# Environmental polycyclic aromatic hydrocarbons, benzo(a) pyrene (BaP) and BaP-quinones, enhance IgE-mediated histamine release and IL-4 production in human basophils

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Kepley, CL, Lauer FT, Oliver JM, Burchiel SW. Environmental polycyclic aromatic hydrocarbons, benzo(a) pyrene (BaP) and BaP-quinones, enhance IgE-mediated histamine release and IL-4 production in human basophils. *Clinical Immunology* 2003; 107(1):10-9.

**Made available courtesy of Elsevier: https://doi.org/10.1016/S1521-6616(03)00004-4**



## Abstract:

Polycyclic aromatic hydrocarbons (PAHs) are major components of diesel exhaust particles found in pollutant respirable particles. There is growing evidence that these fossil fuel combustion products exacerbate allergic inflammation. Basophils contribute to allergic inflammation through the release of preformed and granule-derived mediators. To determine whether allergens and PAHs interact, we incubated human basophils with PAHs and measured the release of histamine and IL-4 with and without added antigen. None of the PAHs induced mediator release by itself and none affected total cellular histamine levels. However, several PAHs enhanced histamine release and IL-4 production in response to crosslinking the high-affinity IgE receptor, FcεRI. The enhancement seen with 1,6-BaP-quinone involved an increase in tyrosine phosphorylation in several different substrates, including the FcεRI-associated tyrosine kinase, Lyn, and elevated reactive oxygen species (ROS) levels detected by dichlorofluorescein fluorescence and flow cytometry. The PAH-induced enhancement of mediator release and ROS production could be inhibited with the antioxidant *N*-acetylcysteine. These data provide further evidence that environmental pollutants can influence allergic inflammation through enhanced FcεRI-coupled mediator release from human basophils.

**Keywords:** Human basophils | Polycyclic aromatic hydrocarbons

## Article:

### Background

Increased industrialization in developed countries has been paralleled by increases in allergic asthma [1], [2], [3]. A number of factors are likely to be important in the increase in mortality and morbidity from this debilitating disease. These include an inverse relationship between

infection and atopy, increased exposure to allergens, a lack of physical exercise in the population, and an increase in airborne materials [3]. Many epidemiological studies have suggested a link among air quality, the increase of atmospheric pollutants, and the increased incidence of asthma [4], [5], [6], [7], [8], [9].

Diesel exhaust particles (DEPs) are important components of pollutant respirable particulate matter (PM) in the range of 2.5–10 μm (PM2.5 and PM10) or less. A major chemical component of DEPs are compounds that contain three to five benzene rings and are classified as polycyclic aromatic hydrocarbons (PAHs). These molecules are ubiquitous in the environment, contaminating air, water, and soil [10]. Diesel exhaust is now one of the major causes of particulate air pollution in urban districts where concentrations of DEPs can fluctuate from 2 to 500 $\mu g/m^3$ during peak automobile traffic. One of the best characterized environmental PAHs associated with DEP and PM is benzo(a)pyrene (BaP). BaP and certain oxidation products formed in the presence of ultaviolet light or by P450s and other oxidative enzyme (e.g., peroxidases), most notably BaP-quinones, activate signaling pathways in lymphoid cells [11].

The evidence linking exposure to DEP and enhanced allergic inflammation is strong [7], [8]. Specifically, Diaz-Sanchez et al. showed that DEPs can enhance the severity of clinical symptoms to allergen by enhancing mast cell degranulation [12]. Similarly, Devouassoux et al. demonstrated enhanced IL-4 production in basophils exposed to DEP extracts [13]. However, it is not clear what component(s) in the DEP milieu is responsible for the observed effects.

To determine whether BaP or any of its known major metabolites may be involved in activation of signaling pathways associated with mediator release from human basophils, we exposed human basophils to BaP and several of its P450 and non-P450 breakdown products. Basophils were exposed to several of these PAHs and IgE and non-IgE-mediated histamine release and cytokine production were examined. We found that BaP and several BaP-quinones (BPQs) could enhance IgE-mediated histamine release and cytokine production by altering specific signaling pathways. In addition, we found that the release of histamine by 1,6-BPQ could be prevented by cotreatment with *N*-acetylcysteine (NAC). Therefore, our data suggest that BPQs or other related oxidative agents present in or on DEP can trigger allergic inflammation through enhanced antigen-induced mediator release from human basophils through oxidative-stress-associated pathways.

## Materials and methods

### Reagents and monoclonal antibodies

PAHs were obtained from commercial sources at greater than 95% purity. BaP and the antioxidant NAC were obtained from Sigma Chemical Company. The 1,6- (1,6-BPQ), 3,6- (3,6-BPQ), and 6-12 (6,12-BPQ) diones (quinones) as well as the 7,8-dihydrodiol (BP-diol) and 7,8-diol, 9,10-epoxide (BPDE) of BaP were obtained from the National Cancer Institute Chemical Repository at Midwestern Research Institute (Kansas City, MO). The 7,8-quinone of BaP (7,8-BPQ) was provided by Dr. Trevor Penning (University of Pennsylvania, Philadelphia, PA). All stock PAH solutions in dimethylsulfoxide (DMSO) were stored under nitrogen gas in the dark at −20°C. Solutions of PAHs were prepared at 100–1000 times their final concentration used for

experiments in DMSO (Sigma Tissue Culture anyhydrous grade). PAH stock solutions were diluted directly into cell culture medium immediately before use. The purity of PAHs was periodically examined by high-performance liquid chromatography. Handling, storage, and exposure of cells to PAHs were done under yellow light or no light conditions. The final concentration of DMSO in cell culture did not exceed 0.1%, and all PAH effects were compared to appropriate DMSO-treated control cultures. Anti-4-hydroxyl-3-nitro-5-iodo-phenylacetyl (NIP) IgE obtained from the cell line JW8 (ECACC No. 87080706) and NIP–BSA were obtained from Serotec (Raleigh, NC). Anti-phosphotyrosine antibodies (PY-99) and anti-Lyn Abs were obtained from Santa Cruz (Santa Cruz, CA). Peroxidase-conjugated anti-mouse Abs were obtained from The Jackson Labs (West Grove, PA). 5-(and 6)-Chloromethyl-2′, 7′-dichlorodihydrofluorescein diacetate (DCF) was obtained from Molecular Probes (Eugene, OR).

