

IL-4 primes human endothelial cells for secondary responses to histamine

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Abstract: Interleukin-4 (IL-4) is a multifunctional cytokine, which is involved in numerous disease states, including atopic asthma. IL-4 not only induces direct responses in cells but can also prime for secondary responses to stimuli. Little is known about the priming effects of IL-4 on endothelial cells; therefore, we chose to examine the ability of IL-4 to prime endothelial cells for platelet-activating factor (PAF) synthesis and prostaglandin E₂ (PGE₂) release. IL-4 alone did not enhance PAF synthesis or PGE₂ release; however, pretreatment with IL-4 primed for PAF synthesis and PGE₂ release in response to subsequent stimulation with histamine. In contrast, tumor necrosis factor α (TNF- α), oncostatin M (OSM), and IL-1 β did not prime endothelial cells for PAF synthesis in response to histamine. The priming effects of IL-4 occurred without any detectable changes in the requirement for signaling pathways upstream of PGE₂ release. IL-4 treatment increased the expression of mRNA for histamine receptor 1 (HR1) and shifted the inhibition curve for pyrilamine, a specific HR1 antagonist. In addition, the dose-response curve for histamine-induced elevations in intracellular calcium was shifted following IL-4 stimulation. Together, these data indicate that HR1 is up-regulated in IL-4-stimulated human umbilical vein endothelial cells (HUVEC) and suggest that this up-regulation may contribute to the enhanced responsiveness of IL-4-stimulated HUVEC to histamine. *J. Leukoc. Biol.* 74: 420–427; 2003.

Key Words: G protein-coupled receptor · HUVEC · platelet-activating factor · prostaglandin E₂

INTRODUCTION

The cytokine interleukin (IL)-4 has been implicated as an important mediator of atopic or allergic asthma. Compared with healthy individuals, atopic asthmatics show elevated levels of IL-4 mRNA and protein in bronchoalveolar lavage samples, as well as higher IL-4 immunoreactivity in T lymphocytes isolated from lung tissues [1–3]. Inhalation of IL-4 protein by atopic asthmatics induces airway hyper-responsiveness (AHR), one of

the hallmark features of asthma, and inhibition of IL-4 preserves respiratory function in atopic asthmatics following discontinuation of corticosteroid treatment [4, 5]. IL-4 has effects on multiple cell types and can induce responses such as class-switching to immunoglobulin E (IgE) in B lymphocytes [6–9], differentiation toward a T helper cell type 2 phenotype in T lymphocytes [10–13], and up-regulation of adhesion molecule and chemokine expression in endothelial cells [14–17].

In addition to its direct effects on cells, IL-4 can also prime cells for subsequent responses to other stimuli. The priming effects of IL-4 have been characterized in a number of hematopoietic cell types, including eosinophils, lymphocytes, macrophages, and mast cells. Preincubation of human peripheral blood eosinophils with IL-4 enhances their chemotactic response to chemokines [18]. In human peripheral blood T lymphocytes, culture in the presence of IL-2 and IL-4 in combination renders cells responsive to eotaxin by inducing surface expression of the eotaxin receptor, chemokine receptor 3 (CCR3) [19]. Human bone marrow-derived macrophages show an enhanced respiratory burst in response to phorbol 12-myristate 13-acetate (PMA) or zymosan following a 48-h incubation with IL-4 [20]. In human mast cells, IL-4 pretreatment primes for the release of histamine, prostaglandins, leukotrienes, and cytokines in response to Fc epsilon receptor I [21, 22], as well as for the release of cytokines and chemokines in response to stimulation with uridine 5'-diphosphate (UDP) or cysteinyl leukotrienes [23]. Despite the extensive data regarding IL-4 priming of hematopoietic cells, little is known about the priming effects of IL-4 on endothelial cells.

IL-4 treatment of human umbilical vein endothelial cells (HUVEC) increases the synthesis and surface expression of the adhesion molecule P-selectin [17]. Subsequent stimulation of these HUVEC with histamine leads to increased P-selectin surface expression and dramatically enhanced neutrophil adhesion [17]. Histamine is a key mediator of allergic responses in several diseases, including atopic asthma [24]. Unlike cytokines, which may require hours to cause functional changes, histamine alone can induce responses in control endothelial cells within a few minutes. Stimulation with histamine alone

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leads to rapid Weibel-Palade body degranulation, resulting in increased P-selectin surface expression [25, 26]. Histamine stimulation of HUVEC also leads to the rapid synthesis of platelet-activating factor (PAF) and prostaglandins [27, 28].

