Extracellular Signal-Regulated Kinases Regulate Leukotriene C4 Generation, But Not Histamine Release or IL-4 Production from Human Basophils

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Human basophils secrete histamine and leukotriene C4 (LTC4) in response to various stimuli, such as Ag and the bacterial product, FMLP. IgE-mediated stimulation also results in IL-4 secretion. However, the mechanisms of these three classes of secretion are unknown in human basophils. The activation of extracellular signal-regulated kinases (ERKs; ERK-1 and ERK-2) during IgE- and FMLP-mediated stimulation of human basophils was examined. Following FMLP stimulation, histamine release preceded phosphorylation of ERKs, whereas phosphorylation of cytosolic phospholipase A2 (cPLA2), and arachidonic acid (AA) and LTC4 release followed phosphorylation of ERKs. The phosphorylation of ERKs was transient, decreasing to baseline levels after 15 min. PD98059 (MEK inhibitor) inhibited the phosphorylation of ERKs and cPLA2 without inhibition of several other tyrosine phosphorylation events, including phosphorylation of p38 MAPK. PD98059 also inhibited LTC4 generation (IC50 ~2 μM), but not histamine release. Stimulation with anti-IgE Ab resulted in the phosphorylation of ERKs, which was kinetically similar to both histamine and LTC4 release and decreased toward resting levels by 30 min. Similar to FMLP, PD98059 inhibited anti-IgE-mediated LTC4 release (IC50 ~2 μM), with only a modest effect on histamine release and IL-4 production at higher concentrations. Taken together, these results suggest that ERKs might selectively regulate the pathway leading to LTC4 generation by phosphorylating cPLA2, but not histamine release or IL-4 production, in human basophils. The Journal of Immunology, 1999, 162: 4198–4206.
activity (22). There have been several attempts to associate the role of ERKs in degranulation, the release of AA, and the production of TNF-α following FcεRI-mediated stimulation (22–24). More recently, Zhang et al. provided evidence that ERK-2 (p42MAPK) activation contributed to the release of AA and the production of TNF-α, but not to the release of hexosaminidase (a marker of degranulation) (24). The causal association of ERK activation with the release of AA and TNF-α was based on studies with the selective MEK inhibitor, PD98059 (24). Thus, it is likely that ERKs regulate the pathways leading to the release of AA (by activating cPLA2) and the production of TNF-α, but not degranulation, in IgE-dependent stimulation in RBL-2H3 cells. Their role in regulating LTC4 formation in RBL-2H3 cells has not been fully elucidated because of the inability of these cells to generate LTC4. However, the opposite results for TNF-α production were obtained in MC/9 murine mast cells (25). Inhibition of ERKs with PD98059 had little effect on FcεRI-stimulated TNF-α production in these cells. In contrast, the MEKK1-regulated JNK pathway activated by FceRI was shown to be mediated by this cytokine production (25). These two conflicting results (RBL-2H3 cell data vs MC/9 murine mast cell clone data) suggest that the role of ERKs may be different in distinct cells (even with the same stimulation; FcεRI-mediated stimulation). Therefore, the studies using normal human cells to examine the role of ERKs in cell functions should be important.

We have recently showed that the activation of cPLA2 is associated with free AA generation and LTC4 release in human basophils (26). Although there is conflicting evidence for the enzymatic source of AA in IgE-mediated stimulation of human basophils (26, 27), our most recent evidence favors the view that activation of cPLA2 is more closely associated with the AA generation and LTC4 formation. This entire procedure was carefully performed at a temperature below 4°C and in the presence of PAG-EGTA (50 μM) to minimize changes in ERKs status. Cells were incubated for 10 min with 1 μg/ml mouse anti-human IgE (TES-19, provided by Dr. Frances Davis, Tanox) in the presence of 4 mg/ml normal human IgG to block FcεRI. After a subsequent 20-min incubation with rat anti-mouse IgG2a and paramagnetic beads (8 μl/107 cells), the cells were passed through a MACS minicolunm (MACS system, Miltenyi Biotec, Sunnyvale, CA). Flow-through cells were collected, and the eluted basophil purities were between 98 and 99.4%.