## Isolation of peripheral blood basophils

Venous blood (100–300 ml) was collected from normal donors who had given informed consent as approved by the Human Studies Committee at the University of New Mexico. EDTA (0.01% v/v) was added as anticoagulant. None of the donors were atopic by history. Basophils were purified by centrifugation over Percoll gradients followed by negative selection using magnetic beads as previously described [14], [15]. Basophil purities were ≥80% in all the experiments as determined with Wrights–Giemsa stain.

## Basophil histamine release and IL-4 production

Basophils (80–95%) were acid stripped to remove surface IgE [16] and suspended in fresh Iscove's medium (Iscove's modified Dulbecco's medium containing 5% heat-inactivated FBS, nonessential amino acids, and 5 μg/ml gentamicin) containing 10 μg/ml of anti-NIP IgE for 4 h at 37°C in a 5% $CO_2$ incubator. PAHs (1 μM final) or DMSO (0.001%) was added at various times (2 h to 15 min) during the sensitization. Cells were washed with $HBSS^{=}$ (Hanks' buffered saline solution without $Ca^{2+}$ or $Mg^{2+}$) [17], resuspended to 0.5–1.1 × $10^6$ basophils/ml in prewarmed $HBSS^{+}$(HBSS with 1.4 mM $CaCl_2$ and 1 mM $MgCl_2$) with NIP–BSA (50 ng/ml for histamine or 10 ng/ml IL-4), and incubated at 37°C for 45 min (histamine) or 4 h (IL-4) at 37°C in a 5% $CO_2$ incubator. Equal amounts of DMSO (final concentration of 0.001%) were included in the non-PAH-challenged cells as a control. Reactions were stopped by centrifugation at 150*g* at 4°C. Supernatants were stored at −70°C. Histamine and IL-4 were measured as previously described [15], [17], [18]. For total cellular histamine levels, basophils were freeze–thawed three times and histamine was measured in the cellular lysates [19].

In some experiments we examined the effect of the NAC on histamine release and IL-4 production. Cells were IgE sensitized as above with or without the addition of NAC (10 mmol) for 1 h at 37°C. This concentration gave maximal inhibition of DEP-induced IL-4 production in human basophils [13]. Following activation, histamine was measured in the supernatants. Alternatively, reactive oxygen species (ROS) levels were measured by FACS analysis (see below).

## Protein tyrosine phosphorylation

Basophils (≥93% pure) were acid stripped and sensitized with IgE with or without PAHs as above. Following activation, basophils (0.5–3 × $10^6$cells/ml) were lysed by 10-min incubation on ice in 50 mM Tris, pH 7.5, 150 mM NaCl, 1% Brij, 1 mM sodium orthovanadate, and 1 μg/ml each of antipain, leupeptin, aprotinin, and PMSF (lysis buffer). Insoluble material was removed by centrifugation at 10,000*g* for 5 min. The lysate was mixed 8:1 (v/v) with 8X Laemmli sample buffer [2% SDS (w/v), 10% (v/v) glycerol, 60 mM Tris (pH 6.8), and 0.1% bromphenol blue], heated at 95°C for 5 min, and applied to a 10% polyacrylamide gel (2.0 × $10^5$ cell equivalents). Molecular weight markers (Gibco–BRL) were included in each gel. Following SDS–PAGE, proteins were transferred to nitrocellulose using a semi-dry apparatus at 400 mA for 1 h. Membranes were blocked for 1 h with Blotto (5% bovine serum albumin and 0.05 M Tween -20 in PBS) and washed three times with Tris-buffered saline containing 0.05% (v/v) Tween 20, pH 7.4 (TTBS). Membranes were incubated overnight at 4°C with 1 μg/ml of PY Abs in PBS/BSA. Membranes were washed three times with TTBS, incubated with peroxidase-labeled goat anti-mouse IgG (1:40,000 dilution; The Jackson Laboratories) in PBS/BSA for 1 h at RT, washed six times with TTBS, and analyzed using ECL as described previously [15]. Blots were stripped for 30 min with 100 mM 2-mercaptoethanol, 2% SDS (w/v), and 0.5 M Tris–HCl, pH 6.8, at 50°C and reprobed with the indicated Abs (1 μg/ml) [15], [17].

## Immunoprecipitation

Basophils (≥95% pure; 2.0–3.0 × $10^5$ cells/condition) were IgE sensitized (±PAHs) and activated as above. Cell pellets were lysed in 400 μl of 50 mM Tris, pH 7.5, 150 mM NaCl, 1% Brij, 1 mM sodium orthovanadate, and 1 μg/ml each of antipain and leupeptin (lysis buffer) and incubated for 10 min on ice. Immune complexes were generated by incubating clarified supernatants with anti-Lyn antibodies preadsorbed to protein A–Sepharose beads (Pharmacia, Uppsala, Sweden) as described [17]. Precipitates were washed 2X with 1 ml of ice-cold lysis buffer (without Brij), followed by the addition of 40 μl of 1X Laemmli buffer and proteins separated by SDS–PAGE on a 10% gel (2.0–5.0 × $10^5$ cell equivalents/lane). Western blotting was performed with anti-PY Abs followed by anti-kinase Abs as described above [15], [17].