PAF (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a potent lipid autocoid, which is involved in multiple physiological and pathological processes [29, 30]. A role for PAF in the pathogenesis of asthma is suggested by studies that show that PAF is able to induce many of the characteristic features of asthma, including bronchoconstriction, AHR, increased vascular permeability, and eosinophil activation [31–33]. Prostaglandin E_2 (PGE_2) is often considered to function as an inhibitory prostaglandin in asthma, as it has been shown to promote airway smooth muscle relaxation and to antagonize the effects of bronchoconstrictors [34]. Endothelial cells synthesize PAF in response to histamine, thrombin, and leukotrienes [29, 30]. Endothelial cells also synthesize and release prostaglandins such as PGI_2 , $PGF_{2\alpha}$, and PGE_2 in response to histamine [35].

In this study, we investigated the ability of IL-4 to prime endothelial cells for PAF synthesis and PGE_2 release in response to histamine. We found that IL-4-stimulated HUVEC were primed for subsequent responses to histamine but not for responses to calcium ionophore. The requirement for signaling pathways upstream of PGE_2 release was not detectably altered by IL-4 priming, as inhibition of the extracellular signal-regulated kinase (ERK)1/2 mitogen-activated protein kinase (MAPK) pathway or intracellular calcium signaling completely blocked PGE_2 release from control and IL-4-stimulated HUVEC. Histamine receptor 1 (HR1), which mediates histamine-induced PAF synthesis and PGE_2 release [27, 35], was up-regulated by IL-4 stimulation, providing a potential mechanism by which IL-4 may prime HUVEC for subsequent responses to histamine.

MATERIALS AND METHODS

Reagents

Human recombinant IL-4, TNF- α , OSM, and IL-1 β were purchased from R&D Systems (Minneapolis, MN). Histamine, ionomycin, pyrilamine, and PAF were from Sigma Chemical Co. (St. Louis, MO). 1,2-Bis(*O*-aminophenyl-ethane-ethane)-*N,N,N',N'*-tetraacetic acid (BAPTA) acetoxyethyl ester (AM) and fura-2 (AM) were purchased from Molecular Probes (Eugene, OR). PGE_2 enzyme immunoassay (EIA) kits were purchased from Cayman Chemicals (Ann Arbor, MI). [^3H] Acetic acid was from NEN Life Science Products (Boston, MA). MAPK kinase (MEK)1 inhibitor PD 98059 was from Calbiochem-Novabiochem Corp. (San Diego, CA). Polymerase chain reaction (PCR) SYBR Green master mix was from Applied Biosystems (Foster City, CA). Qiagen Operon (Mississauga, Canada) synthesized oligonucleotide primers. Hanks' balanced salt solution with Ca^{2+} and Mg^{2+} , Media 199 (M199), and TRIzol reagent were from Gibco-BRL, Life Technologies (Grand Island, NY). Human serum albumin (HSA) was from Immuno US (Rochester, MI). All plasticware was from Becton Dickinson (Franklin Lakes, NJ). All other chemicals were from BDH (Toronto, Canada).

Endothelial cell culture

Primary or first-passage HUVEC were isolated as described [36] and maintained in M199 with 20% human serum. Confluent HUVEC monolayers were washed and incubated for 6 or 24 h in M199 with 0.5% HSA (M199/A) alone or M199/A containing the specified concentration of cytokine. Alternatively, endothelial cells were incubated in depleted media with or without cytokine.

Equivalent results were obtained for both stimulation media; therefore, data from experiments in which either medium was used were combined.

PAF synthesis assay

PAF production by HUVEC was measured as described previously [27]. Briefly, control or cytokine-stimulated endothelial cells were loaded with [^3H] acetic acid (50 μCi) for 10 min at 37°C, after which they were stimulated with 0–10 μM histamine for 5 min. Reactions were stopped by removing the incubation medium and adding acidified methanol. Phospholipids were extracted according to the method of Bligh and Dyer [37]. Isolated phospholipids were separated by thin-layer chromatography, and PAF was identified based on comigration with an unlabeled PAF standard. PAF synthesis was quantified based on the incorporation of [^3H] acetic acid into newly synthesized PAF as described [27]. In some experiments, HUVEC were pretreated with 10 nM or 1 μM pyrilamine for 5 min before histamine stimulation.

PGE_2 assay

Control or IL-4-stimulated HUVEC were stimulated with 0–10 μM histamine for 5 min. Supernatants were collected, and PGE_2 was measured using EIA kits according to the manufacturer's instructions. In some experiments, HUVEC were pretreated with 20 μM PD 98059, 50 μM BAPTA-AM, or 10 nM or 1 μM pyrilamine for the indicated times before histamine stimulation.