Basophils are the only leukocytes in these preparations to release histamine and LTC4 in response to FcεRI and anti-IgE (2). Studies also show that basophils are the major, if not the sole, source of IL-4 following IgE-dependent activation (3). As a result, basophil-enriched leukocyte suspensions were used to evaluate the effect of PD98059 on histamine, LTC4, and IL-4 secretion. Venous blood from consenting donors was anticoagulated with 10 mM EDTA, and basophil-enriched suspensions were prepared by a double-Percol density centrifugation, as previously described (37). The purity of the basophils in the cell suspensions ranged from 2–15%.

**Western blot analysis of whole cell lysates**

High-speed cell pellets (∼14,000 g, 5–10 s) were resuspended at 2 × 107 cells/ml in lysis buffer (50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 10 mM EGTA, 5 mM DTT, 1% Nonidet P-40, 1 mM PMSF, 20 μg/ml leupeptin, 100 μg/ml aprotinin, and 10 mM benzamidine). After 20 s of vortexing, the cell lysates were kept on ice for 20 min and then microfuged for 15 min at 4°C. Supernatant was collected as a protein extract containing lysed cell components without nuclei. Extracts from an equal number of basophils (2 × 107 cells equivalents/lane) were diluted with an equal volume of 2 × loading buffer (0.125 M Tris-HCl (pH 6.8), 4% SDS, 0.005% bromophenol blue, and 20% glycerol; NOVEX, San Diego, CA) containing 0.05% 2-ME and subjected to 10% Tris-glycine gradient gel electrophoresis (NOVEX). Gels were then transferred to pure nitrocellulose membranes (Schleicher & Schuell, Keene, NH) with a Trans Blot (NOVEX). Electrophoresis and transfers were performed according to the manufacturer’s recommendation. After transfer, membranes were immersed in TBST (50 mM Tris (pH 7.5), 0.15 M NaCl, and 0.05% Tween-20) containing 5% nonfat dried skim milk (Carnation, Los Angeles, CA) overnight to block nonspecific binding. Membranes were then washed three times (5 min each time) with TBST. Immunoreactive proteins were detected using anti-ERK-1 Ab, anti-ERK-2 Ab, or anti-p38 MAPK Ab, which were diluted at (0.5 μg/ml) in TBST containing 1% skim milk. After a 4-h incubation, membranes were washed with TBST and then incubated with peroxidase-conjugated anti-rabbit Ig Ab for 1 h. After five 10-min washes, membrane-bound anti-rabbit Ig Ab was visualized with ECL Western blotting detection reagents (Amersham), and HyperECL luminescence detection film (Amerham). The enhanced chemiluminescence film images were converted to digital format with a URL digital camera, and the images were analyzed with National Institutes of Health Image (Wayne Rasband, National Institutes of Health, Bethesda, MD) (26, 36). Although the comparisons were made on the basis of equal numbers of cells, the protein contents of these samples were also found to be equal.
Phosphorylation of ERKs, p38 MAPK, and cPLA₂