## Measurement of reactive oxygen species

ROS production was measured using DCF, a cell-permeant indicator that becomes fluorescent upon oxidation by $H_2O_2$. Basophils (≥92% pure) were sensitized, PAH exposed, and activated as above. After being washed, cells were resuspended in RPMI 1640 medium [19] containing 5 μM DCF and incubated at 37°C for 45 min. Cells were washed twice with basophil medium, and suspended in PBS and the mean fluorescence was measured by FACS analysis. The data are presented as the mean intensty fluorescence (MIF) in which the negative control (DMSO only, see below) was adjusted to approximately the same value (125–150 MIF) for each experiment. All experiments were performed in duplicate.

## Statistical analysis

Histamine release, cytokine production, and ROS formation by PAH and non-PAH-challenged basophils were compared using the unpaired *t* test with Welch's correction or the Mann–Whitney test (Graphpad Prism).

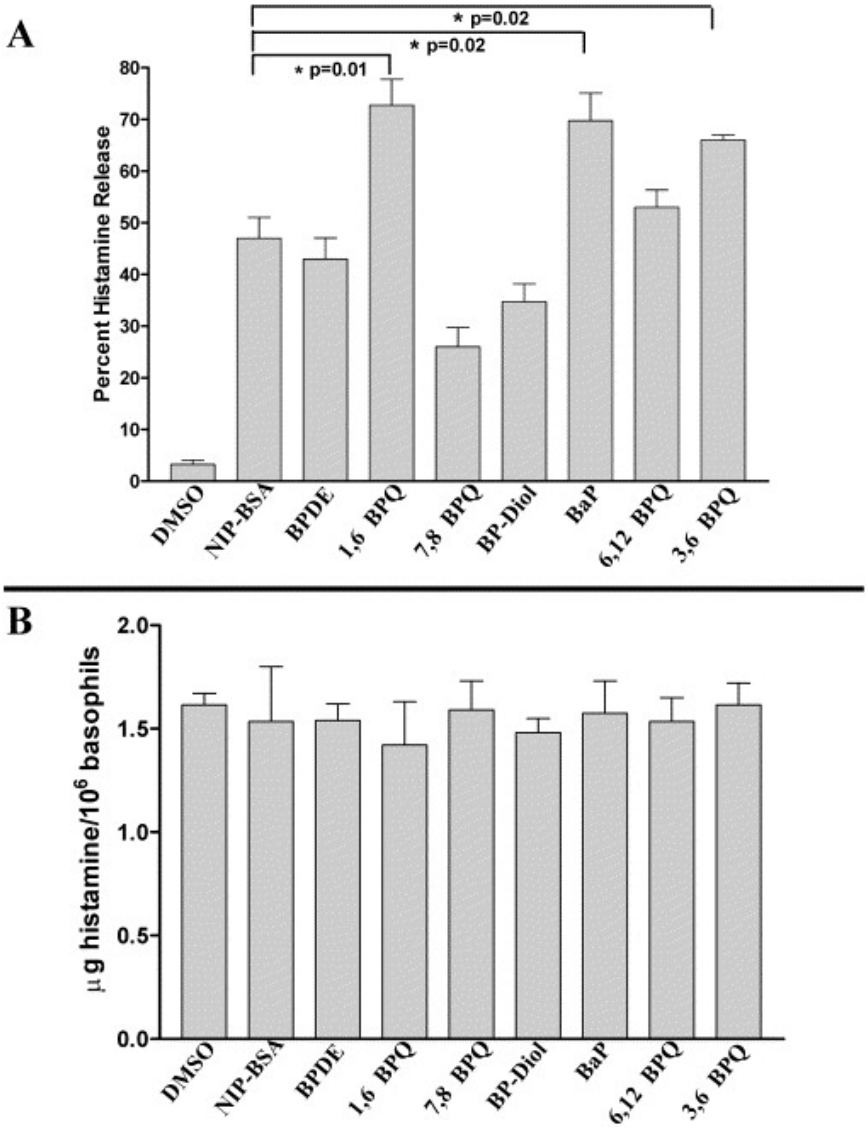



**Fig. 1.** (A) PAHs enhance IgE-mediated histamine release from human basophils. Percoll-enriched, negatively selected human basophils (80% purity) were acid stripped and sensitized for 4 h with anti-NIP–IgE (10 μg/ml). The indicated PAHs were present (1 μM) during the last 2 h. An equal amount of DMSO alone was added as a control. Cells were washed, activated for 45 min with or without 50 ng/ml NIP–BSA, and centrifuged and the supernatant was assayed for histamine by ELISA. Results are representative of three separate experiments, each done in duplicate. The error bars show SEM. *Values significantly increased with *P* values given. (B) PAH exposure does not increase total cellular histamine levels. Purified basophils (83%) were acid stripped, IgE sensitized, and incubated with the same PAHs as in A. Cells were lysed by repeated freeze–thawing and histamine was measured in the lysate by ELISA.

## Results

### PAHs enhance IgE-mediated histamine release

To examine the effects of PAHs on basal and FcεRI-mediated histamine release, acid-stripped basophils were sensitized for 4 h with 10 μg/ml IgE-anti-NIP [15], with the addition of PAHs (1

μM) during the final 2–0.25 h. After being washed, the cells were then washed and activated for 45 min with NIP–BSA. No histamine release was seen when cells were challenged with PAHs alone or with crude DEP extracts (up to 4 h). As seen in Fig. 1A,BaP and its 1,6-BPQ and 3,6-BPQ metabolites significantly enhanced IgE-mediated secretion of histamine from human basophils.

The PAH exposures did not induce cell death as measured by trypan blue exclusion (data not shown), nor were total cellular histamine levels affected. Basophils contained 1.42 to 1.62 μg/histamine per $10^6$ cells, with no significant increases seen with PAH incubation (Fig. 1B).