Real-time PCR for HR1

Endothelial cells were left untreated or stimulated with 10 ng/ml IL-4 or TNF- α for 24 or 6 h, respectively, as described above. Total cellular RNA was extracted using TRIzol according to the manufacturer's instructions. RNA concentrations were determined using a GeneQuant spectrophotometer (Pharmacia, Piscataway, NJ). Reverse transcription (RT) using Superscript II (Gibco-BRL) was performed using 5 μg RNA according to the manufacturer's instructions. Quantitative real-time PCR was performed using an ABI Prism 7000. PCR was performed with a SYBR Green kit using 20% of the RT reaction as template cDNA with the appropriate primer pairs. The primers used were: HR1 forward: 5'-ACCCCCTCATCTACCCCTTGT-3'; HR1 reverse: 5'-CCITCGTCCTCTATTTCCTTGTTG-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward: 5'-CGGAGTCAACGGATTTTGGTCGTAT-3'; GAPDH reverse: 5'-AGCCTTTCTCCATGGTGGTGAAGAC-3'. Each analysis also contained a range of standards. PCR products were confirmed in two ways. First, PCR products were heated and the melt curve was examined. A single peak indicated a single PCR product. We also examined the PCR products by electrophoreses through 2% agarose gels containing 0.5 $\mu g/ml$ ethidium bromide followed by visualization using UV light. Data were analyzed and quantified using ABI Prism 7000 software, and values obtained were compared with a standard curve for each sequence. Data were normalized to GAPDH and represent a ratio of HR1/GAPDH. Data are presented as the fold-change between control and cytokine-stimulated HUVEC.

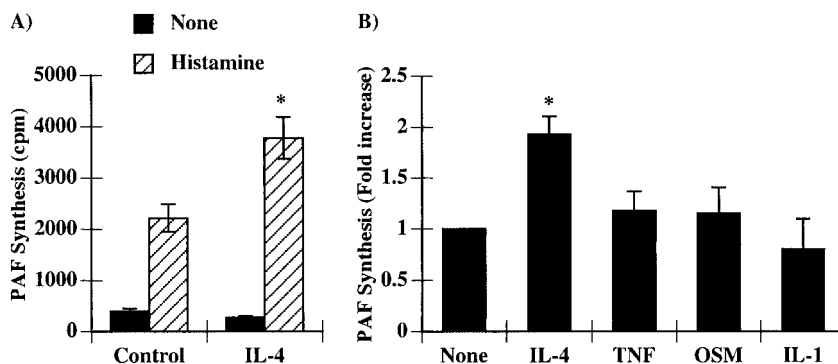
Calcium imaging

Endothelial cells were cultured on gelatin-coated glass coverslips and left unstimulated or stimulated with 20 ng/mL IL-4 for 24 h as described above. Cells were loaded with 5 μM fura-2-AM for 30 min at room temperature, after which fluorescent images were captured at 400 $\times$ magnification using a Zeiss Axiovert microscope as described previously [38]. Cells were excited at 340 nm and 380 nm, and emission was detected at 510 nm. The data are presented as a ratio of the emissions at 510 nm. Data were analyzed using ImageMaster software (Photon Technology International, Monmouth, NJ).

Statistics

All experiments were performed at least three times, and data are presented as mean $\pm$ SEM of all replicates unless otherwise indicated. Alternatively, the data from a single representative experiment are presented as the mean $\pm$ range of duplicate determinations. Statistical differences between experimental groups were evaluated using paired Student's *t*-test, Mann-Whitney *U*-tests, or ANOVA. *P* values $\leq$ 0.05 were considered significant.

Fig. 1. Pretreatment of HUVEC with IL-4 primes for histamine-induced PAF synthesis. (A) HUVEC were incubated for 24 h in medium with or without 20 ng/mL IL-4 as described in Materials and Methods. Cells were subsequently stimulated for 5 min with 10 μ M histamine, after which the monolayers were assayed for PAF synthesis. (A) Data are mean $\pm$ SEM of 23 experiments. (B) HUVEC were incubated for 24 h in medium with or without 20 ng/mL IL-4, OSM, or IL-1 β or for 6 h in medium with 20 ng/mL TNF- α as described in Materials and Methods. Cells were subsequently stimulated for 5 min with 10 μ M histamine, after which the monolayers were assayed for PAF synthesis. The ratio of PAF synthesis by cytokine-stimulated HUVEC to PAF synthesis by control HUVEC was determined. (B) Data are mean $\pm$ SEM of at least three experiments. *, $P < 0.001$, as compared with control plus histamine.