The phosphorylation of ERKs was assessed using three different techniques: 1) anti-phospho-ERK Ab (38), 2) anti-phospho-p38 MAPK Ab (47), and 3) electrophoretic mobility shift, using anti-ERK-1 and anti-ERK-2 Abs (23, 39). The phosphorylation of p38 MAPK was assessed using anti-phospho-p38 MAPK Ab or anti-phospho-p38 MAPK Ab (41, 42). The phosphorylation of cPLA₂ was assessed using electrophoretic mobility shift with anti-cPLA₂ Abs (16, 26). After stimulating basophils in PAGCM buffer, reactions were stopped by adding ice-cold PAG and were microfuged for 5–10 s. Cell pellets were immediately lysed in lysis buffer (20 mM Tris (pH 7.5), 2 mM EDTA, 2 mM EGTA, 5 mM DTT, 1 mM PMSF, 10 mM benzamidine, 100 µg/ml aprotinin, 200 µg/ml leupeptin, 50 mM NaF, 5 mM Na₃PO₄, 1 mM Na₂VO₃, and 1% Nonidet P-40). Extracts containing equal basophil cell numbers (5 × 10⁵ cell equivalents/lane) were diluted with an equal volume of 2× loading buffer and subjected to 10% Tris-glucine gel (NOVEX). After electrophoresis (160 V and 1.5 h for detection of MAPKs phosphorylation, and 160 V and 3.5 h for cPLA₂ phosphorylation), proteins were transferred to nitrocellulose membranes (as described above). The membranes were immersed overnight in TBST containing 2% BSA (for detection by 4G10) or in TBST containing 5% nonfat dry skim-milk (Carnation) for the others. Immunoreactive proteins were detected using 4G10 in TBST containing 1% BSA or anti-ERK-1 Ab, anti-ERK-2 Ab, anti-cPLA₂ Ab, anti-phospho-ERK Ab, or anti-phospho-p38 MAPK Ab, which were diluted in TBST containing 1% skim milk for 4 h. After washing, the membranes were incubated for 1 h with horseradish peroxidase-conjugated anti-mouse Ab (for 4G10) or horseradish peroxidase-conjugated anti-rabbit Ab (for the other Abs). After washing, enhanced chemiluminescence detection was performed as described above.

For kinetic studies, the same membranes were detected by anti-phospho-ERK Ab, anti-ERK-1 Ab, and anti-ERK-2 Ab or anti-phospho-p38 MAPK Ab and anti-p38 MAPK Ab. Between each detection, membranes were stripped with stripping buffer (62.5 mM Tris-Cl (pH 6.7), 100 mM 2-ME, and 2% SDS) for 1 h at 50°C. In preliminary experiments the techniques to assess phosphorylation of ERKs were simultaneously compared using phosphorylated and nonphosphorylated ERK-2 (p42 MAPK) proteins. Both phospho-ERK Ab and 4G10 recognized only phosphorylated ERK-2. Although the anti-ERK-2 Ab recognized both phosphorylated and nonphosphorylated ERK-2, the phosphorylated protein migrated more slowly than the nonphosphorylated protein, indicating that this Ab might also be useful in assessing phosphorylation of ERK-2 by recognizing both the phosphorylated and the nonphosphorylated form.

**LTC₄ and histamine measurements**

Fifty thousand basophils were challenged in a final volume of 100 µl of PAGCM at 37°C. The reactions were terminated with 900 µl of ice-cold PAG-EDTA, and the cells were then centrifuged in a microfuge at 14,000 rpm for 10 s. A RIA was performed using 100 µl of supernatant to determine LTC₄ levels as previously described (1, 43). An additional 500 µl of supernatants was mixed with an equal volume of PAG to measure histamine by automated fluorometry (44). The percentage of total histamine release was calculated for the diluted supernatants after subtraction of spontaneous histamine release (45). Each condition tested was performed in duplicate.

**IL-4 measurements**

Fifty thousand basophils were challenged in a final volume of 100 µl of RPMI 1640 with 0.03% HSA (37°C, 5% CO₂ incubator). After 4 h, the supernatants were harvested by centrifugation of microtiter plates (150 × g, 5 min). The supernatants were stored at −80°C until analysis. IL-4 measurements were performed using an in-house ELISA, as previously described (46). Each condition tested was performed in duplicate.

**AA measurements**

The mass of AA was determined via combined capillary gas chromatography-negative ion chemical ionization mass spectrometry using modifications of previously described procedures (47, 48). One hundred thousand cells were challenged in a final volume of 100 µl of PAGCM at 37°C. The reactions were terminated by the addition of 1 ml of aceton dissolved immediately by the addition of 15–20 ng of octodeuterated AA (as an internal standard). Reaction mixtures were transferred to glass vials and dried under dry N₂. The carboxyl moieties of AA were converted to pentfluorobenzyl esters as previously described (47). Once derivatized, the residues were dissolved in 60 µl of dodecane for injection into the gas chromatograph. The measurement of released AA by gas chromatography-negative ion chemical ionization mass spectrometry was performed as previously described (47, 48). Each condition tested was performed in duplicate.