BPQs enhance IgE-mediated IL-4 production

Increased levels of circulating IL-4 is a hallmark of allergic inflammation. No IL-4 production was observed when basophils were challenged with PAHs alone (data not shown). However, the PAHs 1,6-BPQ and 3,6-BPQ, but not BaP, significantly enhanced IgE-mediated IL-4 production (Fig. 2).These data suggest that some metabolites of BaP and perhaps associated with DEPs and other PM found in air pollution may enhance human allergic inflammation via enhanced mediator release from basophils.

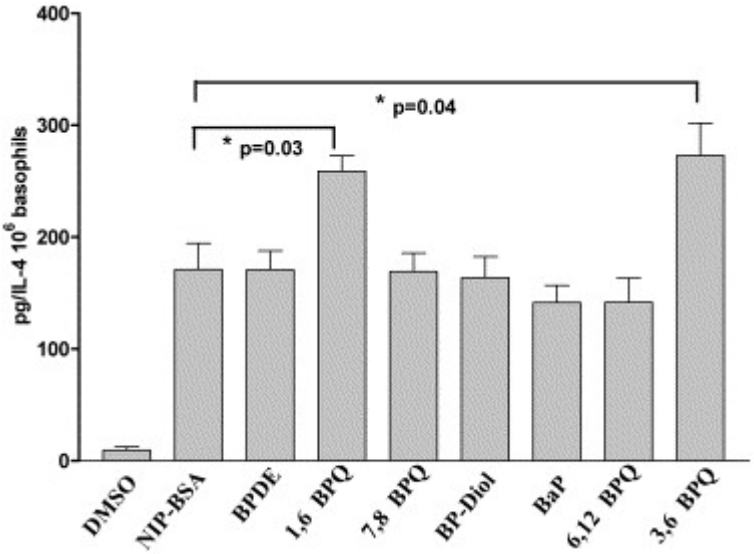



**Fig. 2.** PAHs enhance IgE-mediated IL-4 production from human basophils. Percoll-enriched, negatively selected human basophils (96% purity) were sensitized and activated for 4 h with or without 10 ng/ml NIP–BSA as described under Materials and Methods. The results from one experiment are representative of three separate donors. The SEM is shown by error bars. *Values significantly increased with *P* values given.

Increased ROS levels in PAH-exposed, IgE-activated basophils

ROS are generated at sites of inflammation and injury and can function as signaling intermediates in regulation of fundamental cellular activities [20], [21], [22]. BPQs are well known to induce ROS formation through redox-cycling mechanisms [23], [24]. Previous studies have suggested a link between PAH exposure and increased ROS levels in other cell types [11], [22]. However, the effect of PAH exposure on ROS formation in human basophils has not previously been explored. We chose to focus on the 1,6-BPQ PAH because it was

consistently the most potent enhancer of mediator release in our experiments. As seen in Fig. 3, antigen-activated basophils did not have significantly elevated ROS levels. However, when basophils were first exposed to 1,6-BPQ before antigen activation there was a significant increase in cellular ROS levels. Thus, it appears that the 1,6-BPQ enhancement of mediator release from human basophils involves an increase in ROS.

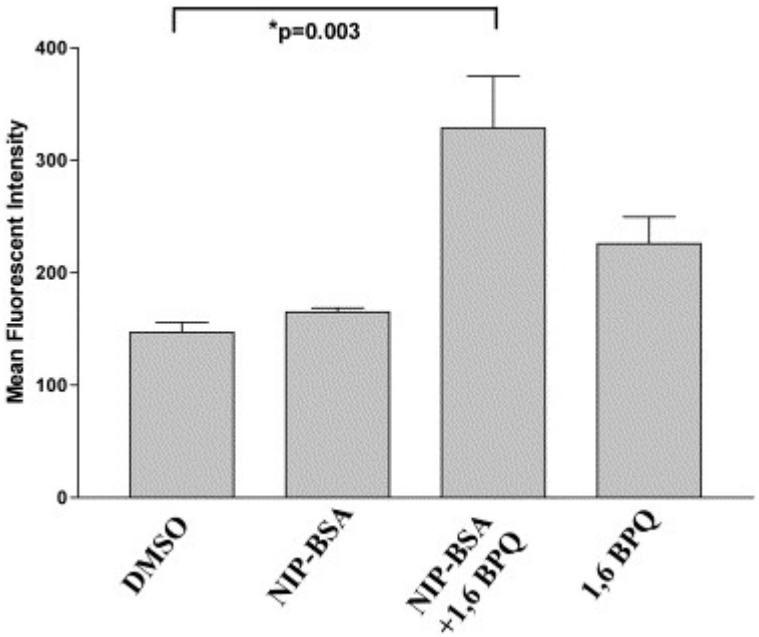



**Fig. 3.** PAH enhances IgE-mediated ROS formation in human basophils. Cells were IgE sensitized and exposed to DMSO or 1,6-BPQ. After being washed cells were incubated in buffer containing 50 ng/ml NIP–BSA or 1,6-BPQ alone (1 μM). Cellular ROS levels were detected by incubating the cells in DCF and detecting the fluorescence by FACS analysis. The results are presented as the mean from three combined experiments (three separate donors). *Values significantly increased with *P* values given.

Pahs alter the tyrosine phosphorylation of specific cellular proteins

IgE-mediated human basophil degranulation is initiated by the sequential activation of two protein tyrosine kinases, Lyn and Syk, that in turn support the tyrosine phosphorylation of specific cellular proteins [17]. To determine whether the enhanced IgE-mediated functional responses we observed were associated with PAH-induced changes in total cellular phosphorylation, IgE-sensitized basophils were challenged for different times with NIP–BSA with or without preincubation with 1,6-BPQ and then lysed and solubilized proteins were separated by SDS–PAGE. Western blots were probed with anti-PY Abs. As shown in Fig. 4A, FcεRI crosslinking stimulated the tyrosine phosphorylation of a number of proteins, including species with molecular masses of 105–110, 84, 74, 56, 53, and 50 kDa. Preincubation with 1,6-BPQ did not affect basal tyrosine phosphorylation, but it strongly increased the FcεRI-mediated tyrosine phosphorylation of several of these proteins.