RESULTS

IL-4 primes HUVEC for PAF synthesis and prostaglandin release in response to histamine

IL-4 has previously been shown to prime for responses to secondary stimulation in a variety of cell types, including endothelial cells [17–23, 39]. To assess the ability of IL-4 to prime endothelial cells for responses to histamine, HUVEC were incubated in media with or without IL-4 for 24 h and subsequently left untreated or stimulated with histamine for 5 min. Following stimulation, the supernatants were collected and assayed for PGE₂ content by EIA and new PAF synthesis was quantified based on [³H] acetic acid incorporation. In the absence of histamine stimulation, levels of PAF synthesis and PGE₂ release were equivalent in control and IL-4-stimulated HUVEC (Figs. 1A and 2), indicating that IL-4 pretreatment alone does not enhance the synthesis of PAF or release of PGE₂ by endothelial cells. Following histamine stimulation, however, levels of PAF synthesis and PGE₂ release were higher in IL-4-stimulated HUVEC than in control HUVEC (Figs. 1A and 2), indicating that IL-4 pretreatment primes endothelial cells for histamine-induced PAF synthesis and PGE₂ release. IL-4 also primed HUVEC for histamine-induced prostacyclin (PGI₂) release (data not shown). These responses were specific

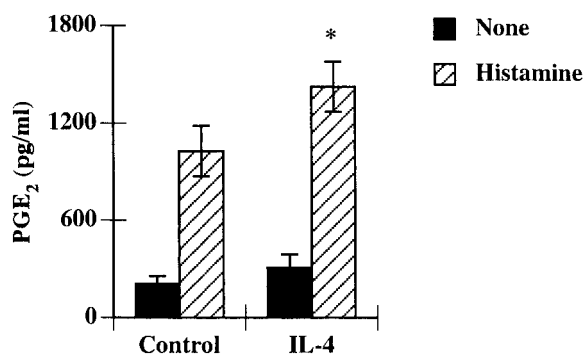


Fig. 2. Pretreatment of HUVEC with IL-4 primes for histamine-induced PGE₂ release. HUVEC were incubated for 24 h in medium with or without 20 ng/mL IL-4 as described in Materials and Methods. Cells were subsequently stimulated for 5 min with 10 μ M histamine, after which the supernatants were assayed for PGE₂ content. Data represent mean $\pm$ SEM of 18 experiments. *, $P < 0.001$, as compared with control plus histamine.

for IL-4 stimulation, as pretreatment with TNF- α , OSM, or IL-1 β for 24 h had no effect on histamine-induced PAF synthesis (Fig. 1B).

Consistent with previous reports [27, 35], we found that histamine-induced PAF synthesis and PGE₂ release were dose-dependent (Fig. 3). PAF synthesis could be detected at histamine concentrations as low as 1 μ M, and a minimum of 5 μ M histamine was required to induce PGE₂ release (Fig. 3). Although IL-4 stimulation increased the total amount of PAF synthesis and PGE₂ release at high concentrations of histamine, IL-4 did not affect the minimum concentration of histamine required to induce PAF synthesis or PGE₂ release (Fig. 3).

Signaling pathways upstream of PGE₂ release in control and IL-4-stimulated HUVEC

The enzyme cytosolic phospholipase A₂ (cPLA₂) cleaves phospholipids at the sn-2 position to release lyso-PAF, which is a precursor of PAF, and arachidonic acid, which is a precursor of PGE₂ [30, 40]. It has previously been shown that cPLA₂ is the predominant PLA₂ isoform responsible for arachidonic acid release in endothelial cells [41]. Levels of cPLA₂ protein in control and IL-4-stimulated HUVEC were analyzed by Western blotting and found to be equivalent (data not shown), suggesting that up-regulation of cPLA₂ expression does not account for the priming effects of IL-4 treatment. The degree of enzyme phosphorylation and the level of intracellular calcium [40, 42–44] regulate the activity of cPLA₂. Histamine has previously been shown to induce phosphorylation of the MAP kinases ERK 1 and 2 in endothelial cells [45]; in turn, these kinases have been shown to phosphorylate cPLA₂, thereby increasing its activity [42, 43]. To examine the role of ERK1/2 in histamine-induced PGE₂ release, we used the compound PD 98059, which is a pharmacological inhibitor of MEK1, an upstream activator of ERK1/2 [46]. HUVEC were pretreated for 30 min with 20 μ M PD 98059 before stimulation with histamine. This time and concentration were found to block ERK1/2 activation in response to histamine in control and IL-4-stimulated HUVEC (data not shown). Pretreatment of HUVEC with PD 98059 before histamine stimulation completely abrogated the release of PGE₂ from control and IL-4-stimulated HUVEC (Fig. 4A), demonstrating that ERK1/2