**Results**

**Expression of ERKs (ERK-1/2) and p38 MAPK in human basophils**

To determine whether ERK-1, ERK-2, and p38 MAPK were present in human basophils, basophils that were very close to 100% purity obtained by anti-IgE positive selection were prepared as described in Materials and Methods. This study included a comparative analysis with the contaminating cells (mainly monocytes and lymphocytes) that typically contaminate enriched basophil preparations (mainly lymphocytes and monocytes), were lysed by 2 × 10⁵ cells/ml. The lysates containing 2 × 10⁶ cells or recombinant-ERK-2 (p42 MAPK) control protein (10 ng) were subjected to Western blot analysis as described in Materials and Methods. The Western blot shown is representative of three separate experiments.

![FIGURE 1. Expression of ERK-1/2 and p38 MAPK in human basophils and contaminating cells. Basophils (purity, 98.9–99.4%) and contaminating cells (basophil purity, <1%), which typically contaminate enriched basophil preparations (mainly lymphocytes and monocytes), were lysed by 2 × 10⁵ cells/ml. The lysates containing 2 × 10⁶ cells or recombinant-ERK-2 (p42 MAPK) control protein (10 ng) were subjected to Western blot analysis as described in Materials and Methods. The Western blot shown is representative of three separate experiments.](http://www.jimmunol.org/)

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of >80% basophils were used to minimize the contribution from contaminating cells.

**Kinetics of phosphorylation of ERKs and cPLA2 and mediator release following stimulation with FMLP**

As noted above, basophils activated with FMLP release histamine and generate free AA/LTC4, much like they do following IgE-dependent activation. We have shown more recently that FMLP also induces the phosphorylation of cPLA2 at a time consistent with free AA/LTC4 generation (26). We therefore examined the relationship between histamine release and free AA/LTC4 generation with the time course of FMLP-induced phosphorylation of ERKs. As shown in Fig. 2A, the phosphorylation of ERK-1 and -2 did occur, as assessed by the three different Abs described in **Materials and Methods**. In these experiments, phosphorylated and nonphosphorylated ERK-2 proteins were used as a reference. Thus, an electrophoretic mobility shift of ERK-1 and -2 was detected by anti-ERK-1 and anti-ERK-2 Abs, respectively. An identical time course in the phosphorylation of ERK-1 and -2 was detected by anti-phospho-ERK Ab (Fig. 2A) and by anti-phosphotyrosine Ab (4G10; data not shown). The phosphorylation of ERK-1 and -2 following FMLP stimulation was modest at 30 s, but a more significant level of phosphorylation was observed after 1 min, with maximal phosphorylation seen after 5 min (Fig. 2A and B). By 15 min, phosphorylation had decreased toward basal levels. The phosphorylation of cPLA2, the generation of free AA, and LTC4 release coincided with or followed the phosphorylation of ERK-1/2. Unlike ERK-1/2, cPLA2 remained phosphorylated at 15 min. On the other hand, histamine release was nearly complete (80 ± 1% of maximum histamine release) within 30 s. These results are consistent with our previous findings that there is no lag time for histamine release, whereas there is a lag time (~1 min) before the generation of free AA/LTC4 following FMLP activation (47).

**Kinetics of phosphorylation of ERKs and cPLA2, and mediator release in response to anti-IgE**

In the next series of experiments, we examined the relationship between the phosphorylation of ERKs and the release of histamine and LTC4 in response to IgE-dependent stimulation. As shown in Fig. 3, A and B, there was no detectable phosphorylation of ERK-1/2 following anti-IgE activation for 1 min. However, both ERKs were maximally phosphorylated by 5 min, with levels decreasing toward baseline by 30 min. Unlike activation with FMLP, histamine release followed the phosphorylation of ERKs. The cPLA2 phosphorylation and LTC4 release also followed or coincided with ERK-1/2 phosphorylation. Anti-IgE-mediated phosphorylation of ERKs was also transient compared with phosphorylation of cPLA2.