We have previously shown that the tyrosine kinase Lyn (55–56 kDa) is tyrosine phosphorylated and activated upon FcεRI crosslinking in human basophils [17]. Certain PAHs have been shown previously to activate Lyn in human B cells [25]. We therefore tested the hypothesis that the Lyn is one of the hyperphosphorylated bands in the 1,6-BPQ plus antigen-treated samples in Fig. 4A.

Cells were activated, with or without 1,6-BPQ preincubation, and lysed, and anti-Lyn (Fig. 4B) immunoprecipitates were analyzed by immunoblotting with anti-PY Ab. Lyn showed basal phosphorylation; this is increased in response to antigen stimulation alone and is further increased by 1,6-BPQ treatment prior to antigen stimulation. Densitometry confirmed a significant increase in the amount of relative signal obtained from IgE-activated cells pretreated with 1,6-BPQ compared to nontreated cells.

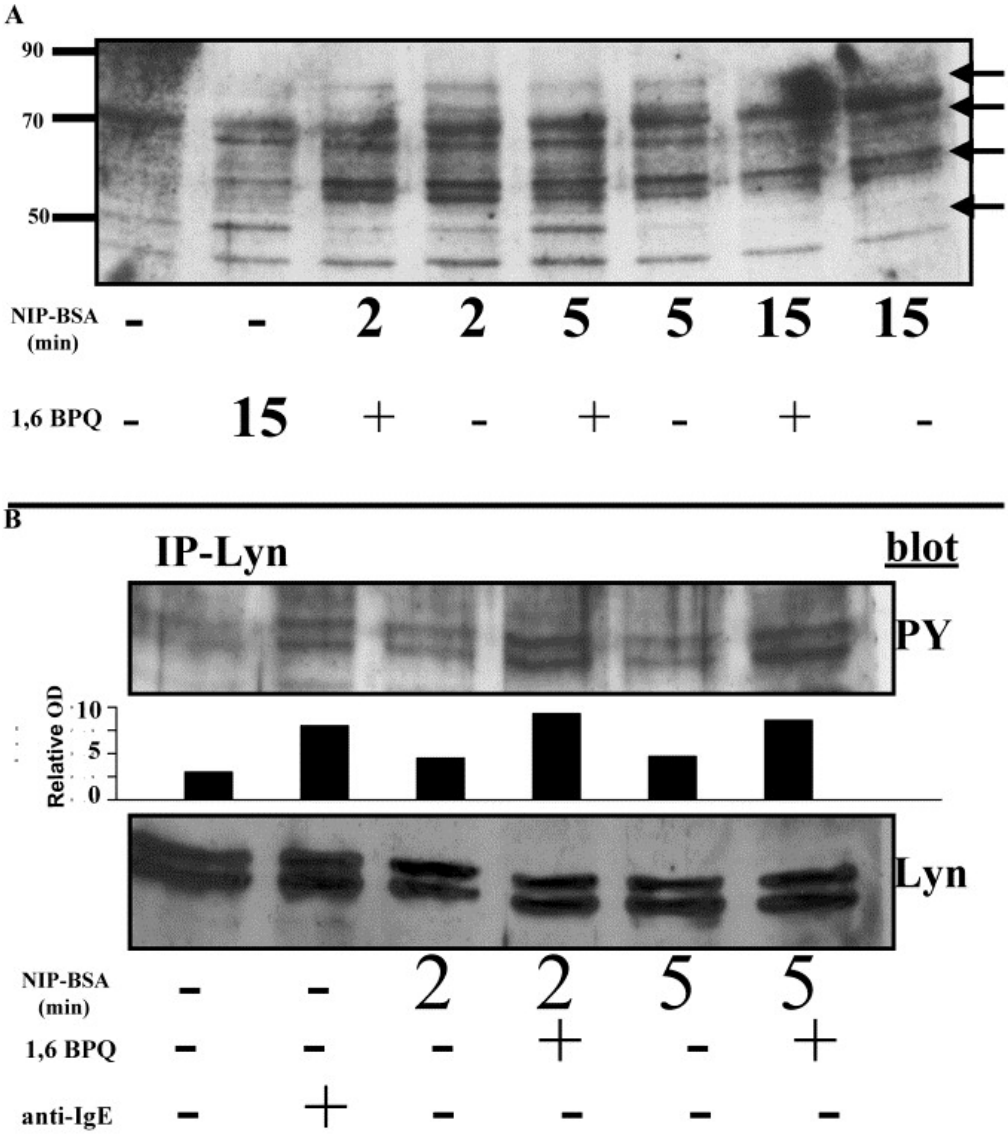
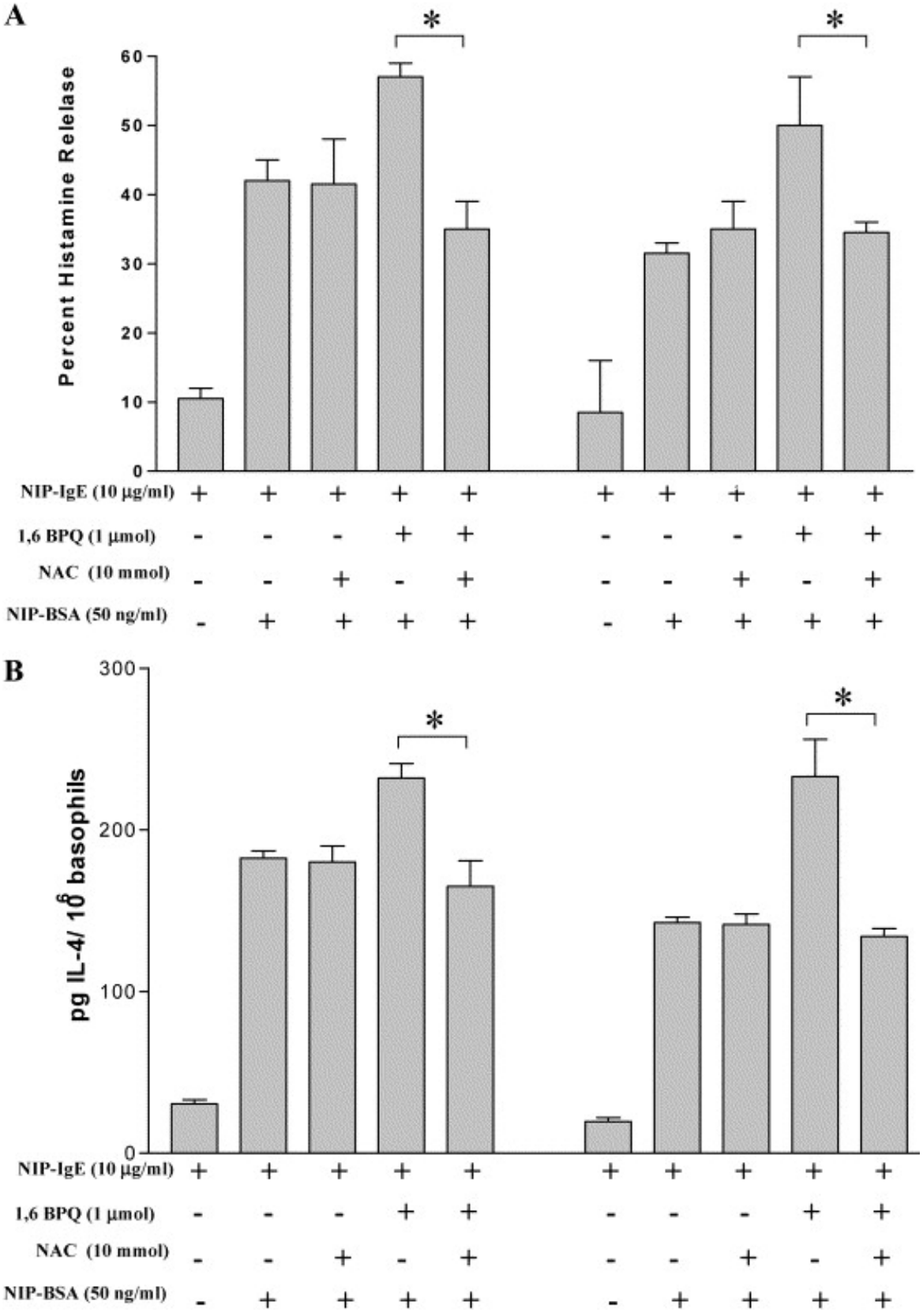