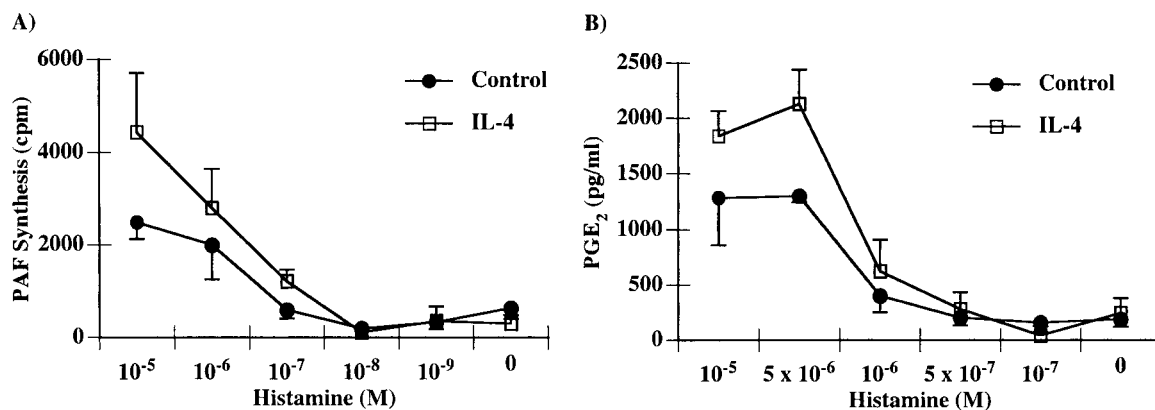


Fig. 3. Pretreatment of HUVEC with IL-4 increases the magnitude but not the dose dependence of responses to histamine. HUVEC were incubated for 24 h in medium with or without 20 ng/mL IL-4 as described in Materials and Methods. Cells were subsequently stimulated for 5 min with 0–10 μ M histamine, after which (A) the monolayers were assayed for PAF synthesis, and (B) the supernatants were assayed for PGE₂ content. Data are mean $\pm$ SEM of at least three experiments.

activation is required for histamine-induced PGE₂ release from control and IL-4-stimulated HUVEC.

To examine the role of intracellular calcium in histamine-induced PGE₂ release, we used the intracellular calcium chelator BAPTA-AM. HUVEC were pretreated for 15 min with 50 μ M BAPTA-AM before stimulation with histamine. Pretreatment with BAPTA-AM before histamine stimulation completely abrogated PGE₂ release from control and IL-4-stimulated HUVEC (Fig. 4A), indicating that intracellular calcium is required for PGE₂ release from control and IL-4-stimulated HUVEC. Taken together, these data suggest that although IL-4 stimulation of HUVEC increases the amount of PGE₂ that is released following histamine treatment, it does not alter the immediate upstream intracellular signaling pathways that are required for PGE₂ release.

As blockade of several signaling pathways downstream of the histamine receptor did not differentiate between control

and IL-4-stimulated HUVEC, we investigated whether IL-4 can prime for responses to nonreceptor-mediated stimuli. Calcium ionophore has previously been shown to elicit PGE₂ release from HUVEC [47]. When control and IL-4-stimulated HUVEC were treated with the calcium ionophore ionomycin, both released similar amounts of PGE₂ and PGI₂ (Fig. 4B and data not shown), indicating that IL-4 does not prime HUVEC for PGE₂ release in response to calcium ionophore. These data suggest that the priming effects of IL-4 are histamine-specific.

IL-4 stimulation causes up-regulation of HR1

Histamine can act at three different receptors; however, PAF synthesis and PGE₂ release in endothelial cells occur exclusively through HR1 [27, 35]. Increased histamine responsiveness in IL-4-stimulated HUVEC could be a result of increased expression or use of histamine receptors. Levels of HR1 protein on control and IL-4-stimulated HUVEC were below levels