Basophil histamine release varies considerably among donor cells undergoing IgE-mediated stimulation (49, 50). Basophils from some donors are essentially unresponsive to IgE-mediated stimulation, the so-called nonreleasing basophils (49, 50). Not included in the data shown in Fig. 3 were the results for a nonreleasing donor. Anti-IgE-mediated phosphorylation of ERKs and cPLA2 was not detected; there was also no LTC4 release (data not shown). Taken together, anti-IgE-mediated ERK activation may be associated with the pathway leading to histamine release and LTC4 generation.

**Kinetics of phosphorylation of p38 MAPK following stimulation with FMLP or anti-IgE Ab**

The kinetics of phosphorylation of p38 MAPK following stimulation with FMLP or anti-IgE Ab were also examined. As shown in Fig. 4A, a significant phosphorylation of p38 MAPK was observed following stimulation with FMLP. The kinetics were slightly faster (a significant phosphorylation was observed at 30 s, with a maximum from 1–5 min and a decrease toward the basal level by 15 min) than those of phosphorylation of ERKs. Similar results were obtained with anti-phosphotyrosine Ab (4G10) blot
Anti-IgE Ab also induced modest phosphorylation of p38 MAPK at 1 min, and maximum phosphorylation was seen from 5–15 min (Fig. 4B). By 30 min, the phosphorylation decreased toward basal levels. Similar results were obtained with anti-phosphotyrosine Ab blot (data not shown). Taken together with previous results, p38 MAPK may contribute to mediator release in human basophils.

**FIGURE 3.** Kinetics of ERKs and cPLA₂ phosphorylation, histamine release, and LTC₄ generation following stimulation with anti-IgE Ab. Basophils were stimulated with anti-IgE (100 ng/ml) for the times indicated. Reactions were stopped with the addition of ice-cold PAG, and the cells were microfuged. Supernatants were collected for histamine and LTC₄ assays. Cell pellets were lyzed and subjected to Western blot as described in Materials and Methods. A, The Western blot is representative of two separate experiments. B, The values are the mean ± range of two experiments. The percent histamine release and LTC₄ release at 30 min were 62 ± 18% and 61 ± 24 pmol/10⁶ basophils. The intensities of the bands detected by anti-phospho-ERK are also plotted.

**FIGURE 4.** Kinetics of phosphorylation of p38 MAPK following stimulation with FMLP or anti-IgE Ab. Basophils were stimulated with FMLP (1 μM; A) or anti-IgE (100 ng/ml; B) for the times indicated. Reactions were stopped by the addition of ice-cold PAG. Cell pellets were lysed and subjected to Western blot as described in Materials and Methods. The anti-p38 MAPK Ab blot indicates equal protein loading. The Western blot is representative of two separate experiments.

As noted, MEKs (MAP kinase kinases) are dual specificity kinases that phosphorylate substrates (ERKs) on both Tyr and Thr residues and increase the catalytic activity of ERKs (51, 52). PD98059 is a well-known inhibitor of MEK (MAPK kinase). This compound blocks the activation (and phosphorylation) of MEK through an allosteric mechanism that does not involve inhibition of ATP binding (53). The potency and specificity of this inhibitor have been extensively documented in a variety of biologic systems (24, 53–55). It has been shown that IgE-mediated phosphorylation of ERK-2 and cPLA₂ are specifically inhibited by PD98059 in RBL-2H3 cells (24).

As shown in Fig. 5A, this also appears to be true in human basophils. The phosphorylation of ERKs induced by FMLP was prevented by PD98059, whereas the phosphorylation of tyrosine in
other proteins, including 38 (p38 MAPK)-, 62-, 100-, and 115-kDa proteins was not significantly affected by this compound. Similar to phosphorylated 38-kDa protein (p38 MAPK) in anti-phosphotyrosine Ab blot, phospho-p38 MAPK Ab blot also confirmed that PD98059 did not affect phosphorylation of p38 MAPK (Fig. 5B). The electrophoretic mobility shift of cPLA2 was also inhibited (Fig. 5A), suggesting that PD98059 specifically blocks the ERK-cPLA2 pathway but not the pathway for p38 MAPK in human basophils.