**Fig. 4.** (A) 1,6-BPQ exposure affects the protein tyrosine phosphorylation profile in human basophils. IgE-sensitized basophils (>90% pure) were incubated with NIP–BSA (50 ng/ml) for the indicated time with or without PAH exposure. Cells were lysed and proteins in the cleared cell lysates analyzed by SDS–PAGE ($0.64 \times 10^6$ cell equivalents/lane). Proteins were transferred onto nitrocellulose membranes and probed with anti-phosphotyrosine (PY) Ab. Immunoreactivity was detected using ECL. As a control, cells were incubated with 1,6-BPQ for 15 min without other stimulation. Similar results were obtained in two experiments. (B) 1,6-BPQ pretreatment enhances IgE-mediated Lyn phosphorylation. Basophils (90% purity) were sensitized and challenged as above. Lyn was immunoprecipitated and analyzed by Western blotting with anti-PY Abs (top) and stripped and reprobed with anti-Lyn Ab (bottom). Each lane shows Lyn immunoprecipitated from $8.0 \times 10^5$ cells. Anti-IgE stimulation (1 μg/ml; 5 min) was used as a positive control for Lyn phosphorylation [17]. The results are representative of two separate experiments. The molecular mass markers (kDa) are indicated.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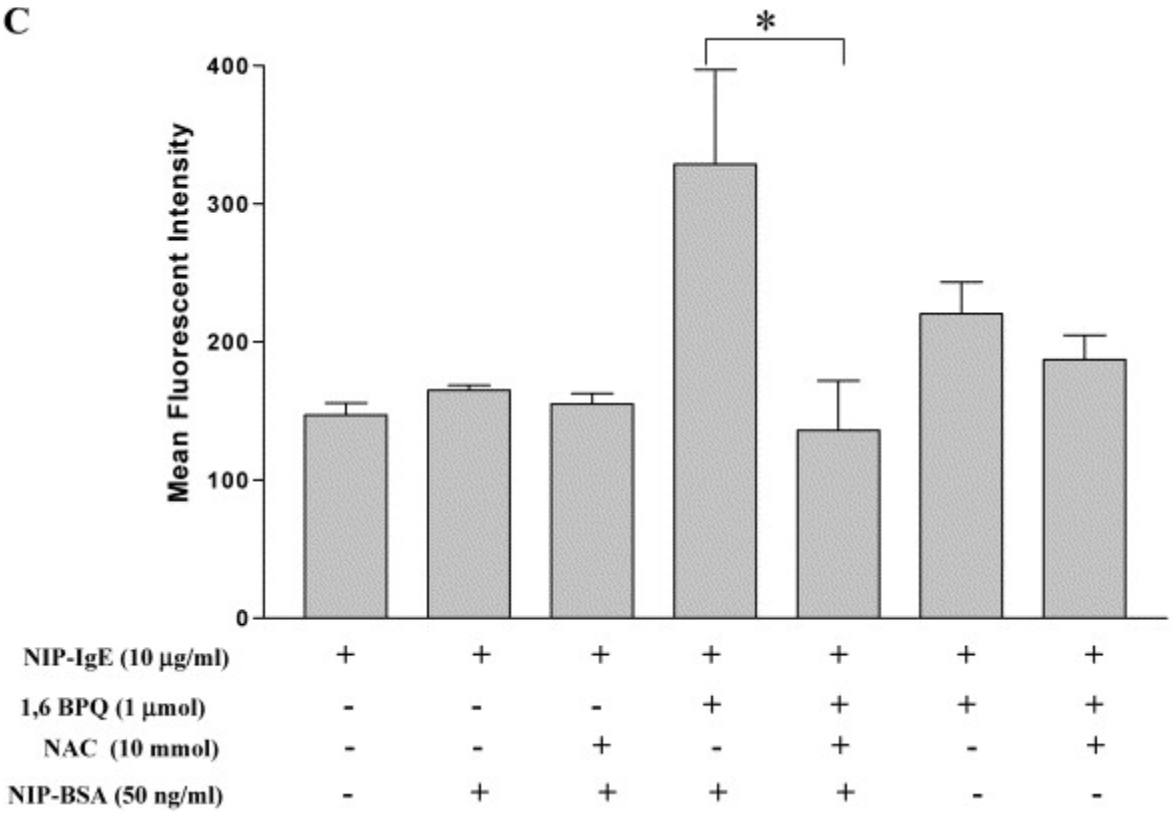