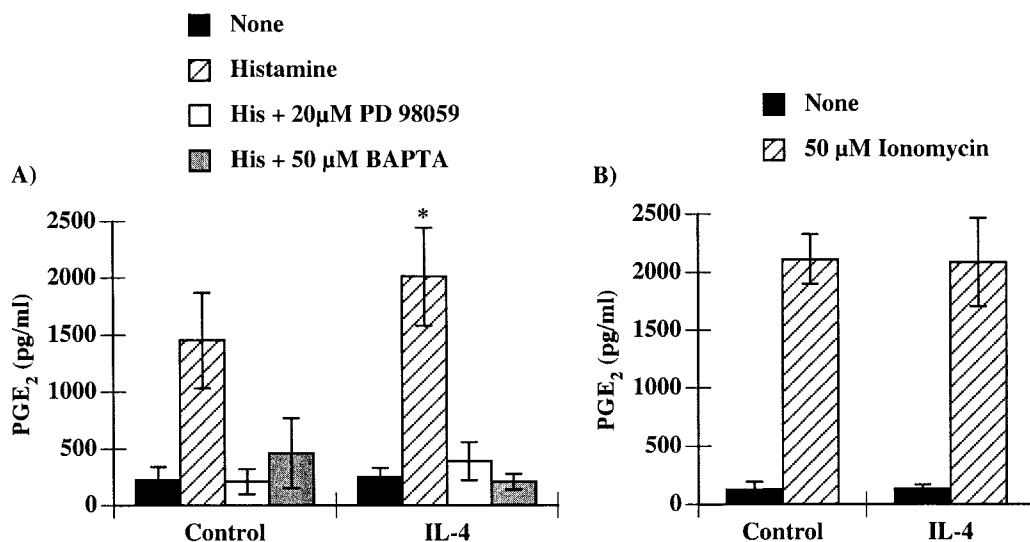


Fig. 4. Role of ERK1/2 and calcium signaling in histamine-induced PGE₂ synthesis. HUVEC were incubated for 24 h in medium with or without 10 ng/mL IL-4 as described in Materials and Methods. (A) HUVEC were pretreated with 20 μ M PD 98059 or 50 μ M BAPTA-AM and were subsequently stimulated for 5 min with 10 μ M histamine (His). (B) HUVEC were stimulated for 5 min with 50 μ M ionomycin. (A and B) Supernatants were collected, and PGE₂ contents were measured by EIA. Data are mean $\pm$ SEM of at least three experiments. *, $P < 0.05$, as compared with control plus histamine.

of detection by cell-surface enzyme-linked immunosorbent assay (data not shown); therefore, we used real-time PCR to determine whether IL-4 stimulation increases expression of this histamine receptor. We found that IL-4 increased steady-state levels of HR1 mRNA in HUVEC as compared with control or TNF- α -stimulated HUVEC (**Fig. 5A**). To further examine IL-4-induced HR1 up-regulation, we used pyrilamine, a competitive inhibitor of HR1. Pretreatment of control or IL-4-stimulated HUVEC with 1 μ M pyrilamine completely blocked histamine-induced PAF synthesis and PGE₂ release (**Fig. 5, B and C**), indicating that these responses are mediated through HR1 under both conditions. When control HUVEC were pretreated with a lower concentration (10 nM) of pyrilamine, PAF synthesis and PGE₂ release were still completely blocked. In contrast, 10 nM pyrilamine was incapable of completely blocking PAF synthesis and PGE₂ release in IL-4-stimulated HUVEC (**Fig. 5, B and C**), suggesting elevated expression of functional HR1 following IL-4 stimulation.

One of the earliest responses to the activation of histamine receptors is an elevation in intracellular calcium levels [48, 49]. We measured intracellular calcium levels in control and IL-4-stimulated HUVEC in response to increasing concentrations of histamine using the calcium-sensitive dye fura-2. Control HUVEC showed elevated intracellular calcium levels following treatment with 10⁻⁶ M histamine (**Fig. 6A**), and IL-4-stimulated HUVEC exhibited elevations in intracellular calcium at histamine concentrations as low as 10⁻⁸ M (**Fig. 6B**). This difference was significant at 10⁻⁸ M and 10⁻⁷ M ($P < 0.05$; $n = 3$). The shift in the dose-response curve for histamine-induced calcium mobilization following IL-4 treatment is consistent with up-regulation of HR1. Together, these data suggest that IL-4-stimulated HUVEC express higher levels of functional HR1 on their surface than control HUVEC, providing a potential mechanism for IL-4-mediated priming of HUVEC for secondary stimulation with histamine.

DISCUSSION

The direct effects of IL-4 on endothelial cells have been well established. IL-4 stimulation of HUVEC increases expression of vascular cell adhesion molecule-1 (VCAM-1) and P-selectin, as well as the chemokine eotaxin-3 [16, 17, 50]. In vitro, VCAM-1 and P-selectin can promote the recruitment of multiple leukocyte subclasses, and eotaxin-3 can facilitate the transendothelial migration of eosinophils [50, 51]. In vivo, IL-4-mediated adhesion molecule up-regulation alters leukocyte recruitment in animal models and is believed to play a role in the recruitment of leukocytes into the lungs of asthmatics [52, 53].

