDN, 0.06 mmol/L). EPO release was also measured from eosinophils and CNBr-coupled beads without cationic coating. Some cationic-coated beads were blocked with heparin (2500 IU/mL) or dextran sulfate (5000 μg/mL). In place of lysine, HSA (0.5%) or aspartic acid (0.037 mol/L) was used for some bead preparations. EPO and EDN release was measured from eosinophils incubated with or without the various types of beads in the presence or absence of the cytokine cocktail.

Cytokine cocktail stimulation

Some eosinophils were incubated with a cocktail of IL-3 (5 ng/mL), IL-5 (2.5 ng/mL), and GM-CSF (5 ng/mL) with or without CNBr-coupled beads. This was compared with calcium ionophore A23187.

Inhibition of intracellular signaling

Eosinophils were preincubated with various concentrations of the PI3 kinase inhibitor wortmannin (0-25 nmol/L), the broad-spectrum tyrosine kinase inhibitor genistein (0-25 μmol/L), the src kinase family inhibitor PP1 (0-100 nmol/L) and the syk kinase family inhibitor piceatannol (0-1000 nmol/L), each for 30 minutes at 20°C before the protocols above. Controls were incubated similarly.

CD18 expression by means of flow cytometry

Eosinophils were incubated with mouse anti-human CD18 IgG2a or isotype control to measure CD18 expression before and after treatment with cytokine cocktail. Goat anti-mouse phycoerythrin-labeled IgG (1:250) was used to quantify CD18 expression by means of FACScan analysis (Becton-Dickinson, Franklin Lakes, NJ).

Blockade of CD18

Eosinophils were preincubated with mouse anti-human CD18 or the isotype control (mouse anti-human IgG2a, sodium azide free) before use in some stimulation protocols.

Live-cell confocal microscopy

By using live-cell real-time photography, eosinophils were observed for morphology and movement during the experimental protocols. Petri dishes with glass coverslip bottoms (MatTek Corp, Ashland, Mass) were precoated with 2.5% HSA. A laser-scanning microscope created differential interference contrast (DIC) images (×160, Olympus FluoView FV1000; Olympus, Markham, Ontario, Canada).

Eosinophil adhesion assay

Eosinophils (5×10^5/well) stimulated with the cytokine cocktail were added to wells precoated with 2.5% HSA and then coated with either lysine or heparin. After washing away nonadherent cells, 3,3′,5,5′-tetramethyl-benzidine (TMB) was added. TMB permeates the cell membrane; thus the color change represents the reaction of TMB with EPO from adherent cells (Softmax, 450-nm wavelength).

CD18 expression by means of confocal microscopy

Eosinophils alone without beads or cytokine-treated eosinophils with beads were incubated with rat anti-human CD18 (1 μg/100 μL) or rat purified IgG before Alexa Fluor 488–labeled chicken anti-rat IgG (H+L, 2.5 μg/100 μL; Molecular Probes, Eugene, Ore). Samples were fixed in 1% paraformaldehyde. CD18 localization and quantification of average fluorescence intensity were determined by using confocal laser scanning microscopy with Fluoview software (×480 images, scanned to a thickness of 0.95 μm with the FV10-ASW, Olympus). Images were selected. Freehand region of interest (ROI) selection was set to contain cell membrane contacting or not contacting the bead surface (see Fig E1 in the Online Repository at www.jacionline.org). Average fluorescence intensity negates he possible effects of small differences in ROI area.

Statistical analysis

Data are expressed as means ± SEMS, with n values representing the number of individual donors and their measured EPO or EDN values within the specified experimental condition. Microplate Manager software (Bio-Rad, Hercules, Calif) was used for linear correlation between optical density and EPO molarity values. Comparisons of mean fluorescent intensity and EPO and EDN activity between the groups were analyzed by using ANOVA (Statview 5.0; SAS Institute, Cary, NC). A P value of less than .05 was considered statistically significant.

RESULTS

Cationic charge induces EPO release from cytokine cocktail–stimulated eosinophils

Individually, IL-5, GM-CSF, and IL-3 are known to increase eosinophil survival and activation.[11,21] Compared with unstimulated control eosinophils (5.1 nmol/L), the cytokine cocktail induced a small but consistent release of EPO (15.3 nmol/L, $P < .02$, Fig 1), which was greater than the release from IL-3, IL-5, and GM-CSF used individually (5.5, 7.5, 9.1 nmol/L, respectively; n = 6 each). In the absence of cytokines, CNBr beads with or without lysine coating did not induce EPO release (7.3 ± 0.86 nmol/L vs 5.5 ± 0.9 nmol/L, respectively). In the absence of lysine coating, CNBr beads did not induce EPO release from cytokine-stimulated eosinophils greater than that seen with cocktail alone (16.7 nmol/L). In contrast, the cytokine cocktail together with lysine-coated CNBr beads caused significant EPO release (51.4 nmol/L) when compared with that seen with the cocktail alone ($P < .0001$). This was similar to the release induced by the calcium ionophore A21387 (51.4 nmol/L). Based on data from lysed eosinophils,[17] this amount of release is equivalent to 14% total EPO release. IL-3, IL-5, or GM-CSF used individually with the lysine-coated beads increased EPO release, although the potency of each cytokine was not equivalent and was less than that seen with the combined cocktail (19.2, 37.5, and 43.4 nmol/L, respectively; n = 6 each).

Other cationic-charged proteins induce EPO release from cytokine-treated eosinophils

To confirm that cationic charge induces eosinophil mediator release, we used compound 48/80, a well-established cationic