**Fig. 5.** PAH-induced enhancement of mediator release and ROS production is abrogated by NAC. Percoll-enriched, negatively selected human basophils (Expt. 1 = 89%; Expt. 2 = 95%) were acid stripped and sensitized for 4 h with anti-NIP–IgE (10 µg/ml). Cells were activated for (A) 30 min (histamine) or (B) 4 h (IL-4) with NIP–BSA (50 or 10 ng/ml) with or without preincubation with NAC (10 mmol for 1 h), 1,6-BPQ (1 µM for 15 min), or both. Supernatants were assayed for histamine or IL-4 by ELISA. Results of two separate experiments, each done in duplicate, are shown. *Significance at $P < 0.001$. (C) IgE-sensitized basophils (85–95%) were exposed to DMSO or 1,6-BPQ with or without NAC preincubation. After being washed cells were incubated in buffer containing 50 ng/ml NIP–BSA or 1,6-BPQ alone (1 µM). Cellular ROS levels were detected by incubating the cells in DCF and detecting the fluorescence by FACS analysis. The results are presented as the mean from two combined experiments (two separate donors). No statistically significant changes in ROS levels are shown.

## NAC abrogates 1,6-BPQ-induced responses

Increases in cellular levels of ROS can be inhibited using the antioxidant NAC. Based on the 1,6-BPQ-induced enhancement of mediator release and increased basophil ROS levels, we tested the effects of NAC on histamine release, IL-4 production, and ROS levels. NAC treated or untreated basophils were challenged with 1,6-BPQ and/or optimal concentrations of allergen and the mediator release and ROS production measured as above. As predicted, preexposure of basophils to 1,6-BPQ enhanced both histamine release and IL-4 production (Fig. 5A and B). However, when the cells were preincubated with NAC no PAH-induced enhancement was seen. Similar to what has been shown in other studies [13], [26], NAC produced no clear effects on antigen-induced mediator release. Cells preincubated with NAC also inhibited increases in basophil ROS levels produced by 1,6-BPQ (Fig. 5C).

## Discussion

Previous studies have clearly shown that DEP exposure leads to the enhancement of allergic inflammation. The purpose of this study was to examine the potential role of PAHs (a major component of DEP) in the regulation of mediator release from human basophils.

DEPs and their PAH components are known to increase IgE production in animals and humans both in vivo and in vitro [27], [28], [29], [30], [31], [32]. They are also known to increase allergic symptoms in animals. In particular, Kobayashi et al. demonstrated increased sneezing frequency, nasal secretion volume, and transnasal airway resistance in DEP-exposed guinea pigs [33], [34], [35]. These changes were accompanied by an exaggerated increase in permeability of nasal vasculature in DEP-challenged animals [36]. Saigai and colleagues [37] studied an "asthma-like" condition that develops in mice after prolonged intratracheal challenge with DEPs. These animals demonstrated an increase in airway resistance, increased mucous production, marked infiltration of eosinophils in bronchi and bronchioles, and a proliferation of goblet cells [37], [38]. In other rodent model studies, DEP challenge produced an induction of Th2-like cytokines [39], [40], an increase in inflammatory cells in bronchoalveolar lavage (BAL) fluid, enhanced airway constriction [39], and eosinophil infiltration, IgE production, and goblet cell hyperplasia [41]. Taken together, animals exposed to DEPs develop disease manifestations that mimic asthmatic disease processes in humans.

In human studies, DEPs sprayed into the nostrils of test subjects enhanced in vivo IgE production in the upper respiratory tract [28]. Using this same model, Saxon and co-workers recently showed that exposure to DEPs can enhance the severity of clinical symptoms to allergen, presumably by mast cell degranulation [12]. DEPs also act as adjuvants to coadministered antigen in nasal provocation challenges. In these studies, nasal lavages from ragweed-sensitized subjects were collected at different times after nasal challenge with ragweed allergen. As compared with challenge with ragweed alone, challenge with both DEPs and ragweed induced markedly higher ragweed-specific IgE but not total IgE levels or IgE-secreting cell numbers. Synergy was also observed between the DEPs and ragweed in altering the profile of epsilon mRNAs generated by alternative splicing, producing mRNAs that code for different expressed IgE proteins [31]. DEP exposure by this method specifically increased total IgE and ε mRNA in nasal lavage fluid [28], [29], [42]. Most recently, DEP extracts have been shown to increase both histamine release and IL-4 production from human basophils [13]. While it is clear that the DEPs and DEP extracts can influence several aspects of the allergic inflammatory process, it is still not clear which components(s) is responsible for these effects.