**FIGURE 5.** A. The effect of PD98059 on phosphorylation of ERKs and cPLA2 stimulated by FMLP. B. The effect of PD98059 on phosphorylation of p38 MAPK stimulated by FMLP. Basophils were preincubated with PD98059 (100 μM) or DMSO (1/1000 dilution) for 1 h and were stimulated with FMLP (1 μM) for 1 min. Reactions were stopped by the addition of ice-cold PAG, and the cells were centrifuged. The cell pellets were lysed, and the lysates were subjected to Western blot analysis as described in Materials and Methods. The anti-p38 MAPK Ab blot indicates equal protein loading. The Western blot shown is representative of two separate experiments. Open, closed, and hatched arrows indicate phosphorylated ERK-2, ERK-1, and p38 MAPK, respectively.

**FIGURE 6.** The effects of PD98059 on histamine release (A) and LTC4 generation (B) following stimulation with FMLP. Basophils were pre-treated with 0–100 μM PD98059 (closed symbols) or the same dilutions of DMSO (open symbols) for 1 h and then incubated in the presence or the absence of FMLP (1 μM) for 30 min. Reactions were terminated by addition of ice-cold PAG-EDTA. The values are the mean ± SEM of three experiments. The control percent histamine release and LTC4 generation (without PD98059 and DMSO) were 48 ± 9% and 53 ± 15 pmol/10⁶ basophils, respectively.

**Effect of PD98059 on histamine release and LTC4 release following stimulation with FMLP**

To evaluate the involvement of the MEK-ERK pathway in LTC4 generation and histamine release, the effect of PD98059 on the secretion of these mediators was tested. For these experiments, basophil-enriched suspensions freshly prepared from blood or cell suspensions derived from leukocyte packs were used (purity ranged from 2–75%). PD98059 did not affect FMLP-induced histamine release (Fig. 6A). In contrast, PD98059 inhibited FMLP-induced LTC4 release (76 ± 5% inhibition at 100 μM and 64 ± 16% inhibition at 10 μM), whereas the same dilution of DMSO did not affect the release of LTC4 (Fig. 6B). The IC₅₀ for the inhibition of LTC4 by PD98059 was approximately 2 μM, comparable to values (2–4 μM) reported for the inhibition of MEK and ERK in other cell types (53–55). The effect of PD98059 on FMLP-induced generation of free AA was also examined. In this series of experiments, the cells were stimulated with FMLP for 5 min, since this amount of time results in maximal free AA generation (Fig. 2B). FMLP-induced free AA generation was also inhibited by PD98059 (57 ± 8% inhibition at 100 μM and 41 ± 7% at 10 μM compared with DMSO control; n = 3). Taken together, these results suggest that FMLP activation uses the MEK-ERK pathway for free AA/LTC4 generation but not histamine release.
Kinetic studies, shown in Fig. 3, suggested that the anti-IgE-mediated activation of ERKs may be associated with histamine release as well as LTC4 release. Further, ERKs also regulate several transcriptional factors (14) that may potentially have a role in cytokine production from human basophils. The effects of PD98059 on anti-IgE-mediated secretion of histamine, LTC4, and IL-4 were examined (Fig. 7). Based on previous experiments to determine the optimal concentrations of anti-IgE to induce histamine and LTC4 release (3), or IL-4 production, 100 ng/ml (for histamine and LTC4 release) and 10 ng/ml (for IL-4 production) of anti-IgE were used. As observed for FMLP activation, anti-IgE-induced LTC4 was inhibited by PD98059 (85 ± 10% inhibition at 100 μM and 80 ± 14% inhibition at 10 μM), with an IC50 of approximately 2 μM. In contrast, histamine release (11 ± 10% inhibition at 100 μM) and IL-4 production (37 ± 7% inhibition at 100 μM vs 14 ± 12% inhibition with DMSO control) were only marginally inhibited by PD98059, suggesting that ERKs are not important in histamine release or IL-4 production.