Although much is known about the direct responses of endothelial cells to IL-4, little is known about the role of IL-4 in priming endothelial cells for responses to secondary stimulation. Studies in hematopoietic cells suggest that IL-4 has priming effects on multiple cell types and can prime for responses to a variety of stimuli [18–23]. We report here the first study of the effects of IL-4 on histamine-induced PAF synthesis and prostaglandin release. We found that incubation of

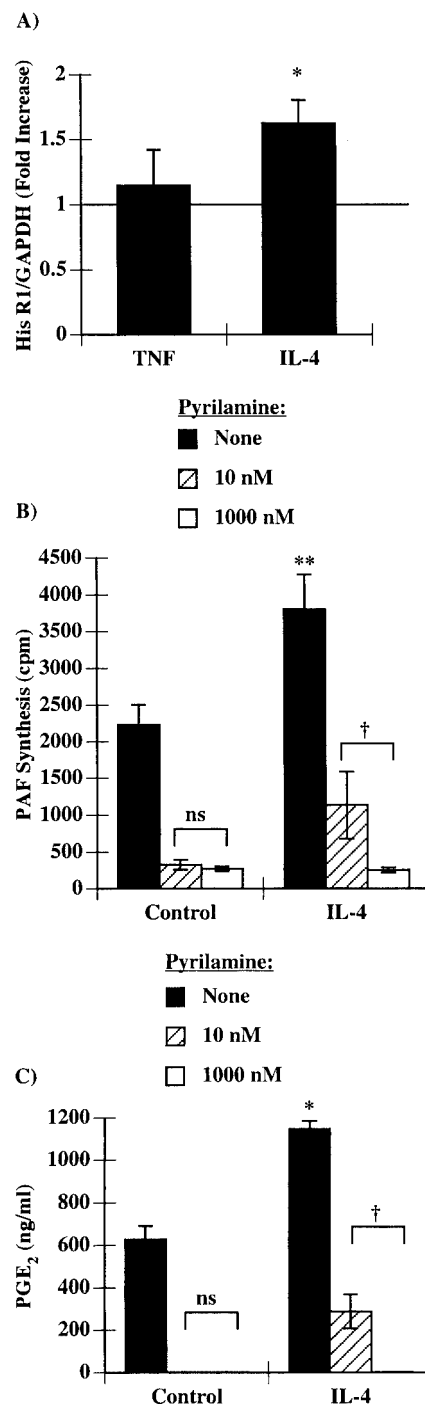


Fig. 5. IL-4 treatment causes up-regulation of HR1. HUVEC were incubated for 24 h in medium with or without 10 ng/mL IL-4 or for 6 h in medium with 10 ng/mL TNF- α as described in Materials and Methods. (A) RNA was isolated from HUVEC using the TRIzol method, and cDNA was generated using a First Strand synthesis kit. Real-time PCR was performed on cDNA using primers specific for HR1. Data were normalized against GAPDH positive controls. (B and C) Cells were pretreated for 5 min with 10 or 1000 nM pyrilamine and subsequently stimulated for 5 min with 10 μ M histamine. (B) The monolayers were assayed for PAF synthesis, and (C) the PGE₂ contents of the supernatants were measured by EIA. The data represent the mean $\pm$ SEM of between three and 10 experiments. *, $P < 0.05$, as compared with control; **, $P < 0.05$, as compared with control plus histamine; †, $P < 0.05$ between 10 and 1000 nM pyrilamine.