Here, we show that preincubation of human basophils with certain PAHs, most notably BaP, 1,6-BPQ, and 3,6-BPQ, enhance histamine release and IL-4 production after short exposure times. This response appears to involve an increase in the tyrosine phosphorylation of certain proteins. Studies examining DEP exposure of human macrophages showed differential phosphorylation of certain kinases in exposed versus nonexposed cells [43]. Specifically, we demonstrate that Lyn, previously implicated in basophil IgE-mediated signaling pathways, is hyperphosphorylated in antigen-activated cells exposed to 1,6-BPQ. In addition, several other proteins were only tyrosine phosphorylated in antigen plus 1,6-BPQ-challenged basophils. Similarly, other studies have determined that PAHs can increase total B cell tyrosine phosphorylation in the phosphorylation of specific molecules including Lyn, Syk, Fyn, ZAP-70, and phospholipase C in human B and T cells [11], [25]. We hypothesize that the increase in protein tyrosine phosphorylation may

enhance the ability of other downstream effectors to efficiently interact and amplify the antigen/receptor signals.

We also observed a significant increase in ROS when basophils were exposed to 1,6-BPQ and antigen challenged when compared to nonexposed cells. A link between ROS production and enhanced allergic mediator release has been suggested. Specifically, blockade of superoxide generation prevents high-affinity immunoglobulin E receptor-mediated release of allergic mediators by rat mast cell line and human basophils [44]. Through this mechanism basophils could contribute to PAH-associated enhancement of allergic inflammation through traditional release of allergic mediators but also through release of ROS. Macrophages exposed to DEP chemicals produce and release ROS, leading to cellular activation or apoptosis which can be abrogated by antioxidants [45]. The effects of ROS on airway function in asthma have been studied with isolated airway cells and tissues and with animal models and patients. These studies show that release of ROS within the airways may contribute to the initiation and worsening of inflammatory respiratory disease [46]. Increased levels of ROS and ROS by-products have been found in asthmatics compared to controls [47], [48], [49]. These studies suggest that oxidative stress contributes to the initiation and worsening of inflammatory respiratory disease.

Our studies differ from that of Devouassoux et al. where organic extracts of DEPs, which presumably include PAHs, induced basophil IL-4 production but not in an antigen-independent fashion [13]. The discrepancies are unexplained but may be due to the complexity of molecules found in the DEP extracts used in the basophil studies resulting in PAH concentrations much lower than the concentrations used in our studies. Our results are more similar to other studies in which synergy of antigen and DEPs on cytokine production was observed in in vivo nasal or bronchial antigen challenge models [8], [12], [30], [31] which implicated mast cells as the source of DEP-induced cytokine production.

We found that 1,6-BPQ-induced enhancement of histamine release, IL-4 production, and ROS formation could be blocked using the antioxidant NAC. DEP-induced expression of IL-4 in human basophils could be inhibited by first preincubating with NAC [13]. Similarly, blocking ROS formation in rodent mast cells inhibits IgE-mediated degranulation [44], [50]. Our data support the hypothesis that ROS generation can influence signaling pathways leading to histamine release and cytokine production through IgE crosslinking. While the evidence showing intracellular ROS levels can be elevated in response to activating signals, it is not known whether human mast cells and basophils are capable of releasing ROS. A recent paper found that murine mast cells did not produce ROS but macrophage-derived ROS inhibited mediator release [51]. Our laboratory has found that mediator release from lung-derived human mast cells is influenced by PAHs through a ROS-dependent mechanisms (C.L. Kepley, unpublished observation). We are currently investigating whether human FcεRI-positive cells can release ROS as well.

It is not clear whether the PAH-induced differential basophil protein tyrosine phosphorylation profile or Lyn phosphorylation is due to a direct BPQ effect or the increase in cellular ROS levels. As mentioned above, certain PAHs directly induce tyrosine kinase activation. However, ROS have been implicated in the regulation of diverse cellular functions including defense against pathogens, intracellular signaling, transcriptional activation, proliferation, and apoptosis. Specifically, generation of ROS, such as $H_2O_2$, has previously been shown to activate protein

tyrosine kinases leading to the stimulation of downstream signaling systems including MAP kinase and PLC-γ in several cellular systems [52].

There is growing evidence that compounds in air pollution may lead to enhancement of allergic inflammation, but the mechanisms are not clear [8], [53]. The data presented here support the hypothesis that particulate matter may directly enhance allergic inflammation through enhanced antigen-induced basophil mediator release after PAH exposure. Mast cells and basophils are involved in the initiation and propagation of allergic inflammation, including asthma. We used a basophil-specific mAb [14] to demonstrate that basophil numbers are significantly increased in the lungs of patents dying from asthma compared to asthmatics dying from nonasthmatic causes [54]. Mast cells are ubiquitously distributed in tissue and may be expected to encounter DEPs and PAHs. Given their involvement in these diseases it is important to understand how environmental pollutants affect their functional responses. Through the observations described here, particulate air pollutants may enhance the allergen/IgE-induced physiologic mechanisms that induce allergic inflammation. They may also contribute to acute asthmatic attack or contribute to the chronic conditions that favor this disease in the lungs of asthmatics.

## Acknowledgements

CLK was supported by a Pilot Project Grant from the NIEHS-sponsored New Mexico Center for Environmental Health Sciences (P20ES09871), and an American Academy of Allergy, Asthma & Immunology Interest Section grant and is a Parker B. Francis Fellow in Pulmonary Research. This work was also supported in part by RO1 ES05495 (SWB). We thank Bethanne Greenawalt for technical assistance.

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