Discussion

ERKs have been shown to phosphorylate and activate cPLA2, resulting in its increased enzymatic activity (16). This phosphorylation of cPLA2 along with its translocation to the membrane (mediated by the increase in cytosolic calcium) are required for its full activity (56, 57). Indeed, our previous studies showed that the accumulation of free AA and the generation of LTC4 followed an increase in cytosolic calcium and the phosphorylation of cPLA2, suggesting that the activation of cPLA2 provides free AA for LTC4 generation in human basophils (26). At that time, we were unable to link cPLA2 involvement, since a selective inhibitor of cPLA2 was not available. In the present study we have provided further evidence to support the concept that cPLA2 has a role in LTC4 generation by implicating a MEK-ERK pathway. These data also suggest that ERKs regulate cPLA2 activation for LTC4 generation in human basophils. First, the phosphorylation of ERK-1/2 preceded or coincided with the phosphorylation of cPLA2 and the release of LTC4 in both FMLP- and anti-IgE-mediated stimulation, demonstrating a kinetic association among ERK phosphorylation, cPLA2 phosphorylation, and LTC4 release. We did not detect a preference for ERK-1 and ERK-2 phosphorylation following stimulation with either FMLP or anti-IgE. It should be noted that cPLA2 remained phosphorylated within the time frame examined (15 min for FMLP and 30 min for anti-IgE), although phosphorylation of ERKs decreased toward the basal level following stimulation with anti-IgE or FMLP. Similar results have been reported for cPLA2, phosphorylation in other cell types. In endothelial cells, fibroblast growth factor-mediated phosphorylation of p42/44 MAPK (ERK-2) is also more transient than phosphorylation of cPLA2 (58). ERKs and cPLA2 might be differentially dephosphorylated by different phosphatases after phosphorylation (58). Second, a selective inhibitor of the MEK-ERK pathway, PD98059, inhibited LTC4 release induced by FMLP and anti-IgE. The phosphorylations of ERK-1/2 and cPLA2 and the generation of free AA induced by FMLP were also inhibited by PD98059, without affecting other tyrosine-phosphorylated proteins (including p38 MAPK). Collectively, these results suggest a causal relationship between ERK activation and LTC4 generation (presumably by the activation of cPLA2) in human basophils. These results also indicate that regulation of cPLA2 for generating AA in human basophils is distinct from that in platelets (30). In collagen-stimulated platelets, p38 MAPK, but not ERKs, might regulate activation (phosphorylation) of cPLA2. PD98059 also fails to inhibit phosphorylation of cPLA2 induced by collagen (30). The activation of cPLA2 by p38 MAPK may be restricted to certain cell types or stimuli. In other cell types, ERKs are thought to regulate activation of cPLA2 (16, 58, 59), which are also supported by the results using PD98059 (24, 60).

FMNL-induced histamine release preceded the phosphorylation of ERKs and was unaffected by inhibition of the MEK-ERK pathway by PD98059. These results strongly suggest that the activation of ERKs is not involved in FMLP-mediated degranulation. In contrast, the release of histamine and LTC4 induced by anti-IgE was consistent with the time course of phosphorylation of ERKs. The kinetics of histamine release induced by anti-IgE are slower than those of FMLP (1, 47). Previous data suggested that the sustained elevation in cytosolic calcium that follows IgE-mediated stimulation was associated with histamine release. However, histamine release only follows elevations in cytosolic calcium after an interval of 2–3 min, suggesting the need for signaling events that are
slower to evolve. This rate-limiting event for histamine release remains unclear, but the slower activation of ERK-1/2 suggested one possibility. However, the inhibition of the MEK-ERK pathway by PD98059 only marginally affected histamine release, suggesting little causal relationship between histamine release and ERK activation. Taken together with the results for FMLP, ERKs appears to have little role in any receptor-mediated degranulation in human basophils. It is useful to note that similar observations have been made in RBL-2H3 cells. PD98059 inhibited the release of AA; however, degranulation (hexosaminidase release) was only slightly affected in RBL-2H3 cells (24).