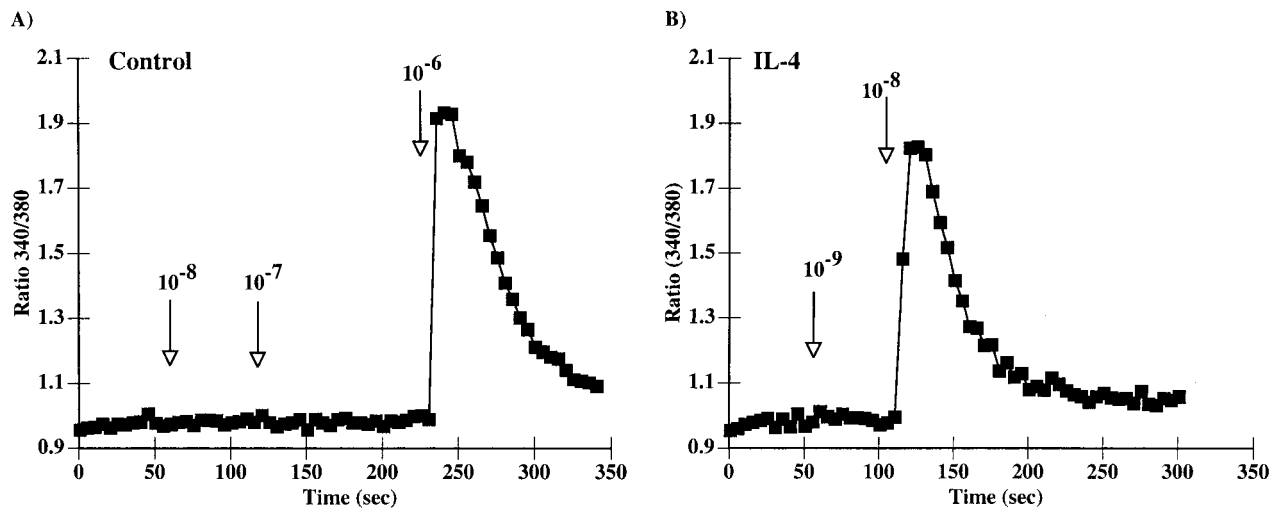


Fig. 6. Pretreatment of HUVEC with IL-4 primes for histamine-induced calcium mobilization. HUVEC were cultured on gelatin-coated glass coverslips and were incubated for 24 h in medium (A) without or (B) with 20 ng/mL IL-4 as described in Materials and Methods. Following stimulation, cells were loaded with 5 μ M fura-2-AM for 30 min, and intracellular calcium levels were measured as described in Materials and Methods. Changes in intracellular calcium were measured following the addition of increasing concentrations of histamine. Data are representative of four experiments. The difference between control and IL-4-stimulated HUVEC was significant at 10^{-8} and 10^{-7} M histamine ($P < 0.05$).

HUVEC with IL-4 for 24 h had no direct effect on PAF synthesis or PGE₂ release. Instead, IL-4 primed HUVEC for elevated PAF synthesis and PGE₂ release in response to subsequent histamine stimulation. It has previously been shown that prostaglandins are rapidly secreted from endothelial cells following synthesis, with little to no measurable prostaglandin remaining associated with the endothelium, suggesting that the observed increases in PGE₂ release were the result of increased PGE₂ synthesis [54]. The priming effects of IL-4 occurred in the absence of detectable changes in the signaling pathways required for PGE₂ synthesis, and priming did not occur when nonreceptor stimuli were used to induce PGE₂ synthesis.

Our data suggest that up-regulation of HR1 is the mechanism by which IL-4 mediates its priming effects for responses to histamine. Other cell types exhibit receptor up-regulation and enhanced responsiveness following IL-4 treatment; for example, costimulation with IL-2 and IL-4 induces CCR3 expression in T lymphocytes, rendering them responsive to eotaxin [19]. Yao et al. [17] have previously investigated the priming effects of IL-4 on HUVEC and found that IL-4 primes for histamine-induced P-selectin surface expression and P-selectin-mediated neutrophil rolling. These effects were attributed to increased storage of P-selectin protein in Weibel-Palade bodies following IL-4 stimulation [17]; however, our results suggest that HR1 up-regulation may also contribute to the ability of IL-4 to prime for increases in histamine-induced P-selectin expression and function in this system. Taken together, this points to the involvement of multiple mechanisms in a single priming response. We are currently investigating whether mechanisms in addition to receptor up-regulation contribute to the priming effects of IL-4 for histamine-induced PAF synthesis and PGE₂ release.

Atopic asthma is characterized by the chronic production of cytokines, such as IL-4. Exposure to allergen leads to the

release of histamine and other fast-acting mediators by mast cells and basophils. In this series of experiments, a 24-h incubation with IL-4 was used to mimic the chronic exposure to IL-4 that is experienced by endothelial cells within the asthmatic lung. Our observation that 24 h of pretreatment with IL-4 primes HUVEC for responses to histamine in vitro suggests that the vascular endothelium of atopic asthmatics, which is chronically exposed to elevated levels of IL-4, may also exhibit enhanced sensitivity to histamine in vivo. The concentrations we used are consistent with those found in vivo following antigen challenge [55]. Histamine is known to have many effects on vascular endothelial cells in addition to the ones examined herein, including increasing vascular permeability and inducing up-regulation of cytokines and chemokines [48, 56, 57]. As such, enhanced responsiveness to histamine within the asthmatic lung may have dramatic consequences for the severity of the response to an allergen challenge.

An improved understanding of the priming effects of IL-4 on endothelial cells brings about a necessary change in our way of thinking about the function of IL-4, which should be viewed not only as a cytokine with long-term effects on endothelial cells but also as a molecule that is capable of modulating rapid responses to secondary stimuli. Given the multiple effects of histamine on endothelial cells, it is likely that the ability of IL-4 to prime endothelial cells for responses to histamine will have far-reaching implications in the area of endothelial cell biology.

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