The activation of ERKs also induces c-Fos expression (which constitutes part of the AP-1 complex) (15), the inactivation of IκB (61), and enhanced binding activity of cytokine transcriptional factors such as NF-κB and activating protein-1 (62). In RBL-2H3 cells, IgE-mediated TNF-α production is inhibited by PD98059, suggesting that the MEK-ERK pathway is involved in the IgE-dependent production of this cytokine (24). In contrast, the same compound does not affect the similar IgE-dependent production of TNF-α in distinct cells (MC/9 murine mast cells), suggesting that the MEK-ERK pathway does not regulate this cytokine. However, the other MAPKs, JNKs, have been shown to mediate this cytokine (25). These conflicting data suggest that roles of ERKs for production of cytokines appear to be dependent on cell type or species. Therefore, it is important to determine the role of ERKs in normal human cells. Human basophils produce IL-4, and there is no evidence of TNF-α production from human basophils. We observed only minor inhibition of IL-4 production by PD98059 at 100 μM in human basophils. Furthermore, the transient activation of ERK-1/2 is not consistent with the time scale of IL-4 secretion. Since disaggregating cell surface cross-links at times greater than 30 min stops IL-4 secretion, there appears to be a need for sustained signaling, and the transient nature of ERK-1/2 phosphorylation is not consistent with this requirement. Taken together, the activation of ERKs may not be involved in IgE-mediated IL-4 production from human basophils. This belief is further supported by the evidence that FMLP, which induces the phosphorylation of ERKs, is a poor stimulus of IL-4 production from human basophils (3). At this time, there is more evidence to suggest that a transcription factor resembling the nuclear factor of activated T cells (NF-AT) has a greater role in IL-4 generation by these cells (63). It remains possible that the activation of ERKs may contribute to the synthesis of other proteins.

We also demonstrated the expression and phosphorylation of p38 MAPK in human basophils. Kinetics studies revealed that phosphorylation of p38 MAPK is slightly faster than that of ERKs following stimulation of either anti-IgE or FMLP, suggesting that p38 MAPK may regulate or modulate mediator release in human basophils. The inhibition of ERK pathway by PD98059 abrogated AA/LTC4 generation without affecting phosphorylation of p38 MAPK. These results may suggest that ERKs rather than p38 MAPK are essential for the pathway generating AA/LTC4. The precise role of p38 MAPK in mediator release remains to be elucidated. We are currently investigating this issue.

In summary, the activation of ERKs appears to be a key step necessary for the phosphorylation and activation of cPLA2, which is important for liberating free AA for LTC4 generation in human basophils. However, ERKs have no apparent role in supporting histamine and IL-4 release.

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19. Hutchcroft, J. E., R. L. Geahlen, and G. G. Deanin. 1992. Phosphorylation of transcription factor NF-AT has a greater role in IL-4 generation by these cells (63). It remains possible that the activation of ERKs may contribute to the synthesis of other proteins.

We also demonstrated the expression and phosphorylation of p38 MAPK in human basophils. Kinetics studies revealed that phosphorylation of p38 MAPK is slightly faster than that of ERKs following stimulation of either anti-IgE or FMLP, suggesting that p38 MAPK may regulate or modulate mediator release in human basophils. The inhibition of ERK pathway by PD98059 abrogated AA/LTC4 generation without affecting phosphorylation of p38 MAPK. These results may suggest that ERKs rather than p38 MAPK are essential for the pathway generating AA/LTC4. The precise role of p38 MAPK in mediator release remains to be elucidated. We are currently investigating this issue.

In summary, the activation of ERKs appears to be a key step necessary for the phosphorylation and activation of cPLA2, which is important for liberating free AA for LTC4 generation in human basophils. However, ERKs have no apparent role in supporting histamine and IL-4 release.

References


